# Telomeres in ecology and evolution: Hypotheses and connections between telomere length, infections and life history trade-offs

Gomez Blanco, David

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## Hypotheses and connections between telomere length, infections and life history trade-offs

DAVID GÓMEZ BLANCO
DEPARTMENT OF BIOLOGY | FACULTY OF SCIENCE | LUND UNIVERSITY

# List of papers

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# Telomeres in ecology and evolution

## Hypotheses and connections between telomere length, infections and life history trade-offs

David Gómez Blanco


LUND
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DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Science at Lund University to be publicly defended on 28th of April at 13:00 in Blue Hall, Ecology Building, Dept. of Biology, Sölvegatan 37, Lund, Sweden

*Faculty opponent*
Prof. Mats Olsson

Göteborgs universitet, Gothenburg, Sweden

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**Abstract:** Studying life history strategies is crucial for understanding the diversity among organisms. The trade-offs in traits related to survival, self-maintenance and reproduction play an important role in the variation of life histories and can be adjusted in response to environmental and physiological factors. Telomeres, can serve as molecular markers linking these factors and ultimately affecting fitness. Telomeres naturally shorten with age and stress, such as diseases, and when they become too short, they trigger cellular senescence and ageing. However, some telomere restoration mechanisms can counteract this process. In this thesis, I have taken advantage of the longitudinal study on the great reed warblers at Lake Kvismaren conducted for 40 years. I measured telomere length using the quantitative PCR method, and to investigate the ecological and evolutionary implications of parasites, I used the multiplex PCR method to detect the presence of avian malaria parasites in the birds' blood. In the first part of this thesis, I and my collaborators present a summary of telomere hypotheses that apply to the fields of ecology and evolution, grouping these hypotheses based on their research context and hierarchical similarities. Furthermore, we identify gaps in knowledge, such as the lack of hypotheses that can clarify telomere elongation patterns in the wild. As a result, we propose and expand on a novel hypothesis that highlights the significance of elongation patterns. The second section of the thesis aimed to test some of the assumptions of the previous hypotheses. The studies were focused on examining the existence of a lower threshold for telomere length and revealed that individuals with critically short telomeres disappeared from the population at a greater rate than those with longer telomeres. Furthermore, life stressors, such as infections, had an impact on these selection patterns. In this thesis, I also found that even in early-life a considerable percentage of individuals can display a net increase in telomere length (telomere elongation), and this prospect was dependent on infection and the current telomere length. Finally, this thesis demonstrates that various factors including age, malaria status, and harem size can predict sex-specific dynamics of telomere length. These telomere dynamics could potentially provide indications of the individual's phenotypic quality. In summary, this thesis has contributed to our understanding of telomere dynamics in the wild, particularly concerning the complex interactions between telomere length, infections and life history trade-offs. It has addressed some gaps in our knowledge of telomere biology and provided insights into important yet under-explored areas, like telomere elongation patterns. The thesis highlights the need for further research into telomere elongation events, the impact of short telomeres on individual life histories, and the potential use of telomeres as a biomarker for individual quality or as a measure of environmental stressors faced.

**Key words:** telomeres, life history, telomere elongation, telomere shortening, early-life telomere length, critical threshold, malaria infection, great reed warbler, Acrocephalus arundinaceus.

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*“Nothing in biology makes sense except in the light of evolution”*

*Theodosius Dobzhansky*

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Furthermore, I have contributed as author of a paper that was published during my PhD studies, but it should be noted that this paper is NOT part of this thesis.

I. **Gómez-Blanco, D.**, Santoro, S., Borrás, A., Cabrera, J., Senar, J. C., & Edelaar, P. (2019). Beak morphology predicts apparent survival of crossbills: due to selective survival or selective dispersal? Journal of Avian Biology, 50 (12).

# Author contributions

I. Conception of the idea – equal (MTo, DH). Data collection – all authors contributed equally. Interpretation and discussion of the results – all authors contributed equally. Writing the first draft – lead (MTo). Edition and revision of the manuscript – lead (MTo, DH), supporting (DGB, AH, ML, JMN, MTa, YX). Acquisition of funding – lead (DH), supporting (AH, MTa).

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# Abstract

Studying life history strategies is crucial for understanding the diversity among organisms. The trade-offs in traits related to survival, self-maintenance and reproduction play an important role in the variation of life histories and can be adjusted in response to environmental and physiological factors. Telomeres, which are repetitive DNA sequences found at the ends of chromosomes, can serve as molecular markers linking these factors and ultimately fitness. Telomeres naturally shorten with age and stress, such as diseases, and when they become too short, they trigger cellular senescence and ageing. However, some telomere restoration mechanisms can counteract this process.

In this thesis, first I have adopted a theoretical approach to review the telomere research field and develop hypotheses for future exploration. Then, I also included an empirical section that aimed to evaluate and test some of these hypotheses' general patterns using data from a wild population of the polygynous bird species, the great reed warbler (*Acrocephalus arundinaceus*). I have taken advantage of the longitudinal study conducted for 40 years at Lake Kvismaren that have collected a comprehensive dataset on life history, behaviours and fitness components, along with a sampling of all the bird born and breeding in the area. I measured telomere length using the quantitative PCR method, and to investigate the ecological and evolutionary implications of parasites, I used the multiplex PCR method to detect the presence of avian malaria parasites in the birds' blood.

In the first part of this thesis, I and my collaborators present a summary of telomere hypotheses that apply to the fields of ecology and evolution. We also classify and group these hypotheses based on their research context and hierarchical similarities. Furthermore, we identify gaps in the knowledge, such as the lack of hypotheses that can clarify telomere elongation patterns in the wild. As a result, we propose and expand on a novel hypothesis that highlights the significance of elongation patterns and encourages further exploration in this area of telomere dynamics.

The second section of the thesis aimed to test some of the assumptions made in the previous hypotheses. The studies were focused on examining the existence of a lower threshold for telomere length and I revealed that individuals with critically short telomeres disappeared from the population at a greater rate than those with longer telomeres. Furthermore, life stressors such as infections had an impact on

these selection patterns. In this thesis, I also found that even in early-life a considerable percentage of individuals can display a net increase in telomere length (telomere elongation), and this prospect was dependent on factors like infection and the current telomere length. Finally, this thesis demonstrates that various factors including age, malaria status, and harem size can predict the dynamics of telomere length in male and female great reed warblers. These sex-specific telomere dynamics could potentially provide indications of the individual's phenotypic quality.

In summary, this thesis has contributed to our understanding of telomere dynamics in the wild, particularly concerning the complex interactions between telomere length, infections and life history trade-offs. It has addressed some gaps in our knowledge of telomere biology and provided insights into important yet under-explored areas, like telomere elongation patterns. The thesis highlights the need for further research into telomere elongation events, the impact of short telomeres on individual life histories, and the potential use of telomeres as a biomarker for individual quality or as a measure of environmental stressors faced.

# Popular science summary

In an ideal world with infinite resources, individuals would maximize all their attributes, live forever and produce many offspring of very high quality. However, life is about making choices, and often these choices involve sacrifice, because investing more in one aspect may come at the expense of another. Those compromises are called “trade-offs” in biology. For example, when it comes to reproduction, an individual may choose to invest heavily in current reproduction and produce many offspring in a short period of time, but this may reduce its chances of survival and reproduction in the future as it depletes its resources and energy reserves. Understanding how individuals make these decisions can be difficult because many complex aspects, such as environmental factors and internal physiological states, can interact to influence these preferences.

One thing that might help us understand this better is the so-called telomeres. These are special parts of DNA that sit at the ends of chromosomes, the condensed structure of DNA, to protect important genetic information from damage or wear, much like the protective caps at the ends of shoelaces. The tricky part is that telomeres get a little shorter each time a cell divides because the enzyme that copies DNA during cell division cannot copy all the way out to the end of the chromosome. Eventually, the telomeres become too short (i.e., below a critical threshold), so the cell can no longer divide and becomes inactive or dies. As we get older or are exposed to stress, our bodies accumulate damaged cells and tissues. This process is one of the main reasons why we age and can lead to diseases like cancer, heart disorders and nerve degeneration.

But wait a minute - not all is necessarily lost. There is some good news: our body has mechanisms to protect and restore telomeres. The most important of these mechanisms is the enzyme telomerase. It is most active during growth in early-life when cells need to divide frequently, but in some species, it can also be activated under certain circumstances in adult life. Some researchers have suggested that adult animals in the wild can increase their telomere length, although the evidence for this is still somewhat controversial.

Restoring telomere length can have many benefits for the individual, but there are also some drawbacks to consider. For example, and here they come into play again the biological trade-offs we have already talked about, restoring telomeres may need

resources that could be used for other important functions. In addition, restoring telomeres and thus making cells “immortal” increases the risk of accumulating mutations that cause cancer, which can be a dangerous problem. Why should individuals consider telomere restoration? When is the right time to do it? Which individuals should restore their telomere length? And how much should they be prolonged?

We hypothesize that individuals will need to restore their telomeres differently depending on the challenges they face, such as hard environmental conditions, different reproductive costs, or infections. It is possible that only some individuals will have access to enough resources to be able to restore their telomeres. Thus, maybe only the very best individuals in a population will be able to elongate their telomeres. Maybe it would be more likely to occur before an upcoming hard time, or after a stressful period when things get better and there are more resources, just like spring follows after winter. Another idea we had is related to what we mentioned earlier: too short telomeres can be problematic for cells and organs, but telomere elongation is costly. Therefore, individuals will only elongate their telomeres when necessary, as a last resort (i.e., when their telomeres are too close to the critical threshold and the benefits compensate the costs). This usually occurs in poor-quality individuals suffering rapid telomere shortening, or more slowly, when individuals get older.

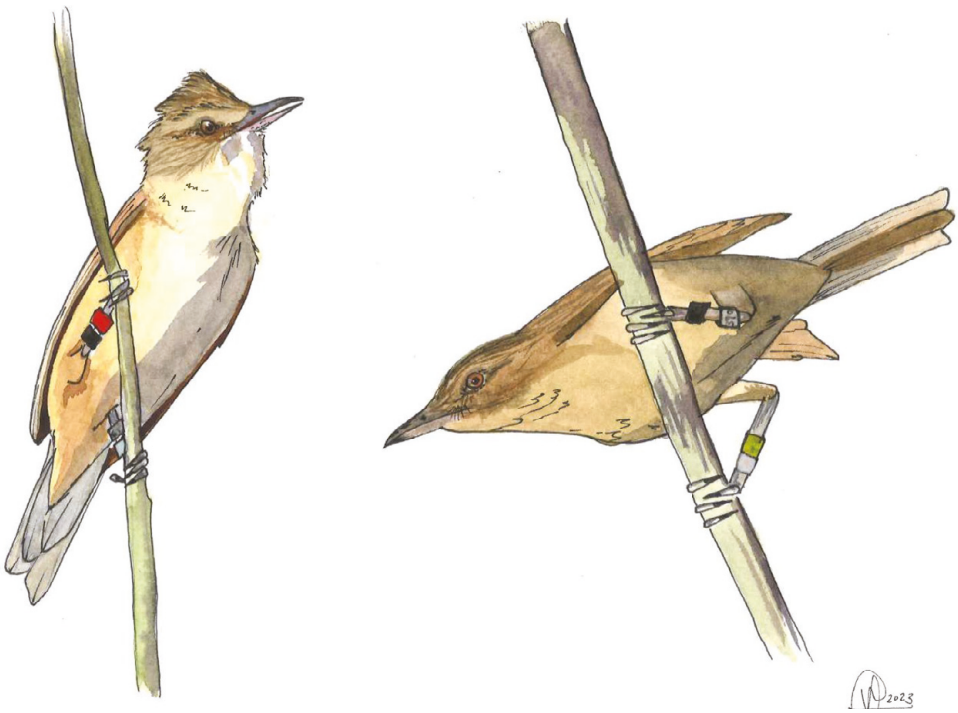
As you can see, our hypotheses rely on the concept of a lower critical threshold in telomere length. However, it is not clear if such a critical length exists... Can we find evidence for a critical threshold in telomere length in natural populations? Also, we have argued that the decision whether to restore telomeres or not might depend on certain circumstances. Can we identify some of the crucial factors that influence these trade-offs? For example, disease! – How can infections with parasites have an impact on how quickly organisms age? Are changes in telomere length over time also related to factors like age, sex, health, or markers of individual quality, like the number of breeding partners? Let us explore all the ideas and questions using actual data from the wild!

To do this, we have used all the information we have gathered over the last 40 years studying the great reed warbler population in Lake Kvismaren (Sweden). Over this long period, the project has helped us to better understand the lifestyle and behaviour of this species, but we have also collected more than 5,000 DNA samples from all adult birds living and breeding in the lake, as well as from their chicks. To measure telomere length, we needed an accurate method that could be used with large sample sizes, which is why we chose quantitative PCR. To check the health status of the birds, we focused on the parasites that cause malaria disease in birds. We detected and identified their presence in the birds' blood using a molecular method called multiplex PCR.

We examined the telomere length of the birds when they were born and found that the birds with shorter telomeres generally did not return to our population after wintering in Africa and migrating to the breeding grounds. This could indicate that they died since the typical pattern of the Kvismaren great reed warblers is that they usually return after migration to breed at their place of birth. Also, even in the birds that return to Kvismaren to breed, we have found that those born with the shortest telomeres disappear from the population much more quickly. This effect is even more severe when birds are infected with malaria parasites. So it could be that there is selection against birds with too short telomeres, i.e., near the critical threshold, and that stressors such as disease increase the risk of reaching this threshold earlier in life. Also, we found that some birds were able to elongate their telomeres between years. It appears that a larger proportion of birds born with short telomeres showed telomere elongation over their first year of life, perhaps as a last resort before reaching the critical threshold. We also found that birds without malaria parasites were able to elongate their telomeres more often than malaria infected birds, possibly a result of that the infected birds had to use their resources to fight the disease. We were intrigued by these findings as they fitted very well with our earlier ideas.

Telomeres do not elongate in all animals. Instead, in most animals, telomeres get shorter and shorter at older life stages. We used statistical models to study the changes in telomere length between years and found that the rate of shortening depends on several factors. For example, we found that age was important because young birds lose telomeres faster than older ones. Moreover, infection with malaria parasites caused telomere shortening to proceed much faster, and this was more pronounced in females. Did you know that the great reed warbler can have more than one pair-mate during the breeding season? Our statistical model predicted that the number of mates a bird has in the current year is related to how much his telomeres shortened from the previous year. Thus, those birds whose telomeres had shortened to a large degree did not manage to get any or just a few breeding mates.

Overall, my thesis work has revealed evidence that there seems to exist a lower critical threshold in telomere length that apparently may affect the lifespan of individuals. The decision to elongate telomeres or not could be a strategy for individuals to improve their chances of survival and reproduction, but this strategy may depend on certain factors, such as infection. In addition, changes in telomere length throughout life are related to age, sex, infection and an index of individual quality as the harem size. However, more research is needed to fully understand the long-term effects of telomere dynamics, including telomere elongation, on patterns of ageing in natural populations.



- Emblematic great reed warblers in Kvismaren (left: 9V-08 male, right: 0V-39 female) - Gift from Violeta Caballero López

# Populärvetenskaplig sammanfattning

I en perfektl värld med oändliga resurser skulle individer maximera alla sina attribut, leva för evigt och producera många avkommor av mycket hög kvalitet. Men livet handlar om att göra val, och ofta innebär dessa val en uppoffring, eftersom investering i en egenskap sker på bekostnad av en annan. Dessa kompromisser kallas "trade-offs" inom ekologi och biologi. Till exempel, när det gäller reproduktion kan en individ välja att göra en stor investering i nuvarande reproduktion och producera många avkommor på kort tid. Men denna stora investering kan minska individens chanser att överleva och reproducera i framtiden eftersom den redan förbrukat en stor del av sina resurser. Det kan vara svårt att förstå hur individer fattar dessa beslut eftersom många komplexa faktorer samverkar, så som miljöfaktorer och interna fysiologiska tillstånd, och påverka dessa preferenser.

En sak som kanske kan hjälpa oss att förstå detta bättre är de så kallade telomererna. Dessa utgör särskilda DNA mönster och är placerade på kromosomernas ändar, dvs. den kondenserade strukturen av DNA, och har till uppgift att skydda den viktiga genetisk informationen från skador, på samma sätt som ändarna av skosnören skyddas av plast eller metall kapslar. Det knepiga är att telomererna blir lite kortare varje gång en cell delar sig eftersom enzymet som kopierar DNA under celldelningen inte kan kopiera hela vägen till kromosomens ände. Så småningom blir telomererna så korta att de hamnar under ett kritiskt tröskelvärde då kromosomerna skadas och cellen därför inte längre kan dela sig utan blir inaktiv eller dör. När vi åldras eller utsätts för stress, ansamlas skadade celler och vävnader i våra kroppar. Denna process är en av de främsta anledningarna till att vi åldras och kan leda till sjukdomar som cancer, hjärtsjukdomar och nervdegeneration.

Men vänta lite, allt är inte förlorat, det finns några goda nyheter. Våra kroppar har mekanismer för att skydda och återställa telomerernas längd. Den viktigaste av dessa mekanismer inbegriper enzymet telomeras. Det är mest aktivt under kroppstillväxt perioden tidigt i livet när celler behöver dela sig mycket. Men hos vissa arter kan telomeras även aktiveras under speciella omständigheter under vuxenlivet. Forskare har föreslagit att vuxna djur i naturen kan öka sin telomerlängd, även om bevisen fortfarande är något kontroversiella.

Att återställa telomerlängden kan ha många fördelar för individen, men det finns också nackdelar. Till exempel, och här kommer de biologiska "trade-offs" in i bilden

som vi nämnde tidigare, kan förlängning av telomererna kräva resurser som kunde använts för andra viktiga funktioner. Vidare, genom att återställa telomerer och göra celler odödliga, så ökar risken för att mutationer som orsakar cancer ackumuleras, vilket kan vara farligt för individens överlevnad. Varför ska någon någonsin överväga att förlänga sina telomerer? När kan det vara rätt tidpunkt i livet att göra det? Vilka typer av individer kan förväntas förlänga sina telomerer? Och hur mycket skall de förlängas?

Vi tror att olika typer individer kan behöva göra olika överväganden när det gäller förlängning av sina telomerer beroende på de utmaningar de står inför, t.ex. vilka miljöförhållanden de lever under, deras kostnader för reproduktion eller om de har infektioner eller inte. Det är möjligt att bara vissa individer har det överskott av resurser som krävs för att kunna förlänga sina telomerer. Kanske är det så att bara de allra bästa individerna i en population kommer att kunna förlänga sina telomerer. Det kan t.ex. ske inför en period med begränsade resurser och svåra miljöförhållanden eller efter en stressig period när saker och ting blivit bättre igen och mer resurser tillgängliga, precis som den milda våren följer efter den hårda vintern. En annan idé som är relaterad till vad som nämndes tidigare, för korta telomerer kan vara problematiskt för celler och organs funktion, men å andra sidan är även förlängning av telomerer en kostsam process. Därför kan man tänka sig att individer bara kommer att förlänga sina telomerer det när det är nödvändigt, till exempel som en sista utväg när telomererna är nära den kritiska tröskeln och fördelarna med telomerförlängning därmed uppväger kostnaderna. Detta borde oftare hända med individer av dålig kvalitet som utsätts för snabb telomerförkortning eller äldre individer som trots långsam telomerförkortning ändå till slut kommer nära den kritiska tröskeln och därför tvingas att förlänga sina telomerer.

Som du kan se bygger våra hypoteser på konceptet att det finns en nedre kritisk tröskel i telomerlängd. Menvi vet ännu inte om en sådan nedre tröskel för telomerläng existerar. Kan vi hitta tecken på att en sådan tröskel existerar i naturliga populationer? Dessutom föreslogs det ovan att beslutet om att återställa telomererna eller inte kan variera beroende på omständigheterna. Kan vi identifiera några av dessa avgörande faktorer som påverkar sådana avvägningar? Till exempel sjukdom påverka dessa överväganden? Hur kan infektioner påverka hur fort vi åldras? Är förändringar i telomerlängd över tid relaterade till faktorer som ålder, kön, hälsa eller hårt fysiskt arbete under uppväxten? Låt oss undersöka dessa idéer och frågor med hjälp av faktiska data från naturliga populationer.

För att göra just detta har jag och mina medarbetare använt information som samlats under 40 års studier av trastsångarpopulationen i sjön Kvismaren (Sverige). Under denna långa period har projektet hjälpt oss att förstå artens livsstil och beteenden, men det har också samlats in mer än 5000 DNA prover från alla vuxna individer

som lever och häckar vid sjön, samt från deras ungar. För att mäta telomerernas längd behövde vi använda en metod som kunde användas storskaligt och som samtidigt var tillräckligt noggrann. Vi valde därför att använda den så kallade qPCR metoden. För att kontrollera fåglarnas hälsa fokuserade vi på förekomst av malariaparasiter. Vi identifierade malariaparasiterna i fåglarnas blod med hjälp av en molekylärgenetiskmetod som kallas "multiplex PCR".

När vi studerade unga trastsångares telomerlängd så fann vi att de med korta telomerer redan vid 9 dagars ålder tenderade att inte återvända till vår studiepopulation efter sin flyttning till och övervintring i Afrika, vilket kan tyda på att de dött eftersom denna art normalt återvänder till sin födelseplats efter flyttningen. Även bland de individer som återvände för att häcka i Kvismaren så upptäckte vi att de som hade kortast telomerer som boungar försvann mycket snabbare från populationen. Denna effekt var ännu tydligare hos de individer som var infekterade av malaria. Dessa studier visar att det verkar finnas selektion mot fåglar med korta telomerer, dvs mot de som är nära den kritiska tröskeln, och att stressfaktorer så som sjukdom ökar risken för att nå detta tröskelvärde tidigare i livet. Men vi fann också att vissa fåglar kunde förlänga sina telomerer från ett år till ett annat. Det var speciellt de individer som hade mycket korta telomerer som boungar som oftare förlängde sina telomerer, kanske som en sista utväg innan de nått den kritiska tröskeln. Vi märkte också att friska fåglar utan malaria förlängde sina telomerer mer än de individer som var infekterade av malaria, kanske beroende på att de malariainfekterade fåglarna behövde använda sina resurser för att bekämpa sjukdomen. Det var verkligen spännande att se att våra studier av de vilda trastsångarna styrkte de idéer vi tidigare föreslagit!

Inte alla förlänger sina telomererna – hos de flesta individer blir telomererna kortare och kortare ju äldre de blir. Vi använde statistiska modeller för att studera hur olika faktorer påverkar förändringarna i telomerlängden hos trastsångare och fann att denna kunde bero på flera faktorer. Till exempel förkortas unga fåglars telomerer snabbare än äldre. Vi upptäckte också att hanar och honor uppvisar samma takt i sin telomerförkortning. Vidare innebar infektion av malariaparasiter att telomererna förkortades mycket snabbare. Visste du att trastsångaren kan ha fler än en partner under häckningssäsongen? Vår statistiska modell visade att en individs antal partners är kopplat till hur mycket deras telomerer förkortades under det föregående året. Så, de fåglar som utsatts för en större telomerförkortning sedan förra året fick ingen eller färre partners medan de utan telomerförkortning oftare fick flera partners.

Sammantaget visar resultaten i denna avhandling att det verkar finnas ett kritiskt tröskelvärde för telomerlängd som kan påverka individernas livslängd. Beslut om att förlänga sina telomerer eller inte kan vara en strategi som används av individer för att förbättra sina överlevnads- och reproduktionschanser, och denna strategi

verkar kunna skilja sig åt mellan olika situationer och t.ex. vara kopplat till infektioner. Dessutom kan förändringar i telomerlängd mellan år vara kopplade till ålder, kön, hälsa och investering i reproduktion. Det krävs dock fortfarande mer forskning för att få en mer djupgående förståelse av de långsiktiga effekterna av telomerers förkortning och förlängning och hur detta kan kopplas till åldrande i naturliga populationer.

- Trastsångare i vassen -
Gåva från Agnes Erland Hanson

# Resumen de divulgación científica

En un mundo ideal con recursos infinitos, los individuos maximizarían todos sus atributos, vivirían para siempre y producirían muchas crías de muy alta calidad. Sin embargo, la vida se trata de hacer elecciones, y a menudo estas elecciones implican sacrificios, ya que invertir más en un aspecto puede tener un coste en otro. Esos compromisos se llaman "trade-offs" en biología. Por ejemplo, cuando se trata de la reproducción, un individuo puede elegir invertir mucho en reproducirse en ese momento y producir muchas crías en un corto período de tiempo, pero esto puede reducir sus posibilidades de supervivencia y reproducción en el futuro, ya que agota sus recursos y reservas de energía. Comprender cómo los individuos toman estas decisiones puede ser difícil porque muchos aspectos, como los factores ambientales y el estado fisiológico interno, pueden interactuar de maneras complejas para influir en esta toma de decisiones.

Una cosa que podría ayudarnos a comprender mejor estos compromisos son los llamados telómeros. Los telómeros son partes especiales del ADN que se encuentran en los extremos de los cromosomas, la estructura condensada del ADN, para proteger la información genética importante de daños o desgaste, al igual que los herretes en los extremos de los cordones de los zapatos. El problema es que los telómeros se acortan un poco cada vez que una célula se divide, porque la enzima que copia el ADN durante la división celular no puede copiar hasta el final del cromosoma. Eventualmente, los telómeros se vuelven demasiado cortos (es decir, por debajo de un umbral crítico), por lo que la célula ya no puede dividirse y se vuelve inactiva o muere. A medida que envejecemos o estamos expuestos a estrés, nuestro cuerpo acumula células y tejidos dañados. Este proceso es una de las principales razones por las que envejecemos y puede conducir al desarrollo de enfermedades como cáncer, problemas cardíacos o neurodegeneración.

Pero espera un momento - no todo está perdido necesariamente. Hay buenas noticias: nuestro cuerpo tiene mecanismos para proteger y restaurar los telómeros. El más importante de estos mecanismos es la enzima telomerasa. Es más activa durante el crecimiento en las primeras etapas de la vida, cuando las células necesitan dividirse con frecuencia, pero en algunas especies también puede activarse en ciertas circunstancias en la vida adulta. Algunos investigadores han sugerido que animales salvajes pueden aumentar la longitud de sus telómeros en la vida adulta, aunque las evidencias todavía son algo controvertidas.

Restaurar la longitud de los telómeros puede tener muchos beneficios para el individuo, pero también hay algunos inconvenientes a considerar. Por ejemplo, y aquí vuelven a entrar en juego los trade-offs biológicos que ya hemos debatido, ya que la restauración de los telómeros puede necesitar de recursos que podrían ser utilizados para otras funciones importantes. Además, la restauración de los telómeros, y por ende la "inmortalización" de las células, aumenta el riesgo de acumulación de mutaciones que pueden ser causa de cáncer, lo que puede ser un problema. ¿Por qué deberían los individuos considerar la restauración de los telómeros?, ¿Cuándo es el momento adecuado para hacerlo?, ¿Qué individuos deberían restaurar su longitud de telómeros?, ¿Y cuánto deberían ser elongados?

Nosotros hipotetizamos que los individuos necesitarán restaurar sus telómeros de manera diferente dependiendo de los desafíos que enfrenten, como condiciones ambientales adversas, el coste de reproducción o infecciones. Es posible que solo algunos individuos tengan acceso a suficientes recursos puedan restaurar sus telómeros. Por lo tanto, quizás solo los mejores individuos en una población serán capaces de elongar sus telómeros. Tal vez es más probable que ocurra antes de un periodo peliagudo que se avecina o después de ese período estresante cuando las cosas mejoran y hay más recursos, como la primavera sigue al invierno. Otra idea que tenemos está relacionada con lo que mencionamos anteriormente: por un lado, los telómeros demasiado cortos pueden ser problemáticos para las células y los órganos, pero por otro lado elongar los telómeros es costoso. Por lo tanto, los individuos solo elongarán sus telómeros cuando sea necesario, como último recurso (es decir, cuando sus telómeros estén demasiado cerca del umbral crítico y los beneficios compensen los costes). Esto suele ocurrir en individuos de baja calidad que están sufriendo un acortamiento rápido de los telómeros, o más lentamente cuando los individuos envejecen.

Como puede ver, nuestras hipótesis se basan en el concepto de un umbral crítico inferior para la longitud de los telómeros. Sin embargo, no está claro si tal longitud crítica existe... ¿Podemos encontrar evidencias de un umbral crítico en poblaciones naturales? Además, hemos argumentado que la decisión de restaurar los telómeros o no puede depender de ciertas circunstancias. ¿Podemos identificar algunos de estos factores cruciales que influyen en estos compromisos? Por ejemplo, ¡enfermedades! – ¿Cómo las infecciones con parásitos pueden tener un impacto en la rapidez con que envejecemos los organismos?, ¿Los cambios en la longitud de los telómeros a lo largo del tiempo también están relacionados con factores como la edad, el sexo, la salud o marcadores de la calidad individual, como el número de pajeras reproductoras? ¡Vamos a examinar todas estas ideas y preguntas utilizando datos reales de poblaciones naturales!

Para hacer esto, nosotros utilizamos toda la información que hemos recopilado en los últimos 40 años estudiando la población de carricero tordal en el lago Kvismaren

(Suecia). Durante este largo período, el proyecto nos ha ayudado a comprender mejor el estilo de vida y el comportamiento de esta especie, pero también hemos recolectado más de 5.000 muestras de ADN de todos los adultos que viven y se reproducen en el lago, así como de sus crías. Para medir la longitud de telómeros, necesitábamos un método preciso que pudiera procesar muchas muestras, por lo que elegimos PCR cuantitativa. Para verificar el estado de salud de las aves, nos enfocamos en los parásitos que causan la enfermedad de la malaria en las aves. Nosotros detectamos e identificamos su presencia en la sangre de las aves usando un método molecular llamado PCR múltiple.

Cuando examinamos la longitud de los telómeros de los pájaros cuando nacieron, encontramos que los pájaros con telómeros más cortos generalmente no regresaban a nuestra población después de pasar el invierno en África y migrar hacia el área de cría. Esto podría indicar que murieron, ya que el patrón típico de los carriceros tordales de Kvismaren es que generalmente regresan después de la migración para reproducirse en el lugar de nacimiento. Además, incluso en las aves que regresan a Kvismaren para reproducirse, hemos encontrado que aquellos que nacieron con los telómeros más cortos desaparecen de la población mucho más rápido. Este efecto es aún más grave cuando las aves están infectadas con parásitos de malaria. Así que podría ser que haya una selección en contra de aves con telómeros demasiado cortos, es decir, cerca del umbral crítico, y que factores estresantes como enfermedades aumenten el riesgo de alcanzar este umbral en etapas de la vida más tempranas. También descubrimos que algunas aves fueron capaces de alargar sus telómeros entre años. Parece que una proporción mayor de aves nacidas con telómeros cortos mostró elongación de telómeros durante su primer año de vida, quizás como último recurso antes de alcanzar el umbral crítico. También encontramos que las aves sin parásitos de malaria podían alargar sus telómeros con más frecuencia que las aves infectadas con malaria, posiblemente como resultado de que las aves infectadas tenían que utilizar sus recursos para combatir la enfermedad. Nos intrigaron estos hallazgos, ya que se ajustaban muy bien a nuestras ideas anteriores.

Los telómeros no se alargan en todos los animales. En cambio, en la mayoría de los animales, los telómeros se hacen más y más cortos en etapas de vida más avanzadas. Utilizamos modelos estadísticos para estudiar los cambios en la longitud de los telómeros entre años y encontramos que la tasa de acortamiento depende de varios factores. Por ejemplo, encontramos que la edad era importante porque las aves jóvenes pierden telómeros más rápido que las mayores. Además, la infección con parásitos de malaria provocó que el acortamiento de los telómeros procediera mucho más rápido, y esto fue más pronunciado en las hembras. ¿Sabías que el carricero de caña de Kvismaren puede tener más de una pareja durante la temporada de reproducción? Nuestro modelo estadístico predijo que el número de parejas que tiene un pájaro en el año actual está relacionado con cuánto se acortaron sus telómeros desde el año anterior. Por lo tanto, aquellos pájaros cuyos telómeros se

acortaron en gran medida no lograron obtener ninguna pareja o solo unas pocas parejas reproductoras.

En general, mi trabajo de tesis ha evidenciado la existencia de un umbral crítico inferior en la longitud de los telómeros que aparentemente puede afectar la esperanza de vida de los individuos. La decisión de alargar o no los telómeros podría ser una estrategia para que los individuos mejoren sus posibilidades de supervivencia y reproducción, pero esta estrategia puede depender de ciertos factores, como la infección. Además, los cambios en la longitud de los telómeros a lo largo de la vida están relacionados con la edad, el sexo, la infección y un índice de calidad individual, como el tamaño del harén. Sin embargo, se necesita más investigación para comprender completamente los efectos a largo plazo de la dinámica de los telómeros, incluida la elongación de los telómeros, en los patrones de envejecimiento en poblaciones naturales.

- Macho de carricero tordal cantando en el carrizal -
Regalo de Andrea Molina Escalada

# Glossary

**Ageing**: or senescence. Gradual deterioration of physiological function that occurs over time, leading to an increased vulnerability to age-related diseases and ultimately death. This process results from a combination of genetic, environmental and lifestyle factors that contribute to cellular damage and impair the body's ability to repair and maintain itself. Common hallmarks of biological ageing include telomere shortening, accumulation of DNA damage, altered gene expression patterns and decline in immune function.

**Early-life telomere length**: length of telomeres during early stages of development, such as during embryonic development or early youth. This telomere length has been found to show associations with lifespan, health and disease risks later in life.

**Fitness**: or Darwinian fitness. It is determined by the relative ability of an individual to survive and reproduce in a particular environment. It is a measure of an individual's genetic contribution to the next generation and thus is estimated by the total reproductive output over an organism's entire lifespan. Fitness is not an absolute measure, as it depends on the specific environment and it is relative to other individuals in the interbreeding population.

**Haemosporidian parasites**: a group of parasitic protozoan organisms that infect red blood cells in animals, including birds. They are commonly known as "malaria parasites" because they are the causative agents of malaria-like symptoms, and include parasites from the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon* in birds. Avian malaria parasites infect a wide range of bird species. For clarity, in this thesis, I will refer to them as malaria parasites or sometimes haemosporidian parasites.

**Life history traits**: characteristics or events that affect the growth, reproduction, maintenance and survival of an organism. These traits are shaped by natural selection and are influenced by genetics, environmental factors and interactions with other organisms. Examples of life history traits include age at first reproduction, reproductive effort, somatic repair mechanism, growth rate, antipredator responses and lifespan.

**Life history strategy**: pattern of allocation of resources and investment in different life history traits, which together influence an organism survival and reproductive success. These strategies reflect the trade-offs an organism must make in order to allocate limited resources between different life history functions: growth, reproduction, maintenance and survival.

**Lifespan**: duration of time for which an organism is capable of living (between birth and death). It varies depending on factors like species, environment, genetic makeup and lifestyle.

**Self-maintenance**: ability of an organism to maintain its own physiological and structural integrity, including processes such as repairing damaged tissues or regulating body temperature. Investing in self-maintenance is important for the survival of the organisms, enabling them to adapt to environmental changes and maintain their health and functioning.

**Trade-off**: situation in which an organism must balance the costs and benefits of different life history traits. For example, an organism may need to trade-off the energy it invests iton growth versus into reproduction. These decisions often involve a trade-off between current and future reproduction, as investing energy in one trait may limit the resources available for other traits that contribute to overall fitness.

**Telomere length**: the total length of a repeated DNA sequence (TTAGGG)n that cap the ends of chromosomes in eukaryotes and protect them from damage or fusion with other chromosomes. When cells divide, DNA cannot be fully duplicated until the end, which causes telomeres to shorten at each cell division. Telomeres can therefore be seen as a disposable barrier to protect the important genetic information on the chromosomes. Telomeres are thought to play a crucial role in cellular senescence and ageing.

**Telomere dynamics**: changes in telomere length that occur as cells divide and age, which can include both shortening and elongation. Telomere dynamics over time can have important implications for cellular health and ageing, as short telomeres can lead to cellular senescence and contribute to age-related diseases.

**Telomere elongation**: net increase in the length of telomeres between time point. It can occur through various mechanisms, most significantly the activity of the enzyme telomerase. Telomere elongation is important for maintaining chromosome stability and could potentially delay cellular senescence and age-related diseases, but it may also entail costs as it may induce malignant cancers.

**Telomere shortening**: gradual loss of telomeric sequences that occurs “naturally” with age as cells divide. Also, several factors can contribute to accelerate this process, including oxidative stress, inflammations, toxins and chronic infections. When telomeres become critically short, cells can no longer divide, leading to cellular senescence, ageing and ultimately, cell death.

- Nest of great reed warbler parasitized by a cuckoo (my worst nightmare) -
Gift from Javier Ábalos Álvarez

# Background

## Ecology and evolution: Life history and fitness

A key aim of ecologists and evolutionary biologists has always been to make sense of the high diversity we see among living organisms. This was made possible by Darwin's theory of evolution by natural selection (Darwin, 1859), which provided a conceptual framework to explain this diversity. Since then, science has made significant progress in the field now called evolutionary biology. In the theory of evolution, Darwin proposed that organisms with traits that are better adapted to their environment are more likely to survive and reproduce, passing on their advantageous traits to their offspring, summarized in the phrase "survival of the fittest". The concept of fitness (i.e., the relative success in producing offspring with high reproductive value) is fundamental to understanding the diversity of life. In order for selection and evolution to occur, it is necessary to have variability in fitness among individuals that is dependent on at least one genetically determined trait (Endler, 1986). Hence, understanding the variation in traits and strategies that shape an organism's fitness is crucial for life history evolution studies (Pianka, 1974; Scheiner and Stearns, 1992; Roff, 1993).

Life history adaptations are the actual currency of fitness in evolutionary biology (Houle, 2001; Losos, 2014). An individual's life history is the range of variables that contribute to its fitness, allowing it to adapt to its environment and therefore gain higher reproductive success (Figure 1). These life history variables include size at birth, growth rate and developmental timing, age and size at maturity, reproductive investment (such as the number of offspring, size and quality of gametes and frequency of reproduction), parental care, age and cause of death (Reznick, 2014). There is an astonishing diversity of life history strategies observed between different species (Wiersma et al., 2007; Darling et al., 2012). For instance, most tropical snakes reproduce all year round, matching their reproductive activity with seasonal changes in resource availability. However, in colder regions, snakes only reproduce during the warmer months when the temperature is ideal for embryonic development (Shine, 2003). This diversity of strategies can be found also within species, among populations and on certain occasions even among individuals within the same population (Sarma et al., 2006; Zimmerman et al., 2015; Hooper et al., 2017; Sepp et al., 2018). Chinook salmon (*Oncorhynchus tshawytscha*) exhibit

at least three different life history-based strategies for migration from their freshwater hatching sites to the sea. Some salmon fries migrate to saltwater almost immediately after hatching, while others remain in the river for several months and a third subset of the same population overwinters in the river mainstream before migrating to the sea. These different strategies have a significant impact on the growth rate and body size of salmon when they leave freshwater (Beamer and Sartori, 2000; Zimmerman et al., 2015). The diversity of life histories is a key focus of evolutionary theory as it provides the raw material for natural selection. While there has been progress in understanding the range of variation in life histories, there is still much to be learned (Roff, 1993; Losos, 2014).

The variation in life history traits is often explained by the expectation that alternative strategies produce similar fitness outcomes (Williams, 1966; Sih et al., 2004; Biro and Stamps, 2008). Depending on their life history trade-offs, species, populations and even individuals can potentially be positioned along a continuum of fast-to-slow pace-of-life, which is sometimes referred to as the pace-of-life syndrome (POLS) hypothesis (Ricklefs and Wikelski, 2002; Dammhahn et al., 2018). These concepts are based on the classical r- versus K-selection models of population ecology (Pianka, 1970). The POLS hypothesis suggests that "fast pace-of-life organisms", at the fast end of the scale, tend to concentrate resources on current reproduction, showing early reproductive maturity, producing a higher number of offspring per bout, low investment per offspring and living shorter lives. In contrast, "slow pace-of-life organisms" take more time to reach reproductive maturity, produce a lower number of offspring per bout, have high investment per offspring and live longer lives. In life history theory, specific ecological settings would favour certain life history strategies affecting a set of reproductive, behavioural and physiological traits that have coevolved within each species (Ricklefs and Wikelski, 2002; Martin et al., 2006; Réale et al., 2010). These strategies are not fixed and can be adjusted in response to changes in environmental factors like predation risk (Reznick et al., 1996), pathogens (Martin et al., 2007), health status (Velando et al., 2006), sex (Tarka et al., 2018), mating system (Ilmonen et al., 2018), habitat quality (Charmantier et al., 2017) or geographic distribution (Wikelski et al., 2003; Wiersma et al., 2007).

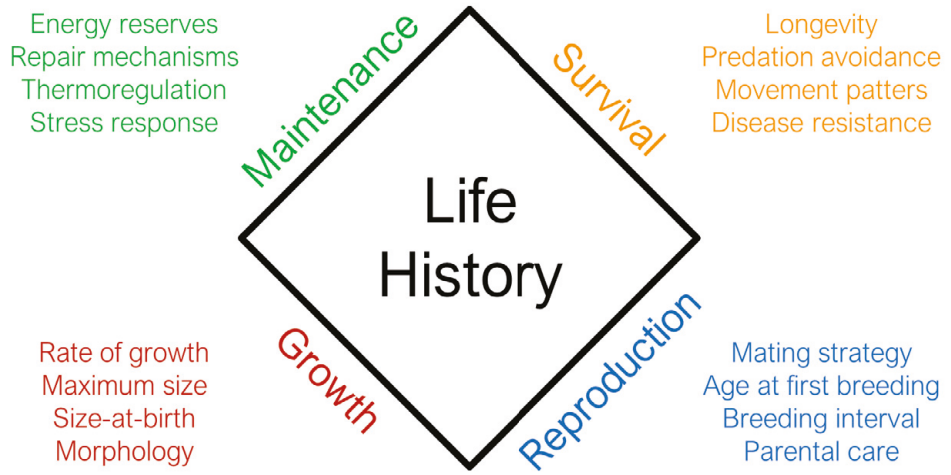



**Figure 1**. Life history refers to the set of traits that an organism exhibits regarding the allocation of resources to growth, reproduction, maintenance and survival. Here listed some examples of how to estimate these life history parameters, which can provide insights into the life history strategies of a particular species.

In the biological context, the concept of "trade-off" is a critical factor in understanding the diversity of life histories within and between species (Scheiner and Stearns, 1992; Roff, 1993; Flatt and Heyland, 2011). In an ideal environment with limitless energy resources, natural selection would favour maximizing all of their life history traits, with immortal organisms that breed from birth and produce an infinite number of high-quality offspring, commonly referred to as "Darwinian demons" (Scheiner and Stearns, 1992; Houle, 2001). However, in reality, resources are typically limited and selection is expected to compromise resource allocation among growth, reproduction, maintenance and survival to maximize fitness (Figure 1, Lack, 1947; Williams, 1966; Weathers and Sullivan, 1989). Classic examples of life history trade-offs are phenotypic correlations between fitness components, like the investment in current reproduction versus survival and future reproduction, or the trade-off between offspring number and size (Williams, 1966; Smith and Fretwell, 1974; Reznick, 1985). However, there are also negative genetic correlations like antagonistic pleiotropic effects (Leroi et al., 2005), that pose limitations that may constrain evolution and prevent it from maximizing fitness beyond certain limits (Scheiner and Stearns, 1992; Houle, 2001). However, despite their significance in the life history theory, documenting life history trade-offs has been challenging and our understanding of how environmental and physiological factors interact to shape these trade-offs is limited (Roff and Fairbairn, 2007; Williams et al., 2010; Montiglio et al., 2018). In this sense, identifying the proximate mechanisms underlying trade-off variation is critical. In recent years, researchers have shown that telomeres can provide valuable insights into the study of life

histories. Telomere length (TL) and changes in TL over time have been suggested as molecular markers that are linked to a range of physiological and environmental factors. Thus telomeres could integrate environmental and physiological information (Eisenberg, 2011; Heidinger et al., 2012; Bateson et al., 2015; Entringer et al., 2018; Young, 2018; Giraudeau et al., 2019), however, they can potentially also have causative functions in transferring these integrated costs to ultimately cause effects on fitness (Monaghan, 2010; Asghar et al., 2015a; Bateson and Nettle, 2018).

# Telomeres

## Telomere structure and function

In the 1930s, scientists Hermann Muller and Barbara McClintock discovered something unique about the ends of chromosomes, naming them telomeres (from the Greek words *telo* [end] and *mere* [part]). They found that the ends of chromosomes were more stable than the rest of the chromosomes when exposing them to X-rays, suggesting that telomeres have a special structure that protects the chromosome ends from damage. Telomeres are specialized regions of non-coding DNA located at the ends of linear chromosomes (as shown in Figure 2). These regions are characterized by tandem repeats of the core sequence $(TTAGGG)_n$ in vertebrates, which can be repeated up to several thousand times in a row (Moyzis et al., 1988). At the 3' end, eukaryotic telomeres have a G-rich single-stranded DNA overhang that ranges from 50–300 nucleotides at which the telomeric DNA is twisted backwards to form a "T-loop" structure (Griffith et al., 1999). Telomeres have a highly conserved structure across eukaryotes, indicating their ancient evolutionary origin (Gomes et al., 2010). They play a critical role in maintaining the structural stability of chromosomes by preventing their degradation and fusion with other chromosomes (Blackburn, 2005). During DNA replication, the lagging strand polymerase cannot fully duplicate the very end of the DNA strands, leading to the attrition of each chromosome ends, shortening the telomeres by a few repeats. Thus, telomeres serve as protective barriers, buffering the effect of chromosomal shortening that inevitably occurs during each cell division, so-called as the "end replication problem" (Watson, 1972), acting as a disposable element that protects the coding sections of the genome.

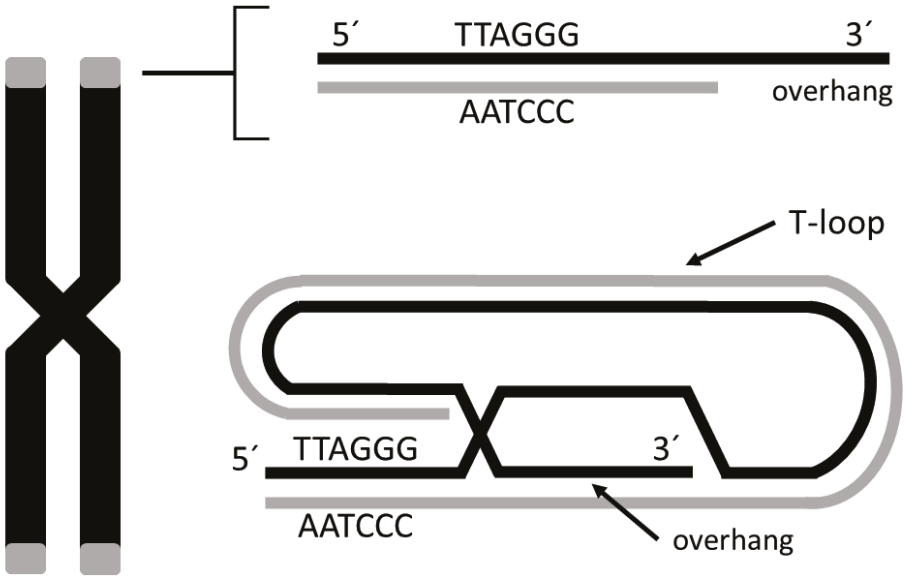



**Figure 2.** The telomere regions are located at the end of each chromosome and serve to protect the DNA from degradation or fusion with other chromosomes. These telomeres consist of repeated sequences, TTAGGG, that terminate in a 3' single-strand overhang. This overhang loops back on itself to form a cap, known as the "T-loop". Adapted from Lai et al., (2018).

## Telomere dynamics: Balance between shortening and restoration

In absence of restoration, telomeres eventually reach a critical (short) length (i.e., "critical threshold"; Monaghan, 2010; Hasselquist and Tobler, 2021; Tobler et al., 2022) where the progressive loss of telomeric DNA can lead to the inhibition of cell division, increased risk of chromosomal instability and ultimately, cell senescence and apoptosis (Stewart and Weinberg, 2000; Cawthon et al., 2003; López-Otín et al., 2013). This process has been suggested to contribute to organismal ageing and even premature death (Allsopp and Harley, 1995; Blackburn, 2005). Furthermore, chromosomal instability can also arise due to environmental factors, resulting in large blocks of telomeric sequences being lost in a single deletion event (Murnane et al., 1994; Nakamura et al., 2009; Muraki et al., 2012). As the soma becomes older and/or there is increased exposure to environmental stressors, dysfunctional senescent cells in tissues start to accumulate and secrete a variety of molecules like proteases, inflammatory cytokines and growth factors (Malaquin et al., 2016). These alterations in the tissue microenvironment can have serious impacts on tissue health and function, including impaired tissue regeneration and repair. Additionally, it can lead to the development of chronic inflammation, which is a hallmark of ageing-associated diseases such as cancer, cardiovascular disease and neurodegeneration (Samani et al., 2001; Sampson and Hughes, 2006; López-Otín et al., 2013).

For many taxa, it is widely documented that telomeres gradually shorten throughout an organism's whole lifetime (i.e., telomere shortening, Figure 3, Ind 1). Thus, until recently, it was believed that telomere protection and restoration only maintained telomere shortening at a reduced or constant level (i.e., telomere maintenance, Figure 3, Ind 2) thus slowing down the inevitable telomere shortening process (Taylor and Delany, 2000; Shay and Wright, 2007; Monaghan, 2010). Nevertheless, there have been several longitudinal studies in wild vertebrates that have observed that a fraction of individuals in a population can have longer telomeres from one time-point to another (e.g., Haussmann and Mauck, 2008b; Nordfjäll et al., 2009; Svenson et al., 2011). At first, these findings were questioned and considered to be errors or statistical effects (including regression to the mean effects; Steenstrup et al., 2013a; Verhulst et al., 2013; Nussey et al., 2014), but as more studies have been conducted, evidence has accumulated that the TL of an individual can become elongated in wild vertebrates, even after accounting for measurement errors (Bateson and Nettle, 2018; Spurgin et al., 2018; van Lieshout et al., 2019; Brown et al., 2021).

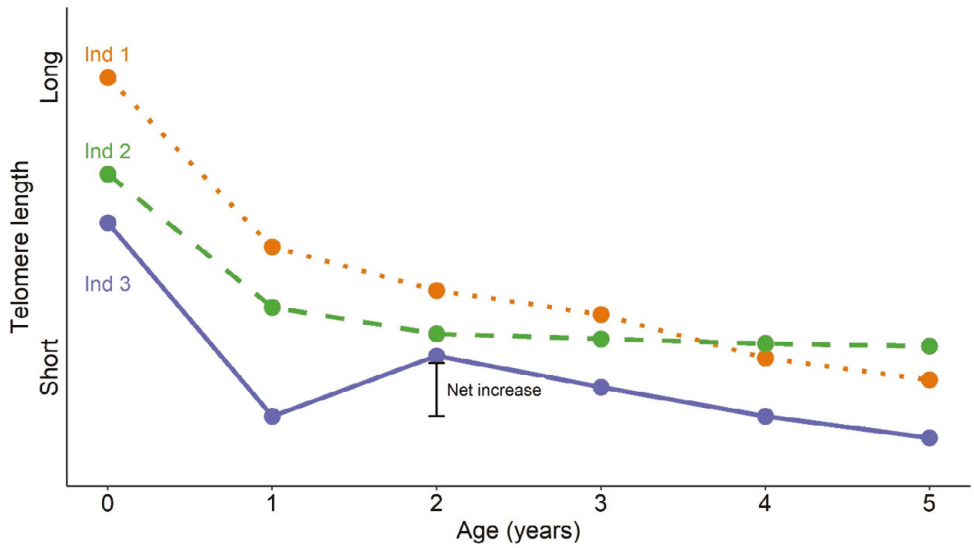



**Figure 3.** Hypothetical example of the relationship between age and telomere length. In general, telomeres tend to shorten over an individual lifespan, although various patterns of telomere dynamics can be observed. Individual 1 experiences continuous telomere shortening throughout its life. Individual 2, maintains a constant TL after reaching 1 year of age, possibly due to telomere protection and restoration mechanisms. Individual 3 invests more in telomere restoration from 1 to 2 years of age, resulting in a net elongation of TL.

## Telomere elongation

There are several mechanisms that can contribute to telomere restoration, repair and protection (in some cases even causing a net increase in TL from time "t" to time "t+1", i.e., telomere elongation, Figure 3, Ind 3), including telomerase activity, recombination and transposable elements (Calado and Chen, 2006; Aubert and Lansdorp, 2008; Capkova Frydrychova et al., 2009; Cesare and Reddel, 2010). Among these mechanisms, telomerase has been found to be the most important for restoring telomeres. Telomerase is a polymerase enzyme that binds to a telomeric complementary-sequence RNA molecule and elongates the overhanging strand of telomeric DNA (Vega et al., 2003; Chow et al., 2012). Then, the DNA replication machinery synthesizes the complementary strand and even though the overhanging strand is still present, it results in a longer total length of the telomere.

The regulation of telomerase activity differs among organisms, stages of life and tissues (Wright et al., 1996; Hornsby, 2007). In embryonic tissues and early-life stages, telomerase activity is high in most animals because cells have a high rate of proliferation, which leads to rapid telomere shortening (Bekaert et al., 2004; Monaghan and Ozanne, 2018). However, in humans and other long-lived vertebrates (e.g., non-human primates, whales and bats) telomerase activity is typically restricted in the adult stage to specific tissues with high proliferation rates (e.g., germline, epithelial and hematopoietic cells) and most somatic cells have low or no telomerase activation beyond the early stages of development (Bekaert et al., 2004; Gomes et al., 2011; Foley et al., 2018). In contrast, telomerase activation has been observed in somatic tissues of typically short-lived non-human vertebrates (like mice and zebrafish), indicating that TL can be actively maintained or even elongated in these tissues (Seluanov et al., 2007; Gorbunova and Seluanov, 2009; Anchelin et al., 2011).

The restoration of TL may have significant implications for the evolution of life history traits, as longer telomeres are potentially associated with higher somatic quality and increased survival, ultimately resulting in higher Darwinian fitness (Monaghan, 2010). However, while the benefits of restoring TL are evident, it has been argued that activating the restoration machinery could entail non-negligible costs (Herborn et al., 2014; Nettle et al., 2015; Young, 2018; Casagrande and Hau, 2019). One potential short-term physiological cost is the trade-offs of resources between the restoration of telomeres against other resource-demanding functions such as reproduction or immediate survival, leading to an adaptive "acceptance" of telomere shortening (Young, 2018; Casagrande and Hau, 2019). Another potential long-term cost of activating the restoration machinery is an increased risk of cell immortalization, which facilitates cancer development. Telomere shortening could be considered a cellular surveillance mechanism to detect cells with high proliferation rates that can indicate dangerous cancer cells (Holt et al., 1996; De

Lange and Jacks, 1999; Shay, 2016; Young, 2018). When telomeres reach a short critical length, it indicates the presence of potential cancer and cell death mechanisms are triggered (reviewed in Shay, 2016). Therefore, it has been hypothesized that there is a trade-off between telomere restoration and the risk of cancer, where the benefit of cancer suppression during the reproductively active stages of life outweighs the costs of age-related pathologies associated with short TL later in life, in agreement with antagonistic pleiotropy models (Kirkwood and Holliday, 1979; Caulin and Maley, 2011; Gomes et al., 2011).

## Variation in telomere length and evolutionary implications

Telomeres vary considerably in length between and within species, populations or even among individuals of the same species and age. This variation is influenced by a variety of factors including age, sex, genetics and environmental pressures. While TL is known to be heritable, the degree of heritability can vary depending on the species and population studied (Olsson et al., 2011; Heidinger et al., 2012; Broer et al., 2013; Asghar et al., 2015a; Factor-Litvak et al., 2016). In humans, TL heritability is estimated to be about 34–82%, meaning that genetic factors can account for a significant portion of the variation in TL among individuals (Broer et al., 2013). In non-human species, heritability estimates range from low to high depending on the species and the method of estimation. For example, the heritability of TL in some birds like king penguin (*Aptenodytes patagonicus*) is estimated to be low, around 20% (Reichert et al., 2015), while in other species, like zebra finch (*Taeniopygia guttata*) or tree swallow (*Tachycineta bicolor*), it can be as high as 70–90% (Atema et al., 2015; Belmaker et al., 2019; Bauch et al., 2022). Variations in TL have also been shown to be influenced by environmental pressures like repeated exposure to stressors (including diseases and physiological stressors), lifestyle factors (such as poor diet, lack of exercise and obesity), habitat quality, reproductive effort and even large-scale climatic processes (Epel et al., 2004; Kotrschal et al., 2007; Heidinger et al., 2012; Angelier et al., 2013; Mizutani et al., 2013; Young et al., 2013; Asghar et al., 2015a; Arsenis et al., 2017; Valera-Gran et al., 2022). These findings provide evidence that TL variation and the underlying mechanisms are essential for identifying potential biomarkers of health and ageing, as well as better understanding the role that telomeres play in life history ecology and evolution.

The importance of telomeres in evolution is a topic of ongoing research and debate. Telomeres are thought to have evolved as a mechanism to protect chromosomes from degradation and fusion during cell division. Therefore, they play an important role in the process of DNA replication and cell division, ensuring that the genetic material is accurately passed down from one generation to the next (Pfeiffer and Lingner, 2013). Nevertheless, the evolution of TL maintenance is complex and context dependent. One role that telomeres are assumed to play is the regulation of

reproduction and development, as they have been linked to lifetime reproductive success and growth (Olsson et al., 2018). In some species, individuals with longer telomeres have been found to produce more offspring or arrive earlier to the breeding grounds (Pauliny et al., 2006; Bauch et al., 2014) and juveniles under faster growth showed shorter telomeres (Olsson, 2016; Salmón et al., 2021). Additionally, TL may be influenced by environmental factors such as stress, diet and exposure to toxins and those can affect reproductive success and fitness (Latifovic et al., 2016; Mathur et al., 2016; Valera-Gran et al., 2022). However, one of the most important evolutionary implications of telomeres is their role in ageing and lifespan (Shammas, 2011; Shay, 2018). Telomere shortening is a natural and inevitable part of ageing and has been linked to a variety of age-related diseases and conditions. In many species, longer telomeres are associated with longer lifespan and better health, while shorter telomeres are associated with reduced lifespan and increased disease risk (Cawthon et al., 2003; Haussmann et al., 2005; Bakaysa et al., 2007; Barrett et al., 2012). Therefore, telomeres may be subject to natural selection as biomarkers of ageing, individuals with longer telomeres may have a survival advantage and be more likely to pass on their genes to the next generation.

# Biological ageing

Senescence, or ageing, is the irreversible accumulation of damage in an organism over time that results in a decline in vital functions and eventually death (Austad, 1997). This is often manifested, both in humans and wild populations, by an increased probability of mortality and decreasing reproductive success with age (Kirkwood and Rose, 1991; Scheiner and Stearns, 1992). Originally, the idea was that senescence is beneficial for the population as it removes old and unproductive individuals, leading to a faster renewal of generations and facilitating adaptation to dynamic environments (Wallace, 1865; Weismann, 1889). However, senescence should not be favoured by natural selection as it is not advantageous for individuals (except in cases of kin or group selection, Bourke, 2007). These misconceptions led to the replacement of these notions with the now classical evolutionary theories of senescence (Medawar, 1952; Williams, 1957; Kirkwood and Holliday, 1979; Kirkwood and Rose, 1991). Senescence theory illustrates that understanding why organisms age, which are the mechanisms and consequences of ageing and how the ageing patterns in natural environments affect life history are fundamental questions in evolutionary biology (Partridge and Gems, 2007; Charmantier et al., 2014; Jones et al., 2014).

In this context, there is a growing understanding of how natural variation in extrinsic environments affects individual senescence patterns (Nussey et al., 2013; Lemaître and Gaillard, 2017; Cooper and Kruuk, 2018). Individuals tend to distribute

resources optimally to achieve higher fitness (Scheiner and Stearns, 1992) and often when reproduction is prioritized, senescence occurs more rapidly (Figure 4, Kirkwood and Rose, 1991). In wild populations of mammals and birds, age at first reproduction has been negatively correlated with age-related declines in survival and reproduction (Charmantier et al., 2006; Reed et al., 2008; Ricklefs, 2010). For example, female red deer (*Cervus elaphus*) with more offspring in early-life exhibit faster reproductive senescence (Nussey et al., 2006), while male red deer with larger harems and rut duration show more rapid ageing (Lemaître et al., 2014). Unfavourable conditions during early-life (e.g., resource competition or low food availability) and unpredictable environmental factors can also accelerate the rate of senescence (Reid et al., 2003; Hammers et al., 2013; Douhard et al., 2016; Campbell et al., 2017). Organisms living in harsh or stochastic environments may prioritize reproduction over somatic maintenance, resulting in shorter lifespans. However, variable environmental conditions may also lead to maladaptation and age-specific disorders (Cotto and Ronce, 2014). In general, measuring variation in senescence patterns can be challenging, but biomarkers can help quantify the effects of extrinsic ecological conditions on individual senescence patterns.

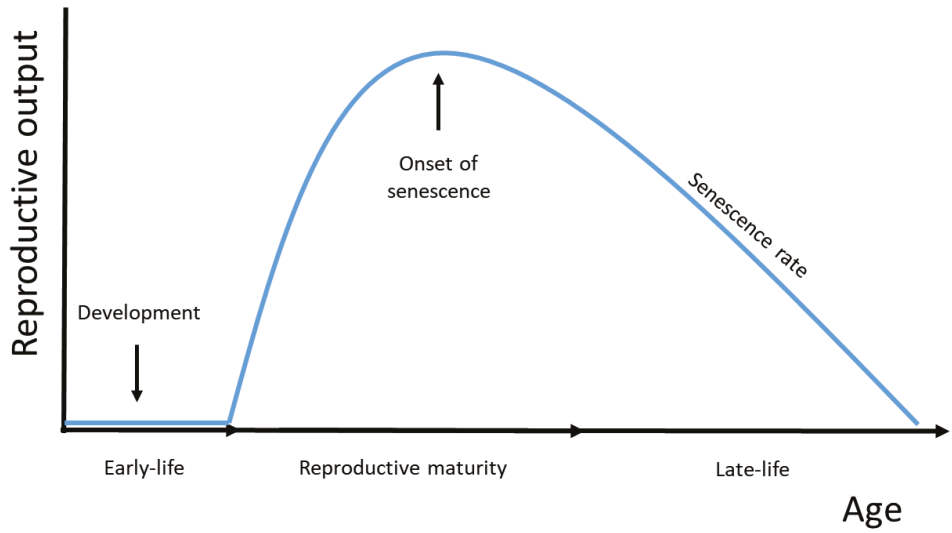



**Figure 4.** Example of age trajectory of individual reproductive output. After development, reproductive output increases in early-life and with a higher investment in reproduction senescence starts. In late-life, senescence occurs more rapidly and the rate of senescence can be determined by the slope of the reproductive output decline.

Multiple genetic and physiological processes play a role in regulating ageing and various biomarkers have been proposed to identify and predict the process of senescence (López-Otín et al., 2013). These biomarkers are used to assess health, susceptibility to age-related disorders and remaining lifespan. TL and telomere shortening are important biomarkers of ageing (Blackburn, 1991; Monaghan and

Haussmann, 2006; Haussmann et al., 2007). In cross-sectional studies, TL and telomere shortening have been linked to lifespan, with shorter telomeres observed in short-lived animals (Haussmann et al., 2003). However, cross-sectional studies may be misleading as individual variation in age-related telomere shortening could obscure the correct interpretation of telomere dynamics, making longitudinal studies necessary to understand the role of telomere dynamics in individual life histories (Sudyka et al., 2016). Longitudinal studies have mostly been conducted on long-lived organisms (Salomons et al., 2009; Barrett et al., 2012) and only recently short-lived species are being considered (Asghar et al., 2015b; Eastwood et al., 2019). By studying both long-lived and short-lived species, it could be possible to obtain a more comprehensive understanding of how TL and shortening influence ageing and how they vary across different species and environments.

# Diseases

## Effect of diseases: ecological and individual level

Parasitic infections are common in most living organisms and understanding their interaction with hosts within the ecological and evolutionary context is a widespread area of research in modern ecology (Hudson et al., 2002). At the ecological level, the effects of diseases on host life history are complex and depend on the specific disease, host species and environmental conditions. In general, diseases can reduce host fitness, altering its behaviour and physiology (Ebert et al., 2000). However, the impact of parasites can scale to affect population size, biodiversity, competition and trophic interactions (Price et al., 1986; Lessios, 1988; Klein, 2003; Tompkins et al., 2003; Lafferty et al., 2006; Delibes-Mateos et al., 2008). For example, the myxomatosis outbreak in rabbits (*Oryctolagus cuniculus*) in the Iberian Peninsula during the 1950s caused mortality rates of up to 90% of the population. This drastic reduction in prey population had a trophic cascading effect on the ecosystem, negatively impacting keystone species such as the highly endangered Iberian lynx (*Lynx pardinus*), for which rabbits represent around 85% of the diet (Delibes-Mateos et al., 2014). This highlights the ecological consequences of parasitic infections and the need of considering diseases when examining life history and population dynamics, as well as developing management strategies for both wildlife and human health.

At the individual level, parasites can harm their hosts both directly, like damaging tissues and organs, and indirectly, for example through immune system overactivation (Bonneaud et al., 2003, 2012; Nunes et al., 2017). In the short term, an infection reduces the resources available for growth, reproduction and other forms of self-maintenance (Viney et al., 2005; Owen et al., 2010). In the long-term,

the costs can be mediated by accelerated ageing through inflammation and telomere shortening, which have until recently been largely overlooked. Most of these costs have been investigated in laboratory studies that often fail to consider the variation of immune responses to infection seen in wild animals (Pedersen and Babayan, 2011) and focus on one type of immune challenge at a time, while multiple infections are common in wild populations (Bordes and Morand, 2011). In humans, chronic infections have been linked to shorter telomeres and increased mortality rates (Zhang et al., 2016), but establishing a causal relationship between immune activation, telomere shortening and longevity remains challenging due to experimental limitations (Asghar et al., 2018). In wild populations, studies on the long-term consequences of infections on telomere dynamics are limited and have produced mixed results. A cross-sectional study on Soay sheep (*Ovis aries*) did not show any correlation between the number of parasites and TL (Watson et al., 2017), while infected birds showed shorter TL and higher telomere shorting rate when compared to uninfected ones (Asghar et al., 2015b; Karell et al., 2017). This mixed outcome of results could potentially be explained by differences in pathogen types, timescales and levels of infection (also, note that cross-sectional studies may have limited power to detect any costs of infection in telomeres given the high variability in TL between individuals). Experimental studies in the wild have also found contradictory results when it comes to the effect of infections on TL. For example, house mice (*Mus musculus musculus*) infected with *Salmonella enterica* experience faster telomere shortening (Ilmonen et al., 2008), while antimalarial treatment had no effect on blue tit telomere shortening rates (Badás et al., 2015). Overall, the long-term consequences of infections on telomere dynamics in wild populations are still largely unclear and it is crucial to conduct more longitudinal and long-term studies on TL to better understand the ageing costs in the context.

## Malaria parasites

One of the most common infectious diseases is malaria which in wild populations can cause illness and mortality in the hosts, leading to changes in population size and composition. In birds, avian malaria can be a chronic and relatively mild infectious disease. It is caused by haemosporidian parasites that infect and complete their life cycle in various bird species from a wide range of different bird families (Valkiunas, 2005; Zehtindjiev et al., 2008; Hellgren et al., 2015). Avian malaria is caused by the protozoan parasitic species belonging to *Plasmodium*, *Haemoproteus* and *Leucocytozoon* genera. These parasites are primarily transmitted by blood-sucking insects such as mosquitoes, biting midges and black flies (Garnham, 1966; Valkiunas, 2005; Ejiri et al., 2011; Santiago-Alarcon et al., 2012). The transmission can for example takes place when a bird is bitten by an infected female mosquito that carries the parasites (for more details see Figure 5). Once the parasites reach the host tissues, they multiply several times, eventually infecting the red blood cells (Valkiunas, 2005). During the acute phase of the infection, parasite intensities can

be high, affecting up to 80% of the red blood cells and causing some birds to become comatose and die (Asghar et al., 2012). However, most birds survive and retain a chronic low-level infection that can last for years or even throughout their lifetime. Despite the lack of direct short-term costs during the chronic phase, the infection can have negative long-term impacts on the fitness of the birds, leading to reduced quality and number of offspring and shortened lifespan (Asghar et al., 2015b).

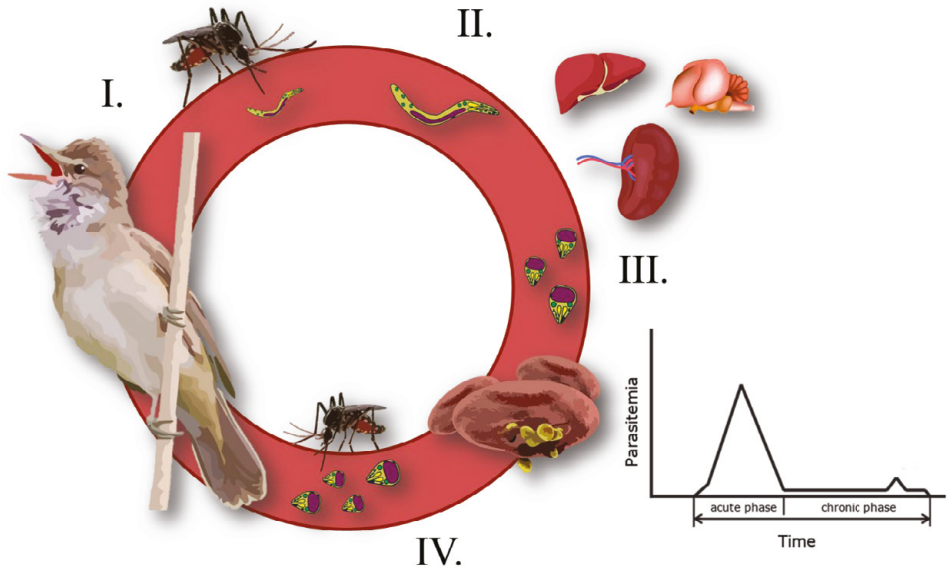



**Figure 5**. Illustration of the life cycle of avian malaria parasites in birds. I. After a mosquito bite, the infection begins with the entry of the parasites (sporozoites) into the bloodstream. II. The sporozoites reach fixed tissues (such as the liver, brain, spleen, kidney and lungs) where they multiply, forming merozoites. III. The merozoites are released into the bloodstream where they infect red blood cells, multiply producing gametocytes and eventually cause them to burst, leading to the acute phase of malaria. Individuals which survive the acute phase can retain a low-intensity level of infection that can persist for years or even throughout life, with the possibility of sporadic relapse events. IV. Eventually, a mosquito can feed on the blood of an infected host and ingests the gametocytes present in the bloodstream to continue the parasite life cycle.

# Aims of the thesis

This thesis is comprised of two sections: a theoretical and an empirical part. In research, these two approaches are both equally important and necessary to achieve a complete understanding of the natural world. In the first part (**Chapter I** and **Chapters II**), me and my collaborators wanted to take a theoretical approach to focus on reviewing the telomere research field and identifying patterns and trends in telomere ecology and evolution, as well as, developing hypotheses to stimulate new research and scientific debate. In the second part of the thesis (**Chapter III**, **Chapter IV** and **Chapter V**), the goal was to evaluate the prediction of some of the telomere hypotheses reviewed in **Chapter I** and **Chapter II**, using empirical data from a wild bird population. Below, I describe the specific objectives of each chapter in more detail:

The study of the variation in TL and telomere dynamics and the relationship with life history traits, survival and Darwinian fitness in animal populations has gained considerable attention, resulting in an overwhelming number of hypotheses and questions (e.g., Monaghan, 2010, 2014; Eisenberg, 2011; Gomes et al., 2011; Monaghan et al., 2018; Sudyka, 2019). However, with the rapidly increasing number of these hypotheses, it can be challenging to evaluate the state of the field, identify gaps in knowledge and relate the results to the relevant hypotheses. In **Chapter I**, the objective was to provide an overview of the telomere hypotheses that are relevant to ecology and evolution, along with a framework to compare these hypotheses, highlighting differences and/or similarities between them. I developed a framework that enables the discrimination and hierarchical grouping of these hypotheses based on their association with different research questions and the assumptions they make (i.e., causality or consequences of telomere shortening on organism performance). Lastly, one aim was also to identify knowledge gaps to inspire new lines of thought in this research field.

In most taxa, the trend is that TL gradually decreases with age after birth and telomere protection and restoration mechanisms are thought to mainly reduce the rate of shortening. Nevertheless, a growing number of studies indicate that telomeres can actually elongate (i.e., a net increase in TL from one point in time to the next) at least during some stages of life (e.g., Steenstrup et al. 2013; Huzen et al. 2014; Fairlie et al. 2016; Hoelzl et al. 2016; Asghar et al. 2018; Spurgin et al. 2018; van Lieshout et al. 2019; Brown et al. 2021; Canestrelli et al. 2021; Seeker et

al. 2021). In **Chapter II**, the aim was to delve deeper into the two hypotheses presented in **Chapter I** proposing explanations for telomere elongation patterns: the "excess resources elongation" and the "last resort elongation" hypotheses. These two hypotheses assume that telomere elongation comes with significant costs. However, they vary in terms of the circumstances under which telomere elongation is more likely to occur and the type of individuals expected to exhibit elongation. The goal was to understand the potential trade-offs of activating the telomere restoration machinery and predict which individuals might invest differently in telomere restoration. I also intended to conduct a review of the current evidence supporting these hypotheses and suggest ways to test them. The ultimate aim was to bring more attention to these particular elongation patterns and to shed light on whether telomere elongation episodes reflect an adaptive strategy to optimize life history strategies or whether they are simply processes that reflect environmental conditions or perhaps even random processes.

Shortened telomeres have been associated with decreased lifespan and reproductive success in wild animals (Monaghan, 2010; Boonekamp et al., 2014; Whittemore et al., 2019). However, it is still uncertain whether telomere shortening is a direct cause or simply correlated with underlying causal mechanisms. As I discuss in **Chapter I** and **Chapter II**, the key assumption of the "critical threshold" models is that once telomeres reach a certain length, further shortening could result in organ dysfunction and senescence. To investigate signs of such a critical threshold in the wild, in **Chapter III** the aim was to explore the distribution of TL in my study population, with a specific emphasis on the lower end of the distribution. My objective was to examine whether individuals with critically short TL would disappear from the population disproportionately more than individuals with longer telomeres. Additionally, to investigate whether the presence of life stressors, such as infections, can influence these patterns.

Somatic maintenance processes that reduce the degradation or promote restoration of TL have been identified as potentially important factors influencing life history decisions and ageing (Eisenberg, 2011; Young, 2018; Casagrande and Hau, 2019). In **Chapter IV**, the focus was on investigating individual differences in telomere net elongation patterns in a wild bird population. Specifically, my aim was to analyse the relationship between telomere elongation versus shortening patterns and to relate this to two factors that previously have been linked to telomere dynamics: the prevalence of chronic avian malaria infection and early-life TL. By examining these relationships, the aim was to better understand how the results could be related to the predictions and life history associations laid out in **Chapters I** and **Chapters II**.

In order to comprehend the significance of telomeres in life history ecology is crucial to establish the factors that impact the telomere shortening and elongation.

**Chapter V** aimed to investigate predictors of telomere dynamics between years, using a comprehensive dataset of life history data. Individual telomere dynamics have the potential to serve as an indicator of quality. Those with superior health and body condition may have a better ability to handle environmental stressors or maintain and repair somatic tissues, resulting in less telomere shortening or more telomere elongation. Therefore, the objective was to develop models that could predict the associations between changes in TL and several indicators of quality, including age, physiological status and breeding performance. Finally, it was aimed to examine whether there are sex-specific differences in telomere dynamics and how sex interacts with the above factors.

# Material and Methods

## Systematic review

Producing a high-quality review manuscript requires selecting a specific topic within the field that presents gaps in research or requires further conceptualization. After compiling relevant literature (from articles, books and other publications) a comprehensive review should be done to identify trends and areas where further studies are necessary. It is essential to remain objective and unbiased throughout the writing process, using only high-quality and credible sources to support the review. For the overview in Chapter I, key articles were scanned as well as reviews related to telomere biology in ecology and evolution. We used the search string "(telomer*) AND (loss or shorten* OR length OR dynamics) AND (hypothes* OR model*)" and restricted the search to the Web of Science database categories of evolutionary biology, ecology, zoology, plant sciences and biology. We located and screened 328 articles potentially relevant to our scope. From there, a total of 23 name-given hypotheses were identified and grouped based on (i) the research question to which they were linked and (ii) the assumptions these hypotheses made about the causality of telomeres in evolutionary processes and in the regulation and the development of physiological and behavioural traits that can affect Darwinian fitness determining factors.

## Study species

Birds are, for several reasons, an ideal model system to investigate ecological and evolutionary questions and to test specific hypotheses about life history trade-offs. First, the diversity of this taxonomic group, with over 10,000 species (Barrowclough et al., 2016), provides an opportunity to study a wide range of life history traits such as clutch size, migration, social behaviour and parental care that can have significant impacts on survival, reproductive success and population dynamics (Ar and Yom-Tov, 1978; Klomp, 2002; Goodson, 2005; Hedenström, 2008; Lyon and Montgomerie, 2012; Gómez-Blanco et al., 2019; Sokolovskis et al., 2023). Birds are also relatively easy to observe and study in the wild, with well-established methods to capture and handle them and collect life history data (Stamm et al., 1960). Most bird species have a relatively short generation time, which allows for

studying them over multiple generations. Also, birds can be easily manipulated in the laboratory and in the field, so researchers can experimentally manipulate specific traits to study the effects on growth, survival and reproduction.

For this thesis, I focused on a wild population of a single species, the great reed warbler (*Acrocephalus arundinaceus*). The great reed warbler is a migratory songbird that belongs to the family of old-world warblers (*Sylviidae*). It is a medium-sized bird, weighing around 30 grams and it has a sexually monomorphic plumage, so males and females present no obvious difference in appearance (Cramp and Perrins, 1994). It is widely distributed over the temperate latitudes of the Palearctic and breeds in wetland habitats like reed beds, marshes and swamps (Cramp and Perrins, 1994). During the summer, its breeding range extends from Spain in the west to Mongolia in the east (Dyrcz, 2020). Then during winter, it migrates to sub-Saharan Africa, where it spends the non-breeding season (Figure 6).

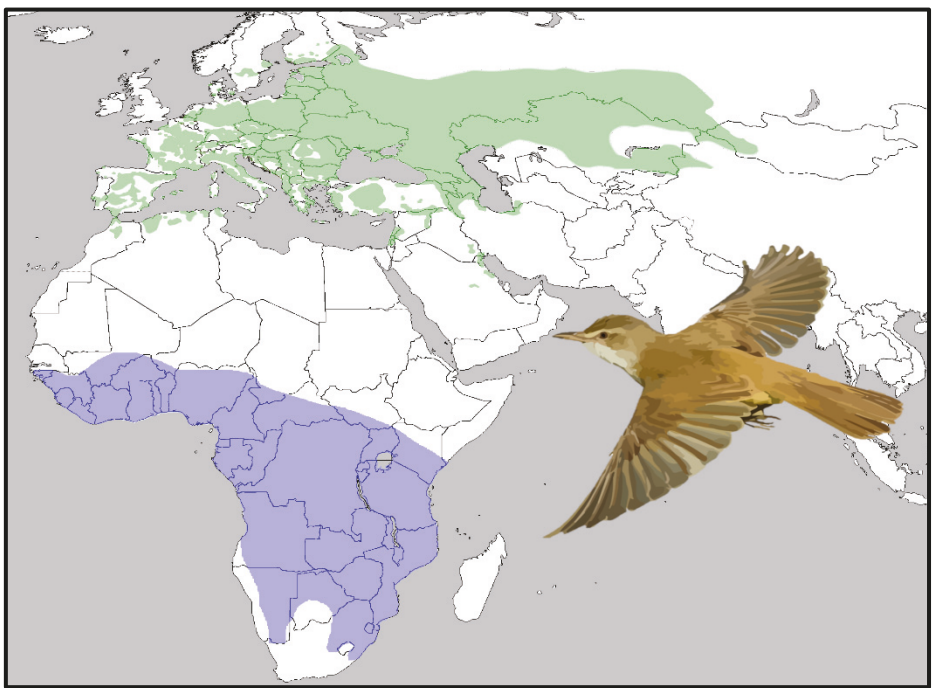


**Figure 6.** Range of distribution of the great reed warbler (Acrocephalus arundinaceus). This species migrates seasonally between Europe and the west Palearctic during the summer breeding season (green) and sub-Saharan Africa during the winter (blue). In green are represented the passage area between migratory flights. Distribution information from BirdLife.

The great reed warbler exhibits a facultatively socially polygynous mating system, which means that males have the potential to mate with multiple females. In this mating system, males establish territories in prime nesting areas in dense reed beds near waterbodies and attract females by singing and displaying (Figure 7). Females base their mate choice on territory quality and the male's song-repertoire size, both of which predict harem size and reproductive success (Catchpole, 1986; Bensch and Hasselquist, 1992; Hasselquist, 1998). During the breeding season, from May to the beginning of August, males display two distinctive types of songs. Unmated males produce loud and melodious, long songs of about 4-10 seconds, constituted by multiple syllables and performed to attract females. Once a female is attracted to a male's territory, males switch to produce short songs of about 1-2 seconds in length that contain few syllables and that are used when males are mate-guarding a fertile female (Ezaki, 1987; Hasselquist and Bensch, 1991). However, around the time when females lay the first egg, males resume singing the long song to attract another female (Hasselquist and Bensch, 1991). Males provide less parental care to nests of secondary females than to primary females (Sejberg et al., 2000). However, despite available non-mated males, females can prefer becoming a secondary female in a harem of a polygynous male that shows better genetic quality, territory quality and/or food supply than those of its competitors, to increase their reproductive success (Bensch, 1996).

It takes the female 3-5 days to build a cup-shaped nest (Figure 7), usually in a dense stand of reeds, weaving together dried grasses, reeds and other plant material into the desired shape and size. Once the nest is complete, females lay a clutch of 3-7 eggs and incubate them for approximately 14 days. After the eggs hatch, both parents care for the chicks at least to some extent, bringing food and protecting them from predators for 12-14 days (Figure 7). After that period parents encourage the chicks to leave the nest and three weeks later, they become independent (Cramp and Perrins, 1994).

As a migratory bird, the great reed warbler is a particularly suitable species for my research since it is exposed to a wide range of parasites, that can impact its health and survival. One of the most well-known parasites that these birds meet is haemosporidian parasites, which cause avian malaria. Typically, birds encounter these parasites for the first time at their wintering quarters in Africa where they may become infected. Many of them survive the initial acute phase and maintain a low-level chronic infection in their bodies for a period, which can range from one to several years or up to their entire life (Bensch et al., 2007). This chronic period of low-level infection provides the opportunity to evaluate the cost of relatively mild disease within an individual bird's lifetime.

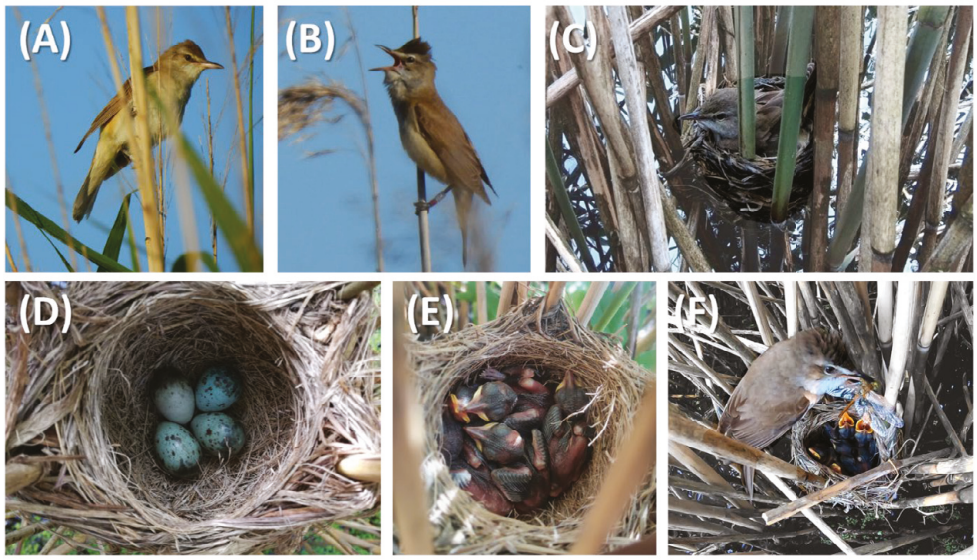



**Figure 7.** Illustration of various aspects of the biology of the great reed warbler, including (A) a male on top of the reed of his territory and (B) a male singing to attract females, (C) a female constructing the nest, which is a little cup made of plant material (D) fully finished nest with four eggs (E) five-days old nestling (F) female feeding the nestling.

In the context of the ecological and evolutionary consequences of these parasites-host interactions, great reed warblers have been found to show phenotypic, physiological and life history trait differences between individuals. Some examples are associations of MHC alleles and protection against malaria infections (Westerdahl et al., 2005), genetic variation in the dynamics of parasitaemia (Zehtindjiev et al., 2008) and delayed fitness effects mediated by accelerated telomere degradation (Asghar et al., 2015b).

## Study area

The longitudinal study on the great reed warbler is based at Lake Kvismaren (59°10' N, 15°25' E, Figure 8) in the southern central region of Sweden, situated approximately 150 kilometres west of Stockholm (Bensch and Hasselquist, 1991; Bensch, 1993; Hasselquist, 1994). The study area involves several small eutrophic lakes, namely Rysjön, Nyängen/Löten, Fågelsjön, Åslasjön and Sörbysjön, within a 2 x 3 km area. Since the beginning of the last century, the lakes were entirely covered by dense reed beds (*Phragmites australis*). However, over the past five decades, numerous zones have undergone management and restoration, leading to

the emergence of shallow waterbodies with a diverse mosaic of reeds intertwined with tussocks of Iris (*Iris pseudacorus*) and sedges (primarily *Carex acuta* and *Carex riparian*).

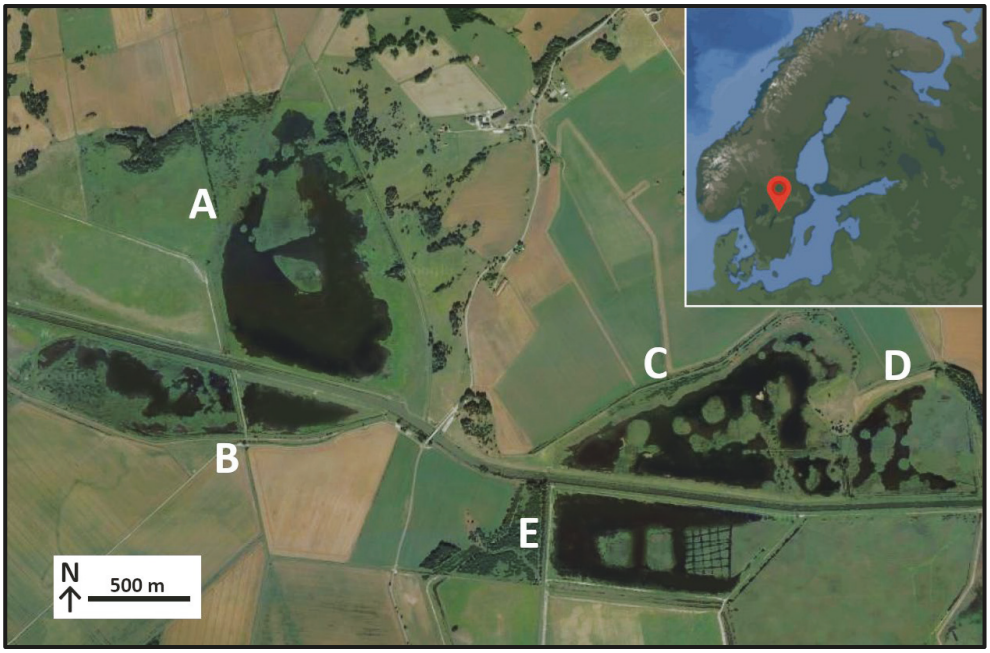



**Figure 8.** Map of the study area, Google Earth satellite image. The map shows the boundaries of Lake Kvismaren, which includes two major reed areas (over 1.5 km$^2$), East and West Kvismaren, that constitute the nature reserve hosting a bird station where over 250,000 birds of approximately 160 species have been ringed. The study area is delimited by Rysjön (A), Nyängen/Löten (B), Fågelsjön (C), Åslasjön (D) and Sorbysjön (E). The lakes are connected by the Kvismaren canal, which empties into Lake Hjälmaren to the east. The geographic location of Kvismaren is shown in the upper right corner of the map.

The great reed warbler population in Kvismaren has increased from its founding by two singing males in 1978 (Bensch, 1987) to approximately 15-25 territorial males and 20-35 breeding females that occupy territories in the lake each year. This population shows a high rate of return between years for both juveniles and adults (Bensch and Hasselquist, 1991; Hasselquist, 1995). The great reed warbler population in Kvismaren, despite being a long-distance migrant, exhibits a high degree of philopatry and of the birds that disperse the majority settle at nearby breeding sites (Bensch and Hasselquist, 1991; Bensch et al., 1998; Hansson et al., 2002a). In Sweden, there are about ten locations where the vast majority of great reed warblers breed and the dispersal patterns have been studied by visiting all these sites in order to identify dispersing individuals born in Kvismaren (Bensch et al., 1998; Hansson et al., 2002a, 2002b). These studies have estimated that approximately 30% of nestlings survive their first year of life and that roughly 50%

of these become recruits into the study population, i.e., their place of birth (Hansson et al., 2002b). Among adults, between 35-65% survive from year to year and of these individuals, approximately 80-92% return to breed within the same part of Kvismaren as the year before (Bensch et al., 1998; Hansson et al., 2002a).

Every year since 1983, daily visits were made to each of the lakes throughout the breeding seasons, which spanned from late April to late July. With few exceptions (< 5%), all territorial males and breeding females were caught using mist nets and individually marked with numbered aluminium rings, as well as a unique combination of three colour rings (Figure 9). During capture, biometric measurements and blood sampling from the brachial vein were taken before releasing the birds at the capture sites. Tracking male and female mating behaviours, 95% of the nests were located during the nest-building period. Each breeding attempt was registered by visiting the nest repeatedly at intervals of 3 days (range 1-6 days) until the chicks fledged or the nest failed. Nestlings were marked with individually numbered aluminium rings and a single colour ring when they were 8-10 days old. Biometric measurements and blood samples were also taken from each fledgeling (Figure 9).


**Figure 9.** Fieldwork procedures for studying wild great reed warblers in Kvismaren. (A) Setting up a net in the field. (B) Ringing with numbered aluminium rings and a unique combination of three colour rings. (C) Taking morphometric measurements, including wing length and body mass. (D) Canoeing on the lake. (E) PhD student (rowing the canoe) and supervisor (commanding). (F) Blood sampling a nestling using a microcapillary. These procedures are commonly used to study wild bird populations.

The comprehensive data set obtained in this long-term study of the great reed warbler during the breeding season provides a multitude of information about the

life history, behaviours and components of fitness. For instance, reproductive success (number of eggs, number of chicks hatched and the number of chicks that survive to adulthood), parental care, territory size and defence, mating systems (breeding status, number of females that a male has in his harem), life history traits (age at first breeding, the number of offspring produced over a lifetime and lifespan) or environmental conditions (food availability, temperature), host-parasite interactions, immune system, dispersion and migration patterns and others (Bensch, 1987; Hasselquist, 1994; Westerdahl et al., 2000; Hansson et al., 2002a, 2002b; Hasselquist et al., 2007; Tarka et al., 2010; Lemke et al., 2013; Asghar et al., 2015b; Roved et al., 2017; Malmiga et al., 2021; Sjöberg, Malmiga et al., 2021).

# Laboratory analysis

## Data selection

The data collection occurred in each year from 1987 to 2019, specifically during the breeding season which spans from late April to late July. Each bird that was captured during this time period had a blood sample taken, which consisted of 20–100 µl of blood that was stored immediately in SET buffer and stored at a temperature of -20 °C for 1–3 months and then -40 °C until DNA extraction. The collection of samples summed nearly 4000 nestling blood samples and over 1200 adult samples from individuals who were both born and later bred in Kvismaren. Individuals for which life history data were collected and were blood sampled, both as nestlings and throughout their adult life, were selected for the following longitudinal studies included in this thesis. Additionally, we included also individuals born in Kvismaren that never returned to the breeding population after their first wintering in Africa (non-returning). This latter group was selected based on the following criteria: (i) one non-recruiting sibling (at random) from broods where there was one recruiting nestling in our data set, (ii) all remaining non-recruiting siblings from broods where there were at least two recruiting nestlings, and (iii) all nestlings from 17 nests where there were zero recruiting individuals. These 17 nests were matched to nests containing at least two recruiting nestlings, in terms of hatching year, sub-area within Kvismaren, brood size and hatching date. In the end, a collection of 1,415 blood samples from 678 individuals was obtained for the purpose of this thesis.

## Blood samples and DNA extraction

The processing of blood samples was done at the Molecular Ecology and Evolution Laboratory at Lund University, Sweden. DNA extraction was conducted using the organic extraction method (phenol-chloroform) described by Sambrook et al.,

(1989), with slight modifications. The extracted DNA was then quantified and evaluated for purity using a NanoDrop 2000 spectrophotometer (Thermo Scientific). In order to assess the quality of the extracted DNA for further experimentation, a benchmark of good quality was established, where samples with A260:A280 absorbance ratios within the range of 1.8 to 2.2 and A260:A230 ratios ranging between 1.8 and 2.0 were considered acceptable. Then, the DNA samples were diluted with TE buffer to a concentration of approximately 50 ng/µl and stored at a temperature of -40 °C.

## Telomere measurements

Currently, there are several molecular methods available to measure TL (Aubert et al., 2012; Montpetit et al., 2014; Nussey et al., 2014). Among the most widely used, the Telomere Restriction Fragment (TRF) and quantitative Real-Time Polymerase Chain Reaction (qPCR) have been highlighted for their applicability in animal field studies (Nussey et al., 2014).

The TRF assay involves fragmenting DNA into small parts using restriction enzymes, which cut outside of the telomeric sequence (Harley et al., 1990; Nakagawa et al., 2004; Haussmann and Mauck, 2008a). Then those fragments are size sorted by pulsed-field gel electrophoresis and visualized by Southern blotting to measure average fragment lengths (Harley et al., 1990). TRF is a reliable method that measures absolute TL, in kb and can be compared between different populations and species (Haussmann et al., 2003; Flanary and Kletetschka, 2005; Criscuolo et al., 2009). However, the method has some limitations in capturing the full distribution of TL (especially the shortest telomeres, which may be critical for cell arrest and apoptosis), measurements can vary depending on the restriction site (Nakagawa et al., 2004) and requires expertise and it contains a certain level of subjectivity in the interpretation of the results. The main limitations of the TRF method in long-term studies with large sample sizes are the amount of genetic material required and the time-consuming assay method (Haussmann and Mauck, 2008a).

As an alternative, the qPCR method is a variant of PCR that uses fluorescent labelling to quantify the number of amplified DNA fragments over time (Cawthon, 2002). It measures TL by comparing the abundance of (T) telomere sequences to (S) a non-variable single-copy gene (Figure 10). The T/S ratio represents an average measure across multiple chromosomes and is a relative value compared to a given reference sample (Smith et al., 2011). Therefore, the qPCR assay provides TL values that are relative within the study and the results cannot be compared between different populations or studies. The qPCR primers can also amplify interstitial telomeric sequences (TTAGGG) in the genome, which are not part of the telomeres (Meyne et al., 1989; Nanda et al., 2002; Ruiz-Herrera et al., 2009). However, these

sequences often have a stable copy number within species and do not change with age within individuals (Delany et al., 2003). Some advantages that qPCR has over TRF are that it requires less DNA and is less time-consuming, making it highly applicable in ecological and evolutionary studies where large numbers of individuals are sampled repeatedly. Despite this, both TRF and qPCR methods show highly reproducible results as demonstrated high correlations when comparing TL measurements of the same individuals estimated by both methods (Cawthon, 2009; Criscuolo et al., 2009; Aviv et al., 2011; Barrett et al., 2012).

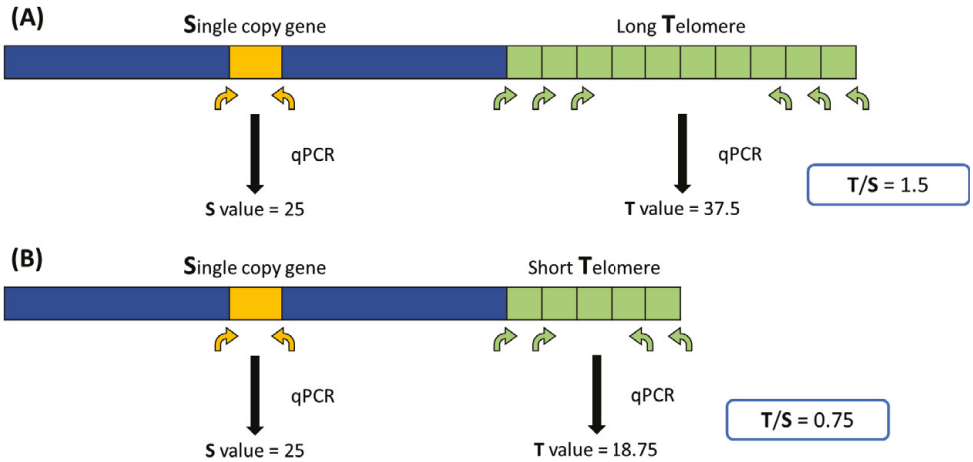



**Figure 10.** Illustration of the qPCR method for telomere length (TL) measurement as outlined by Cawthon, (2002). The qPCR technique is a widely used approach for TL measurement that does not require a large amount of DNA input and is less time-consuming, however, it provides relative measurements. The T and S values for samples (A) and (B) correspond to the number of thermal cycles at which the product amplification crosses a predetermined fluorescence threshold and then the T/S ratio is calculated for each sample. Adapted from Lai et al., (2018).

In chapters III, IV and V, we measure TL using the qPCR method published by Cawthon, (2002), using a non-variable single-copy gene (SFRS3) to account for the concentration of DNA in each qPCR reaction (Bejerano et al., 2004). The qPCR assay was performed using a CFX96 Real-Time PCR Detection System (BIO-RAD). Each reaction contained 5 ng of DNA, 12.5 µL Supermix (Platinum® SYBR® Green qPCR SuperMix-UDG, Invitrogen), 0.1 µL ROX and either 200 nM of each telomere primer or 400 nM of each SFRS3 primer and ddH2O to complete 25 µL reaction volume (Asghar et al., 2015a). Samples were assayed in triplicate, with the mean of the three replicates used for all calculations. A reference "golden" sample from a single individual was included in each plate as a standard to account for between-plate variation, along with a negative control and a serial dilution (2X from 4 to 0.25 ng × µl−1 of DNA per well) of one individual, different than the "golden" sample. The average qPCR efficiency was 100.6 % ± 2.9 (SD) for telomere and 112.2 % ± 4.1 (SD) for SFRS3. TL was estimated for each sample based on the

telomere to single-copy gene ratio (T/S ratio; Cawthon, 2002) using the formula provided here:

$$2^{\Delta\Delta Ct}, \text{ where } \Delta\Delta Ct = (Ct^{telomere} - Ct^{SFRS})_{golden\ sample} - (Ct^{telomere} - Ct^{SFRS})_{sample}$$

The repeatability of Ct values of intra-plate sample triplicates was high (intraclass correlation coefficient for telomere 0.979, 95% CI: 0.975–0.983; and for SFRS 0.982, 95% CI: 0.979–0.985). The inter-plate repeatability was calculated using a dataset consisting of 116 DNA samples obtained from 43 randomly selected great reed warblers with between 1–3 samples from each individual at different ages (separated by at least one year). These samples were collected and processed during the same time period as the main datasets of this thesis. Each of the 116 samples was measured twice, on different qPCR plates and at different time points, but still within the same time period as the main datasets. Therefore, there is no reason to suspect that the subset of samples used in the inter-plate repeatability analysis behaved differently from those in the main datasets. The intraclass correlation coefficient for these 116 samples was 0.79 (95% CI: 0.71-0.85).

## Measurement errors

In ecological studies, qPCR is a convenient and high-throughput method widely used in the field of in vivo telomere dynamics (Pepper et al., 2018). However, there has been considerable debate on the impact of measurement error on the reliability of qPCR-based TL measurement (Martin-Ruiz et al., 2015; Verhulst et al., 2015; Tarik et al., 2018) and the best laboratory practices when using it (Lindrose et al., 2021). The analysis of changes in TL over time is susceptible to a well-known measurement error-related issue known as “regression to the mean” (RTM), which can lead to potential errors in the analysis (Yudkin and Stratton, 1996; Barnett et al., 2005). RTM is a statistical phenomenon that occurs when measuring an extreme value of a variable because such a value is likely to be followed by a value closer to the actual mean in the next sampling of the same variable (Stigler, 1997). In this case, when measuring TL on the same population over time, it is expected that some of the individuals initially measured to have a long TL would be more likely to be measured to have a shorter TL the second time. And vice versa for individuals that were measured to have short TL on the first occasion. The RTM effect depends on the random variation in the measurements due to methodological errors. To address this issue, it is important to use appropriate statistical methods to check the sensitivity of the analyses to RTM effects and account for them. This can involve adjusting for the baseline TL measurement or using a repeated measures analysis to look for significant changes in TL over time (Nettle et al., 2019). Additionally, it is important to use standardized protocols for sample preparation, qPCR analysis, and data interpretation to minimize assay variability (Lin et al., 2019; Lindrose et al., 2021).

We examined to which degree our TL changes over time (i.e., observing the change between a baseline TL and the follow-up time point one year later) could be attributed to measurement errors. First, we performed a Levene's test (using 116 samples analysed twice for TL; see above for details), to compare within-sample TL measurement variation to within-individual TL change. We found that the change in TL within individuals had significantly higher variance compared to the variation in TL measurement within samples (Levene's test: $F = 38.81$; $p < 0.001$), so measurement error caused by the qPCR machine was smaller than the individual TL change between consecutive samplings. After that, we calculated the standard deviations (SD) of the mean within-sample TL measurement variation by dividing the samples into quartile groups, based on their first TL measurement (Table 1). This helps us to test the sensitivity of the results to possible measurement errors, such us RTM. The SD values for the two extreme quartiles showed the largest values.

**Table 1.** The mean difference in telomere length and standard deviation (SD) were obtained when the same sample was analysed two times on different qPCR plates and dates (N = 116). Values were calculated for each TL quartile (each quartile had N = 29 individuals) based on the first measurement of each sample. The two extreme quartiles, i.e., the shortest TL (Q1) and the longest TL (Q4) showed the largest mean difference and SD values, which is expected and caused by the effects of "regression to the mean" (RTM) measurement errors.

| *Group* | *Range of 1st TL measure* | *Mean TL difference* | *SD* |
|---|---|---|---|
| Shortest TL quartile ($Q_1$) | 0.27 – 0.48 | 0.09 (22.2 %) | 0.114 |
| Medium short TL quartile ($Q_2$) | 0.49 – 0.61 | 0.09 (16.4 %) | 0.064 |
| Medium long TL quartile ($Q_3$) | 0.62 – 0.85 | 0.13 (17.8 %) | 0.107 |
| Longest TL quartile ($Q_4$) | 0.86 – 1.55 | 0.20 (18.5 %) | 0.124 |

We utilized these quartile-specific estimates to determine the expected variance in TL "change" caused by measurement errors. We employed the estimated quartile-specific SD to compute the error bars ($\pm 2 \times$ SD) for each measured TL change in our main data set (Sokal and Rohlf, 1973; Cumming et al., 2007; Rosner, 2015) to visualize the RTM effects through the error bars of the individual samples in relation to their baseline measurement (Figure 11). Our analyses confirmed that the results obtained with our raw main data compared to the data set where we excluded all data points where SD whiskers reached the zero-change line, i.e., samples that could not, with statistical confidence, be grouped as elongating or shortening (for more details about this approach see Chapter IV).

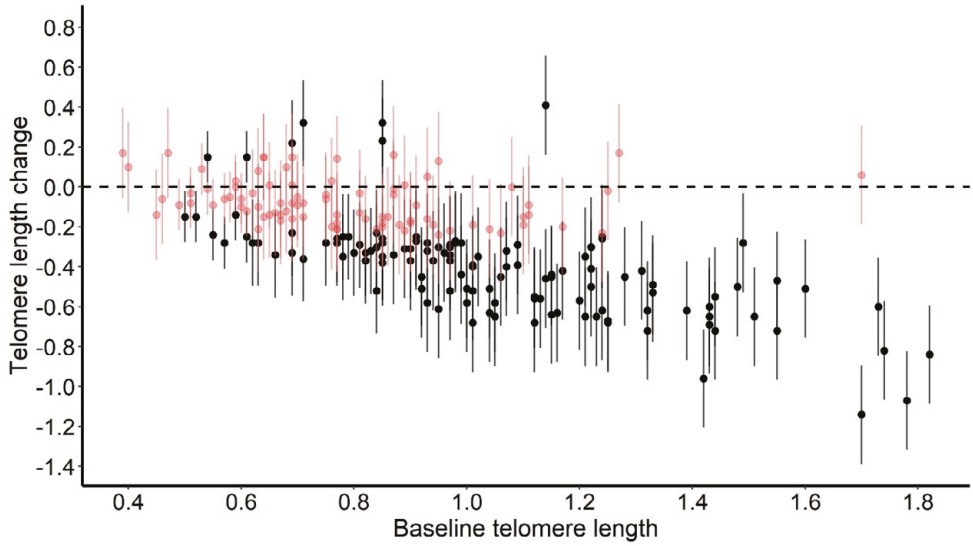



**Figure 11.** Example dataset of change in telomere length (TL; measured as T/S ratio) of great reed warblers. Negative values represent the shortening of TL and positive values indicate TL elongation. Error bars represent estimated measurement errors of TL change for the sample (±2×SD). Samples in light shading in red are those where the 2xSD error bar overlaps TL change = 0 (for more details see Chapter IV).

## Parasite detection method

To investigate the ecological and evolutionary implications of avian malaria parasites, it is important to use reliable methods for the detection and identification of these pathogens. Traditional microscopic methods have limitations and require expertise in taxonomy and developmental stages, as low levels of parasitaemia can otherwise easily be overlooked (Paperna et al., 2007; Valkiunas et al., 2008; Chavatte et al., 2009; Savage et al., 2009; Garamszegi, 2011; Ellis et al., 2014). Molecular methods, in contrast, especially PCR-based techniques, have several advantages as high sensitivity and simple implementation. These methods allow for the identification of species and mitochondrial lineages and comparison with publicly available catalogues, such as the MalAvi database (Bensch et al., 2009). For this reason, over the last two decades molecular methods have routinely become the common approach for detecting and characterizing avian malaria parasites (Bensch et al., 2000; Hellgren et al., 2004; Waldenström et al., 2004; Ricklefs et al., 2005; Ciloglu et al., 2019). However, molecular methods cannot determine the developmental stage of the parasite and may underestimate mixed infections (Valkiunas et al., 2006). Therefore, a combination of molecular and morphological analyses is necessary for the accurate detection and identification of avian malaria parasites.

In chapters III, IV and V, the blood samples of all birds were screened for malaria parasites of the *Plasmodium* and *Haemoproteus* genera using the multiplex PCR method developed by Ciloglu et al., (2019). This one-step PCR assay amplifies unique sequences of different lengths for each genus (377-379 bp for *Plasmodium* and 525-533 bp for *Haemoproteus*, Figure 12). To confirm the successful amplification of the parasite DNA, an electrophoresis gel was run to detect the presence of parasites by observing bands of different lengths under UV light. Each sample was then classified as either infected or uninfected. In total, out of the 704 adult samples that were analysed, 35% were infected by at least one genus of malaria parasites and among the 389 individuals, 55% were infected at least once during their lifetime. Over the period of 1988 to 2019, the prevalence of the *Plasmodium* (18%) and *Haemoproteus* (15%) parasites showed fluctuations between years (Figure 13).

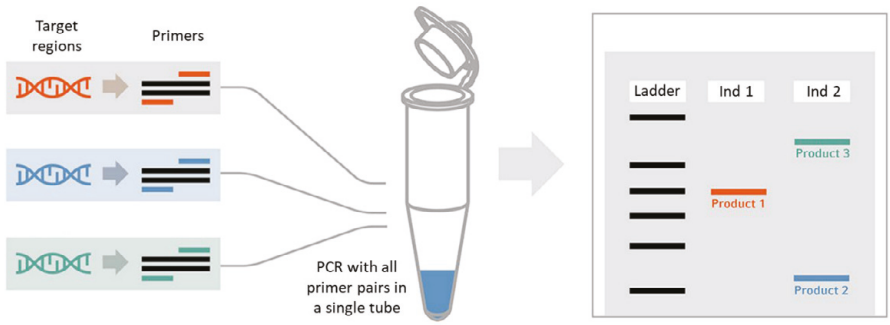



**Figure 12.** Multiplex PCR approach as described by Ciloglu et al., (2019) to detect the presence of haemosporidian parasites. This method amplifies unique sequences of each genus in a single-step PCR assay. The final PCR product is loaded onto an agarose gel. Positive samples are identified by the presence of bands of different lengths, allowing the identification of parasite genera. Adapted from Eppendorf SE.

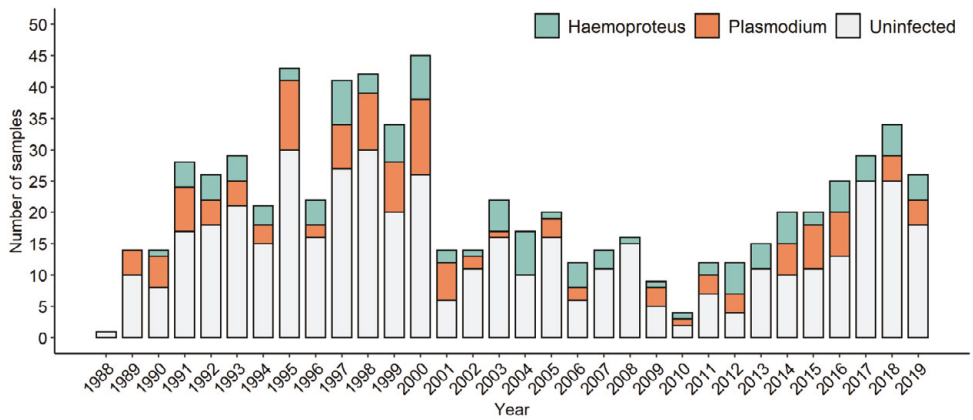



**Figure 13.** Annual prevalence of malaria parasites in the great reed warbler population at Lake Kvismaren, 35% of the individuals (n = 704) were infected by at least one genus of parasites. The total prevalence of *Plasmodium* (18%) and *Haemoproteus* (15%) parasites.

# Results and discussion

## Chapter I

The goal of categorizing and grouping hypotheses was to facilitate comparisons between them and identify similarities and differences. This would be very useful due to the increasing number of hypotheses in the rapidly growing research field of telomeres in ecology and evolution, which can make it challenging to identify related or overlapping hypotheses.

Our first grouping (Figure 14) presents each hypothesis in relation to its research context, considering the role of telomeres in ageing, as well as their potential as markers of individual health and quality. We also group them according to the role that the hypothesis gives TL in regulating life history strategies while taking into account the physiological constraints that shape telomere-mediated life history trade-offs. This helps to provide a brief overview of the current state of research. Also, it helps to identify controversies in the different fields, such as the different ideas about the rate of telomere shortening (constant vs. variable) and its potential impact on organism performance. Within these research questions, we discuss the controversial relationship between telomeres and ageing, and whether telomeres play a causal role in determining lifespan (Figure 14, aging). While short TL is associated with increased mortality risk, the debate continues over whether telomeres actually cause ageing or simply reflect overall individual quality, health and performance (Figure 14, marker of quality). We also explore the role of telomeres in regulating life history strategies, with some hypotheses suggesting that early-life TL can "program" individual life trajectories, while others propose that telomeres function as a signal, regulating life history trade-offs during adulthood (Figure 14, mediator of trade-offs). Finally, we address the physiological constraints that regulate telomere restoration and maintenance and how life history trade-offs are supposed to be resolved with an acceptance of telomere shortening, however, the importance of energetic costs is still unclear (Figure 14, physiological currency).

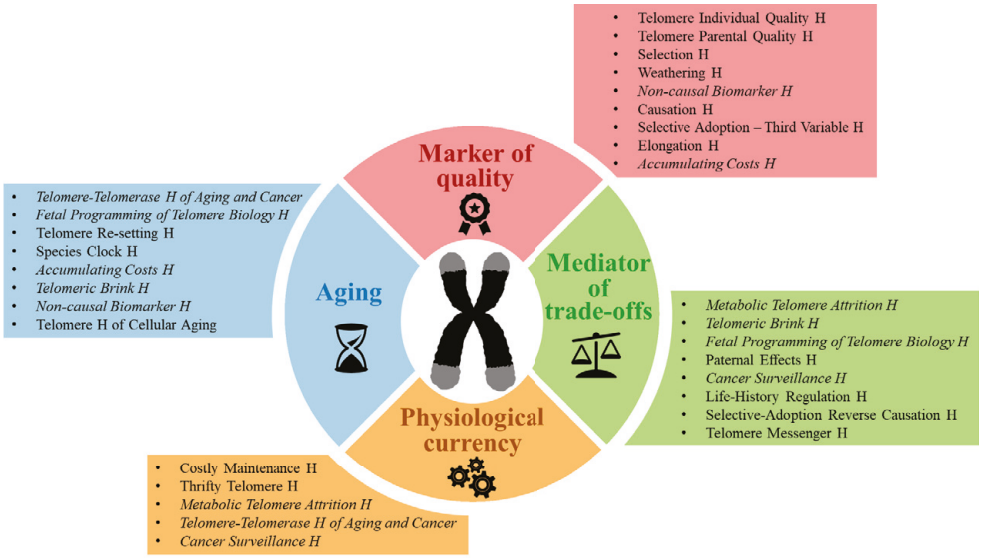



**Figure 14.** Grouping of hypotheses based on research questions (Grouping I). The hypotheses in italics occur under more than one research question as they are broadly formulated, encompassing multiple contexts.

The second grouping (Figure 15), the hierarchical categorization, orders hypotheses independently of the research context to illustrate associations and similarities between them, creating clusters that are not immediately obvious. The first dichotomic classification (Figure 15, I. vs II.) separates out models that do not assume telomere function or generate predictions about causality or not (or its direction) of TL and telomere shortening (i.e., the "no assumption models" group). The authors of these models have not been specific about the role of telomeres as a causal mediator of life history strategies or not. Our second dichotomic classification (Figure 15, II.A vs II.B) divides models into those assuming or not assuming causal effects of telomere dynamics on fitness. It highlights that whether telomere dynamics affect an organism's life history and fitness remains a critical question in telomere biology. Overall, however, most hypotheses assume a causal link between TL and fitness, suggesting that TL may act as a mediator of life history trade-offs, and those hypotheses are the ones we consider from now on. The third dichotomy of our classification (Figure 15, II.B.1 vs II.B.2) separates between the "disruption of function" and "adaptive regulation" models. The "disruption of function" models focus on the negative effects of telomere shortening, both physiological and fitness costs. In contrast, "adaptive regulation" models build on the basic idea that telomere shortening could be used as a signal of the internal state of an organism or environmental conditions that potentially could be used by the organism to change behaviours and/or life style. We recognise that the signalling hypotheses (i.e., the shortening of telomeres acts as signals thus allowing for adaptive regulation of organismal function) require further testing to be proposed as

alternatives to the models proposing purely negative effects of telomere shortening. A step forward in this sense could be to manipulate or disrupt the factors that are considered to be monitored by the telomeric signal.

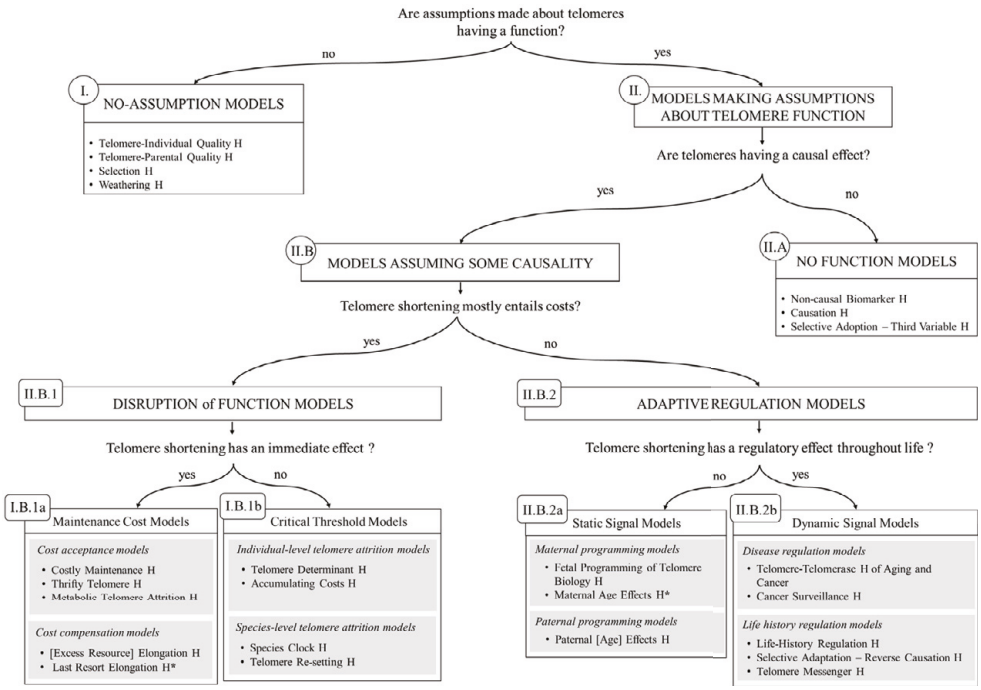



**Figure 15**. Hierarchical classification of the telomere hypotheses (Grouping II). The causality or causal effect refers to the consequences of telomere length or attrition *per se* on organism function or performance.

Our classifications provide an overview of the many different telomere hypotheses, without favouring any specific one, and highlight the need for further research in the field. For instance, one such sub-field of interest that we identified was telomere elongation hypotheses. Besides the only previously proposed hypothesis (the "[excess resources] elongation hypothesis" (Haussmann and Mauck, 2008b), we propose a new hypothesis, the "last resort elongation" hypothesis, to further inspire conceptualization and testing of hypotheses in the field of telomere ecology and evolution.

# Chapter II

The focus of this manuscript is to present and elaborate upon two hypotheses that have been proposed to explain telomere elongation patterns within ecology and evolution: the "excess resources elongation" hypothesis (EREH) and the "last resort elongation" hypothesis (LREH). Both these hypotheses assume that there are some types of significant costs to restore TL, e.g., in terms of energy, resources or cancer risk. However, these hypotheses differ in terms of which individuals are expected to show telomere elongation. The EREH hypothesis (Haussmann and Mauck, 2008b; Tobler et al., 2022), suggests that telomere restoration that results in net elongation of telomeres can only be achieved by high-quality individuals with excess resources (Figure 16). According to this model, telomere elongation may occur before or after a period of stress (see e.g., Hoelzl et al., 2016, Asghar et al., 2018).

According to the LREH, we predict telomere elongation to occur in individuals of lower quality and/or older individuals as a "last resort" to prevent them from reaching a critically short TL inducing severe somatic costs (Figure 16). These individuals are facing a critical situation affecting the trade-off between current and future investments. However, this "terminal investment" may benefit individuals in the short term by preventing severe somatic problems and allowing them to complete ongoing or reach additional reproductive events, resulting in an overall positive effect on fitness. The two hypotheses we presented here are not mutually exclusive and telomere elongation may more frequently occur when individuals reach a critically short TL and at the same time experience favourable environmental conditions.

When longitudinal studies have reported telomere elongation, these cases were often treated as a single quantitative process (i.e., "negative shortening"), resulting in a lack of attention to the patterns of telomere elongation. This is likely due to a lack of scientific framework and misconceptions about telomere elongation as a methodological artefact (e.g., Chen et al., 2011; Steenstrup et al., 2013b; Seeker et al., 2021). We argue that it is important to evaluate telomere elongation patterns explicitly, rather than treating them as a small and negligible part of telomere dynamics. In this line, recent studies provide evidence for both the EREH and LREH hypotheses, with telomere elongation in response to excess resources or more benign circumstances (Hoelzl et al., 2016, Brown et al., 2021). Moreover, there are a number of studies showing negative correlations between baseline TL measurement and subsequent telomere shortening (i.e., higher incidence of telomere elongation in individuals with short telomeres) in line with the LREH hypothesis, (Elmore et al., 2008; Nordfjäll et al., 2009; Shalev et al., 2013).

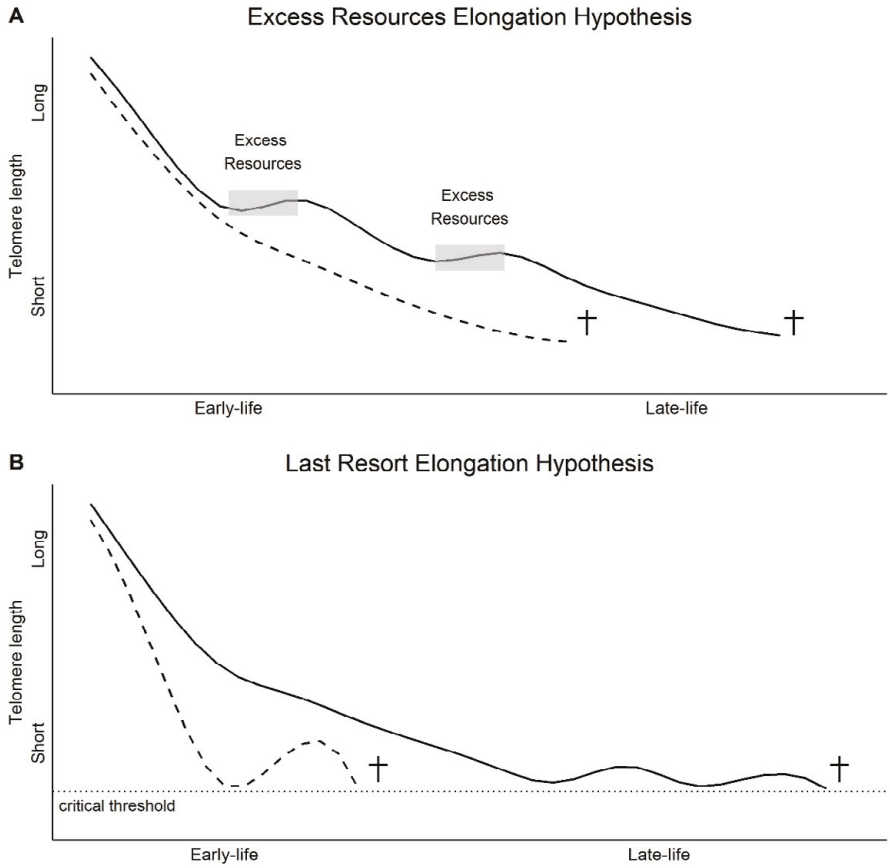



**Figure 16.** Illustration of a hypothetical scenario of telomere dynamics with ageing. In (A) the "excess resources elongation hypothesis" predicts that only high-quality individuals (solid line) with access to excess resources will invest in costly telomere restoration, leading to net telomere length elongation. Poor-quality individuals (dashed line), on the other hand, do not have access to these resources and therefore will not invest in telomere restoration. In (B), the "last resort elongation hypothesis" predicts that telomere elongation is expected in individuals in a "desperate" state (i.e., with telomeres close to the critical threshold), which may occur: (i) at an early chronological age in low-quality individuals (dashed line) with high TL shortening rates, (ii) later in life when biologically older individuals with short telomeres.

Finally, we made some suggestions on how the EREH and LREH could be specifically tested, for example by experimentally manipulating resource availability and telomerase expression. Additionally, indirect evidence based on correlational studies using proxies of individual quality (such as body condition or species-specific quality indicator traits) could be used to investigate positive relationships with the occurrence of telomere elongation.

# Chapter III

In this study, we investigated the existence of a lower threshold for TL, below which further shortening cause individuals to be removed from the population. Also, we explored whether exposure to life stressors, such as avian malaria infections, could increase the risk of reaching the critical threshold at an earlier age. The estimated value of the lower critical threshold for TL in our population of great reed warblers was found to be 0.24 T/S ratio. This lowest TL value was observed in a 9-day-old nestling (Figures 17 and 18), which is surprising given the fact that TL generally decreases with age and that we measured a large number of individuals 1-9 years old.

We examined the impact of post-fledging selection (defined as the time between fledging and 1 year of age) by comparing the eTL of all 9-day-old nestlings (pre-selection) to the sub-sets of nestlings that became recruits at 1 year of age (post-selection, recruiting uninfected and infected with malaria parasites). The mean eTL of the nestlings in the post-selection sub-set was significantly longer (mean eTL = 0.94 ± 0.02 SE) than the mean eTL of all 9-day-old nestlings in the pre-selection data set (mean eTL = 0.89 ± 0.01 SE), which could indicate a "selective disappearance" effect of nestlings with short eTL. We noted that we cannot exclude that "selective dispersal" out of the study area of individuals with short TL also could have played a role, but its impact is likely minimal as the population has a high between-year return rate (Bensch and Hasselquist, 1991; Bensch et al., 1998; Hansson et al., 2002a).

The eTL distribution of all 9-day-old nestlings (pre-selection) appeared to be normally distributed, but the short end of the eTL distributions of both post-selection datasets (uninfected and infected) seemed to be truncated. Our statistical examination analysis confirmed a significantly lower frequency of data points in the post-selection data subset within range A (i.e., delineated by the short-end limits of the eTL distributions of the pre-selection and the post-selection data sets, see Methods in Chapter III) than expected from the pre-selection data set ($p = 0.005$, Figure 17). Furthermore, infected recruits showed a significant reduction in the frequency of data points of the eTL distribution within range B (i.e., delineated by the short-end limits of the eTL distributions of the uninfected and the infected data sub-sets, see Methods in Chapter III) compared to what was expected from the pre-selection data set ($p = 0.037$, Figure 18).

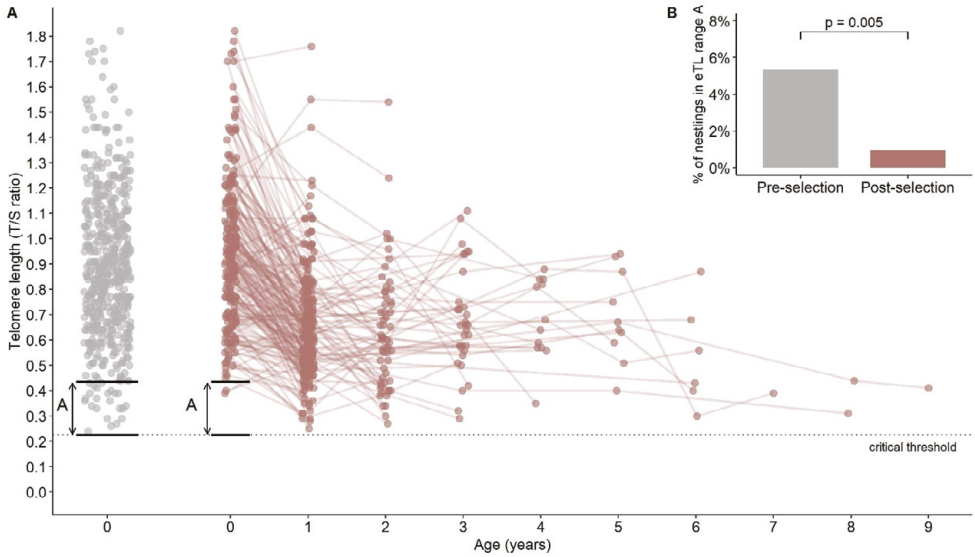



**Figure 17. A.** Longitudinal data showing telomere length (TL, measured as T/S ratio) in wild great reed warbler individuals. The individuals were born at Lake Kvismaren and followed over their lives when later returned to breed, at least when 1 year old (age range: 9 days (0 years) – 9 years; n = 839 measurement points from 507 individuals born from 1988 to 2019). Presented in grey on the left is the early-life TL (eTL) of all 9-day-old nestlings in the data set before post-fledging selection occurred (pre-selection). On the right is shown the sub-set of individuals that survived post-fledging selection (post-selection) and became recruits in the breeding population at least when 1 year old, in blue (uninfected recruits) and orange (infected recruits). The dotted line indicates our empirically derived estimate of the "critical threshold" in TL (i.e., the shortest TL value of any individual in the study population based on the whole data set = 0.24 T/S ratio). **B.** Frequency (%) of data points within range A (the "critical zone" of eTL in nestlings) in all 9-day-old nestlings (the pre-selection data set) as compared with in the recruits data sub-set (post-selection). Range A was identified as the truncated range at the short end side of the eTL distribution in the recruits data sub-set as compared with all 9-day-old nestlings (see Methods in Chapter III).

In contrast, the uninfected recruits did not differ from that of the pre-selection data set in range B ($p = 0.42$, Figure 18). This study suggests that there is a lower critical threshold in TL that predicts first-year survival in great reed warblers, which may indicate a link between TL and systemic cell and organ dysfunction. The selection against nestlings with short eTL was stronger in the infected data sub-set, indicating that infections and stressors can accelerate telomere shortening and lead to negative consequences for health and survival. These findings may explain the association between short TL, reduced lifespan and increased susceptibility to disease found in previous studies (Ilmonen et al., 2008; Effros, 2011; Boonekamp et al., 2014; Asghar et al., 2015b; Karell et al., 2017; Whittemore et al., 2019). The selection against nestlings with short eTL was stronger in the infected data sub-set, indicating that infections and stressors can accelerate telomere shortening and lead to negative consequences for health and survival. These findings may explain the association between short TL, reduced lifespan and increased susceptibility to disease found in

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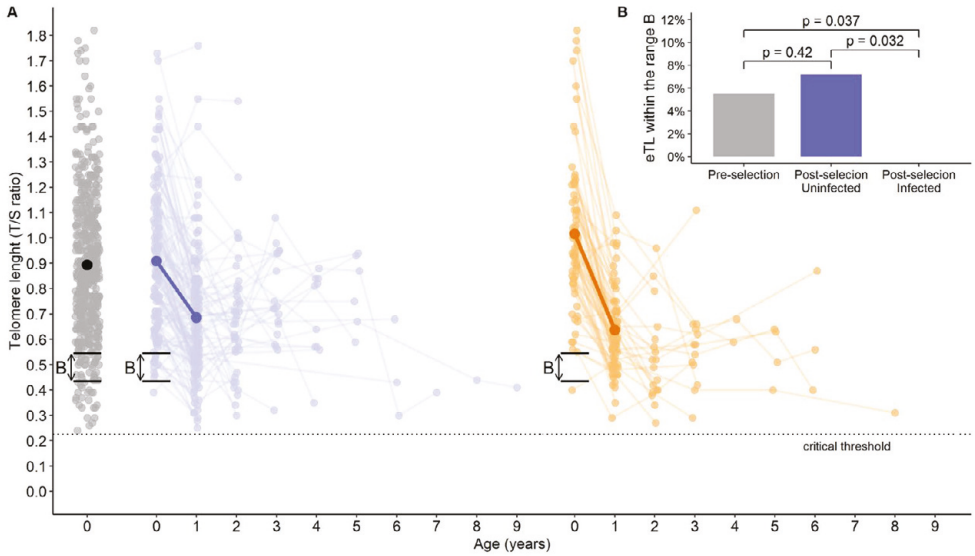


**Figure 18. A.** Longitudinal data on telomere length (TL, measured as T/S ratio) of wild great reed warbler individuals followed throughout their lifespan, that were born and later returned when ≥1 year old to breed at Lake Kvismaren (age range: 9 days (0 years) – 9 years; n = 839 measurement points from 507 individuals born from 1988 to 2019). On the left in grey, we show the early-life TL (eTL) of all 9-day-old nestlings in our dataset, before post-fledging selection had occurred (pre-selection). On the right, we present the early-life TL and TL of the subset of individuals that survived post-fledging selection (post-selection) and subsequently became recruits in the breeding population, at least, when 1-year-old. Blue represents uninfected recruits, while orange represents infected recruits. Mean telomere shortening rates over the first year of life for each group (uninfected recruits = -0.22 T/S ratio change; infected recruits = -0.38 T/S ratio change) is represented by the thick solid lines. Our empirical estimate of the "critical threshold" in TL (i.e., the shortest TL value of any individual in the study population based) is represented by the dotted line, which is 0.24 T/S ratio. **B.** Frequency (%) of data points within range B (the increased "critical zone" by the effect of infection) in all 9-day-old nestlings (pre-selection) as compared with both the recruit data sub-sets (post-selection). Range B was identified as the truncated range at the short end side of the eTL distribution of the uninfected and the infected data sub-sets (see Methods in Chapter III).

We then calculated the mean telomere shortening rate over the first year of life and subtracted the mean shortening rate from the lower eTL limit for each recruiter sub-group, we found that the predicted TL at 1 year of age would be below our established critical threshold of TL (0.24 T/S ratio). Thus, all 9-day-old individuals within ranges A (if uninfected) and range A+B (if malaria infected) were, based on the mean TL shortening rate over the first year, predicted to reach the critical threshold in TL by the time they reached 1 year of age. Each data subset had one outlier data point within the "critical zones" that survived to recruits at 1 year of age. Both these individuals showed a net increase in TL (i.e., telomere elongation) during their first year of life, indicating that telomere elongation may have contributed to their survival (as it moved their TL away from and above the critical

threshold). It is possible that most nestlings with eTL in the "critical zone" cannot afford the potential costs associated with telomere elongation (Gómez-Blanco et al., submitted - Chapter II, submitted - Chapter IV).

## Chapter IV

In this study, it was found that a significant proportion of individuals (13% of 209) in a wild population of great reed warblers showed a net increase in TL from early-life (9 days old) to 1 year of age. The likelihood of these telomere elongation events was higher in individuals without chronic avian malaria infection (uninfected) and with shorter eTL (Figure 19 and Figure 20). These findings remained statistically significant after accounting for measurement error, including regression to the mean effects.

Interestingly, we found that chronically malaria infected birds had a lower likelihood of telomere elongation (Figure 19). Here two possible explanations were proposed. The first explanation suggests that telomere elongation comes with considerable costs (see Young, 2018; Gómez-Blanco et al., submitted - Chapter II), and individuals with increased stress, such as malaria infections, may refrain from activating the restoration process since they may need to redirect resources in order to fight the infection (Eisenberg, 2011; Shay, 2016; Casagrande and Hau, 2019). The second explanation is that telomere elongation costs are relatively low allowing all individuals to restore their TL equally much, but malaria infected birds suffer stronger telomere shortening than uninfected (Von Zglinicki, 2002; Asghar et al., 2015b, 2016). This may result in an increased incidence of telomere shortening in infected birds and telomere elongation in uninfected individuals.

We found that individuals with shorter eTL were more likely to show telomere elongation (Figure 20), which supports the "last resort elongation" hypothesis (Tobler et al., 2022 Gómez-Blanco et al., submitted - Chapter II). This hypothesis suggests that individuals whose telomeres are too short (i.e., close to the "critical threshold") will activate their telomere restoration processes to substantially elongate their TL, which would take them away from the critical threshold and thus avoid senescence. However, the "excess resources elongation" hypothesis was not supported, as individuals with long telomeres (proposed to signal high-quality) less frequency elongated their telomeres (Haussmann and Mauck, 2008b; Tobler et al., 2022; Gómez-Blanco et al., submitted - Chapter II).

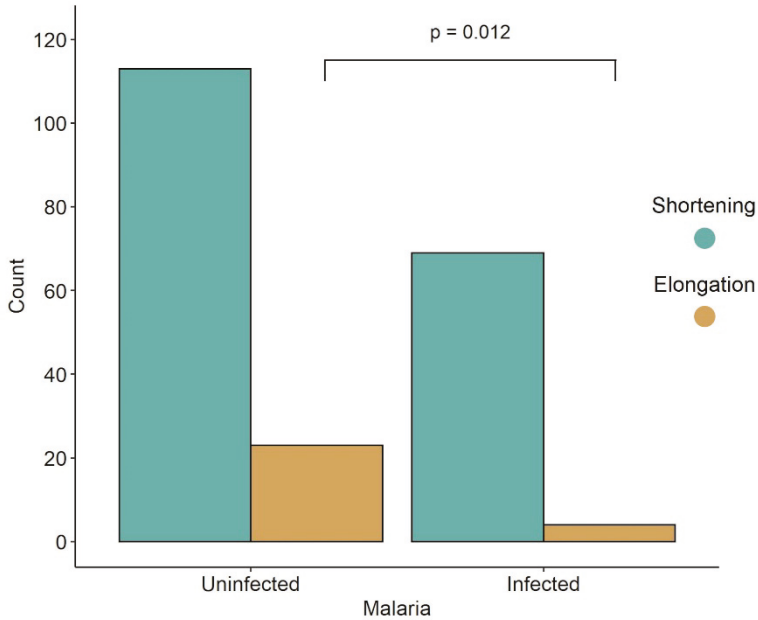


**Figure 19.** Impact of malaria infection prevalence (uninfected n = 136; infected n = 73) on the likelihood of telomere length shortening (blue) or elongation (brown) over the first year of life in great reed warblers.

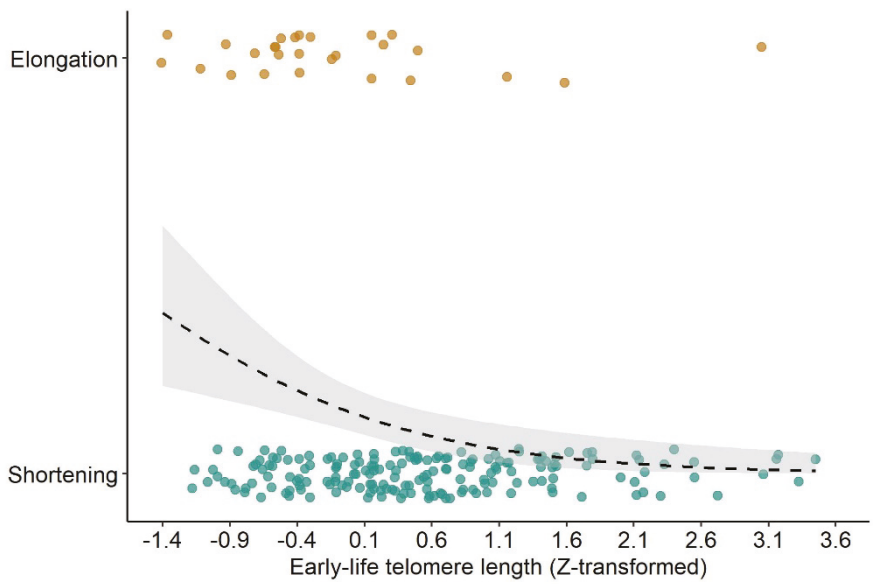


**Figure 20.** Relationship between changes in telomere length (TL) over the first year of life (elongation vs. shortening) and early-life TL (measured as T/S ratio) in great reed warblers (n = 209). The predicted probability of elongation in relation to early-life TL is presented with a 95% confidence interval shown by the shaded area.

Previous studies have suggested that the rate at which telomeres shorten may be more significant than TL itself in explaining the connection between telomeres and organismal processes like ageing, growth, reproductive success, and survival (Haussmann et al., 2003; Boonekamp et al., 2014; Tricola et al., 2018; Whittemore et al., 2019). However, the results of this study indicate that reaching a critically short TL per se may be a key factor inducing telomere elongation.

A take-home message from this study, as well as other recent studies of wild animals (Hoelzl et al., 2016; Olsson et al., 2018; Brown et al., 2021; Canestrelli et al., 2021), is that telomere elongation (at least in blood cells) may be much more common than previously thought. Therefore, changes in TL over an individual's life may be a more continuous and dynamic (not just an erosive) process that depends on environmental and physiological conditions, as well as genetic and phenotypic quality.

# Chapter V

In order to understand the importance of TL in life history ecology, it is essential to identify the factors that impact telomere shortening and elongation. Therefore, we investigated some possible predictors of telomere dynamics in a wild population, such as age, malaria status and harem size, which could be a measure of an individual quality phenotype.

Our study found that older birds experience less telomere shortening or more telomere elongation than younger birds. This potentially connects age and individual quality, since the ability to survive to older age and overcome environmental challenges may indicate the presence of advantageous traits (Nussey et al., 2013). These high-quality individuals may be capable of coping with environmental stressors better, resulting in less stress and improved self-maintenance, slowing the rate of telomere shortening. Also, younger birds are exposed to accelerated telomere shortening during growth and development, due to higher cellular proliferation and metabolic activity, and their immune system, less developed, makes them more vulnerable to disease and infection (Bekaert et al., 2004; Noreen et al., 2011; Monaghan and Ozanne, 2018). Another added effect could be that inexperienced birds (typically younger) may also struggle more to cope with environmental stressors (Lack, 1966; Hasselquist, 1998; Dingemanse and De Goede, 2004; Vergara et al., 2007).

Interestingly, our results suggest that the positive effect of age on telomere dynamics is more pronounced in females than males. This could be attributed to the differences in reproductive effort, e.g., the cost of male competition for females and defending breeding territory (Muller et al., 2021), sexual selection, such as energic

expenses of mate-attracting song displays (Hasselquist and Bensch, 2008) and increased risk of predation (Clinchy et al., 2013), and sex hormones like the immune suppress effect of testosterone (Folstad and Karter, 1992). Those factors can lead to increased stress and metabolic costs in males, especially during the breeding season. As a result, despite experiencing less telomere shortening with age, males may have reduced opportunities for self-maintenance when compared to females.

Telomeres may play also play a role in mediating the costs of diseases and immune responses. Here we found that malaria infected individuals had more telomere shortening compared to uninfected birds. This is consistent with previous research showing that disease and physiological stress can lead to faster telomere shortening with severe fitness effects (Epel et al., 2004; Asghar et al., 2015b). The negative of malaria on TL was larger in females than in males, which could be due to fundamental sex differences in the immune responses and hormonal profiles. Females exhibit stronger immune responses than males, which can lead to lower vulnerability to infections but higher costs in terms of immunopathology and autoimmune reactions (Zuk and Stoehr, 2002; Roved et al., 2017), potentially resulting in greater telomere shortening.

In males, we found that birds in larger harem sizes had experienced less telomere shortening until the current year than those with zero or fewer social partners (Figure 21). The harem size can indicate breeding effort, as males with more social partners invest more time and energy into mating activities while females in larger harem sizes may need to provide more parental care to their offspring (in our study system socially polygynous males provide less help; (Sejberg et al., 2000). However, individuals were sampled at the beginning or middle of the breeding season, so the impact of the current-year breeding effort on telomere shortening is likely not significant. On the contrary, the results of this study suggest harem size is probably an indicator of individual quality, since individuals in larger harem sizes may hold superior traits (e.g., body condition or access to resources) that make them preferred (Hasselquist, 1998; Nowicki and Searcy, 2004; Xia et al., 2021). Those traits may allow them to invest more in self-maintenance, increasing telomere elongation or reducing telomere shortening.

In females, the relationship between TL change and harem size is dependent on malaria status in females (Figure 22). We observed greater telomere shortening in non-breeding females, but exclusively in cases where they were malaria infected. In great reed warblers, being a female in a larger harem may not necessarily indicate individual quality to the same extent as in males. It is uncertain whether high-quality females prefer to breed in smaller harems (possibly with a lower-quality male but becoming the primary female in the territory) or in larger harems (with an already mated higher-quality male but becoming a non-primary female, thus receiving less help in paternal caring the offspring), weakening the association between harem size

and TL change. However, malaria-infected females showed a difference in TL change between individuals breeding and non-breeding, so it can be an indication of lower quality females that may skip breeding to improve survival and reproductive performance in the future (Seward et al., 2014; Seress et al., 2020). Individuals exposed to high levels of stressors, such as malaria infection, may be particularly affected by these patterns.

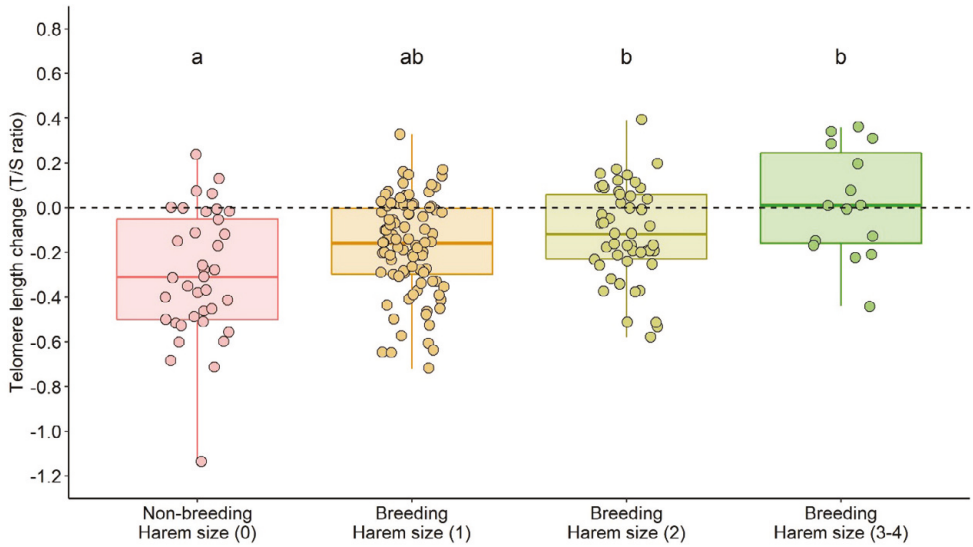


**Figure 21.** Relationship between harem size and telomere length (TL) change (negative values: telomere shortening, positive values: telomere elongation) in male great reed warblers. The boxplots indicate the median, the 25th and 75th percentiles, and the range of 9-91% of the data set. Individual data points are also included with a compact letter display used to indicate significant differences.

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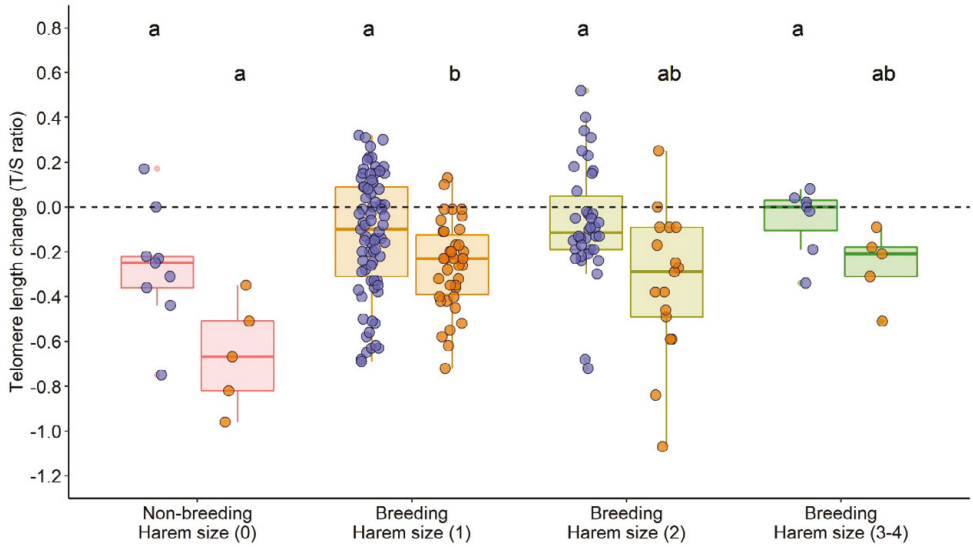


**Figure 22.** Relationship between harem size and telomere length (TL) change in female great reed warblers. Here negative TL change values indicate that telomeres have become shorter between measurements, whereas a positive value indicates a net increase of TL (i.e., telomere elongation). Boxplots show: median, 25th percentile, 75th percentile and range between 9–91% of the dataset. The individual data points are also shown (blue for uninfected and orange for malaria infected individuals), with significant differences indicated by a compact letter display comparing each malaria status separately.

# Conclusions and future perspectives

In this thesis, I intend to contribute to the current comprehension of telomere dynamics in the wild, specifically regarding the intricate interplay among TL, infections and life history trade-offs. Through an in-depth investigation of these relationships, I aspire that this thesis has contributed towards bridging some gaps in our knowledge of telomere biology and provided insights into crucial, but yet under-explored in many cases, facets of the role that telomeres play in life history ecology and evolution. At the same time, I highlighted in this thesis the need for additional investigation into some of the key areas of telomere research including the significance of telomere elongation events, the influence of short TL on shaping individual life histories and the potential of telomeres as a biomarker for individual genetic quality or as a measure of environmental stressors faced. It is imperative that to address all of these questions, a wide range of species (with different life history strategies) are studied using both captive and wild populations, considering both ecological and evolutionary perspectives. These answers may hold great potential to improve our understanding of the fundamental biological mechanisms regulating ageing processes and fitness across the phylogenetic tree.

This thesis presents evidence indicating that the increasing number of telomere hypotheses over the past five years, suggest a greater understanding of concepts related to telomeres in the context of ecology and evolution, aligning with the rapid proliferation of empirical and theoretical studies. However, there are numerous ongoing research directions in telomere biology and overlapping lines of thought and some of the main controversies between this hypothesis remain unresolved. Hence, I anticipate a promising future for telomere research in ecology and evolution and I hope that this thesis will inspire further investigation and collaboration to verify or refute the various hypotheses in this field.

Also, I provide insights into the existence of a critical threshold in short TL at the organismal level, an argued question key for the “critical threshold” models proposed in the fields of medicine and telomere ecology and evolution. I also suggest that TL is a trait that is subject to natural selection against short TL and I emphasize the association of TL with a lifespan and how stressors, such as malaria, increase the likelihood of reaching the critical TL threshold at an earlier age. I believed that the use of novel approaches that measure the very shortest telomeres of each individual cell and chromosome could offer important insights in the near

future in order to gain a better understanding of the significance of critically short telomeres for overall organism function and fitness.

In addition, I emphasized in this thesis the importance of telomere elongation patterns (i.e., where telomeres net increase in length). Despite operating throughout the same mechanisms as other telomere restoration processes that typically slow down telomere shortening, it is a biologically relevant phenomenon that deserves more attention from ecologists and evolutionary biologists. While there are benefits of telomere restoration, the potential associated costs may result in the acceptance of telomere shortening in some individuals. These episodes of telomere elongation may be particularly interesting as they may represent individual decisions to optimize life history strategies and maximize fitness, associated with stressors, like infections, or internal states, such as short TL. So, it may not be evident if we only look at the overall telomere dynamics pattern, as vertebrates typically exhibit telomere shortening over their lifetime. Thus, I believe that it is crucial to conduct more long-term studies and analyse longitudinal data using the framework described in this thesis, directly comparing individuals with telomere elongation versus shortening. I hope that the hypotheses described here will encourage this type of research, as it can shed light on whether telomere elongation reflects an adaptive strategy or merely environmental conditions, ultimately leading to a better understanding of the mechanistic foundations of life history trade-offs and how selection might act upon them.

This thesis identified several factors that can predict sex-dependent telomere dynamics, which may be indicative of an individual's phenotypic quality. While telomere dynamics can serve as a proxy for individual quality, it's important to note that the results of this thesis suggest that it is TL itself, which may ultimately trigger organismal trade-offs affecting ageing, self-maintenance, reproductive success, and survival. Additional research on the link between telomere dynamics and other ecological factors could enhance our comprehension of individual ageing trajectories in wild populations.

There have been numerous studies demonstrating the association between TL and various aspects of life history. However, there is still debate regarding whether TL triggers certain behaviours or trade-offs, or whether a third variable affects both TL and life history decisions, and telomeres simply run parallel to this variable without playing a decisive role. Several candidates for this third variable have been proposed, for instance, oxidative stress, which can cause DNA damage, shorten TL, and lead to cellular senescence and decline in overall health. However, this thesis has shown that individuals with shorter TL are more likely to exhibit telomere elongation and disappear disproportional more from the population compared to those with longer TL. If one of these candidates, like oxidative stress, were the sole factor causing accelerated ageing and death, and TL was only affected as a

consequence of oxidative stress, then it would not make sense for individuals to invest in potentially costly self-maintenance mechanisms such as telomere restoration. Therefore, it is probable that TL itself plays a regulatory role in triggering changes in an individual's life history and, under favourable conditions, individuals tend to elongate their telomeres above a critical length, which highlights that the regulation of TL is a critical and biologically relevant phenomenon.

Should be recognized and appreciated the efforts made by many members of our research community to identify and bring attention to the common mistakes in the telomere field. By highlighting these pitfalls, we can prevent similar errors from being repeated and promulgated. Throughout this thesis, I have learned the importance of carefully considering the potential sources of measurement error (e.g., contamination, pipetting, unoptimized protocols or other sources of variability between assays) and implementing appropriate correction methods to minimize their impact on the results of telomere studies(Barnett et al., 2005; Verhulst et al., 2013; Bateson and Nettle, 2017; Lin et al., 2019; Lindrose et al., 2021). In this sense, statistical corrections are essential in estimating and attempting to correct the artificial variance in our data produced by measurement errors. Although, it is crucial to exercise caution when designing statistical error correction measures, as unfortunate approaches may inadvertently introduce additional variation, aggravating rather than improving these errors, which can obscure true biological and potentially highly interesting patterns and relationships. In this thesis, I dedicated substantial effort to suggesting and carefully implementing alternative methods to deal with measurement errors.

The selection of a suitable method for measuring telomeres in a given setting or system cannot be just prescribed, since to date each available method has distinct advantages and limitations. Numerous studies using the qPCR method to enable a rapid evaluation of TL in a larger dataset have found strong correlations between average TL and life history traits and fitness. Nonetheless, mean TL estimates obtained through qPCR do not capture the full variation in TL that exists within a given sample. Since there is increasing evidence highlighting the importance of this TL variation and the cellular function (particularly the presence of critically short telomeres), alternative methods which offer a distribution of TLs within a sample (such as STELA, TeSLA, Flow-FISH or Q-FISH), should be considered. In order to comprehensively investigate the relationship between very short telomeres and other factors of variation in TL, it may be crucial to supplement the advantages of qPCR with other methods. Despite the need for a significant investment of time and resources to establish and validate these new techniques, it is crucial to recognize their potential to offer valuable additional insights in the near future of the study of telomeres in ecology and evolutionary biology.

After the accomplishment of this thesis, it is clear to me that the investigation of telomere dynamics in evolutionary and ecological contexts holds a promising future. Future research on telomere dynamics should build upon the questions addressed in this thesis, such as gaining a detailed understanding of the biological mechanisms underlying telomere dynamics, examining their evolutionary implications, investigating the relationship between telomere dynamics and environmental stressors and studying telomere dynamics in a wider range of non-model organisms to gain a more comprehensive understanding of the role of telomeres in ecology and evolution.

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# Reference List


Allsopp, R. C., and Harley, C. B. (1995). Evidence for a critical telomere length in senescent human fibroblasts. Exp. Cell Res. 219, 130–136.

Anchelin, M., Murcia, L., Alcaraz-Pérez, F., García-Navarro, E. M., and Cayuela, M. L. (2011). Behaviour of Telomere and Telomerase during Aging and Regeneration in Zebrafish. PLoS One 6, e16955. doi: 10.1371/JOURNAL.PONE.0016955.

Angelier, F., Vleck, C. M., Holberton, R. L., and Marra, P. P. (2013). Telomere length, non-breeding habitat and return rate in male American redstarts. Funct. Ecol. 27, 342–350. doi: 10.1111/1365-2435.12041.

Ar, A., and Yom-Tov, Y. (1978). The Evolution of Parental Care in Birds. Evolution (N. Y). 32, 655. doi: 10.2307/2407731.

Arsenis, N. C., You, T., Ogawa, E. F., Tinsley, G. M., and Zuo, L. (2017). Physical activity and telomere length: Impact of aging and potential mechanisms of action. Oncotarget 8, 45008–45019. doi: 10.18632/ONCOTARGET.16726.

Asghar, M., Bensch, S., Tarka, M., Hansson, B., and Hasselquist, D. (2015a). Maternal and genetic factors determine early life telomere length. Proc. R. Soc. B Biol. Sci. 282, 20142263. doi: 10.1098/rspb.2014.2263.

Asghar, M., Hasselquist, D., Hansson, B., Zehtindjiev, P., Westerdahl, H., and Bensch, S. (2015b). Hidden costs of infection: Chronic malaria accelerates telomere degradation and senescence in wild birds. Science (80). 347, 436–438. doi: 10.1126/science.1261121.

Asghar, M., Palinauskas, V., Zaghdoudi-Allan, N., Valkiūnas, G., Mukhin, A., Platonova, E., et al. (2016). Parallel telomere shortening in multiple body tissues owing to malaria infection. Proc. R. Soc. B Biol. Sci. 283. doi: 10.1098/rspb.2016.1184.

Asghar, M., Westerdahl, H., Zehtindjiev, P., Ilieva, M., Hasselquist, D., and Bensch, S. (2012). Primary peak and chronic malaria infection levels are correlated in experimentally infected great reed warblers. Parasitology 139, 1246–1252. doi: 10.1017/S0031182012000510.

Asghar, M., Yman, V., Homann, M. V., Sondén, K., Hammar, U., Hasselquist, D., et al. (2018). Cellular aging dynamics after acute malaria infection: A 12-month longitudinal study. Aging Cell 17, e12702. doi: 10.1111/acel.12702.

Atema, E., Mulder, E., Dugdale, H. L., Briga, M., van Noordwijk, A. J., and Verhulst, S. (2015). Heritability of telomere length in the Zebra Finch. J. Ornithol. 156, 1113–1123. doi: 10.1007/s10336-015-1212-7.

Aubert, G., Hills, M., and Lansdorp, P. M. (2012). Telomere length measurement-Caveats and a critical assessment of the available technologies and tools. Mutat. Res. - Fundam. Mol. Mech. Mutagen. 730, 59–67. doi: 10.1016/j.mrfmmm.2011.04.003.

Aubert, G., and Lansdorp, P. M. (2008). Telomeres and aging. Physiol. Rev. 88, 557–579. doi: 10.1152/physrev.00026.2007.

Austad, S. N. (1997). Why we age: what science is discovering about the body's journey through life. John Wiley & Sons Incorporated.

Aviv, A., Hunt, S. C., Lin, J., Cao, X., Kimura, M., and Blackburn, E. (2011). Impartial comparative analysis of measurement of leukocyte telomere length/DNA content by Southern blots and qPCR. Nucleic Acids Res. 39, e134. doi: 10.1093/nar/gkr634.

Badás, E. P., Martínez, J., Rivero de Aguilar Cachafeiro, J., Miranda, F., Figuerola, J., and Merino, S. (2015). Ageing and reproduction: antioxidant supplementation alleviates telomere loss in wild birds. J. Evol. Biol. 28, 896–905. doi: 10.1111/JEB.12615.

Bakaysa, S. L., Mucci, L. A., Slagboom, P. E., Boomsma, D. I., Mcclearn, G. E., Johansson, B., et al. (2007). Telomere length predicts survival independent of genetic influences. Aging Cell 6, 769–774. doi: 10.1111/j.1474-9726.2007.00340.x.

Barnett, A. G., van der Pols, J. C., and Dobson, A. J. (2005). Regression to the mean: What it is and how to deal with it. Int. J. Epidemiol. 34, 215–220. doi: 10.1093/ije/dyh299.

Barrett, E. L. B., Burke, T. A., Hammers, M., Komdeur, J., and Richardson, D. S. (2012). Telomere length and dynamics predict mortality in a wild longitudinal study. Mol. Ecol. 22, 249–259. doi: 10.1111/mec.12110.

Barrowclough, G. F., Cracraft, J., Klicka, J., and Zink, R. M. (2016). How many kinds of birds are there and why does it matter? PLoS One 11, e0166307. doi: 10.1371/journal.pone.0166307.

Bateson, M., Brilot, B. O., Gillespie, R., Monaghan, P., and Nettle, D. (2015). Developmental telomere attrition predicts impulsive decision-making in adult starlings. Proc. R. Soc. B Biol. Sci. 282, 20142140. doi: 10.1098/rspb.2014.2140.

Bateson, M., and Nettle, D. (2017). The telomere lengthening conundrum – it could be biology. Aging Cell 16, 312–319. doi: 10.1111/acel.12555.

Bateson, M., and Nettle, D. (2018). Why are there associations between telomere length and behaviour? Philos. Trans. R. Soc. B Biol. Sci. 373. doi: 10.1098/rstb.2016.0438.

Bauch, C., Becker, P. H., and Verhulst, S. (2014). Within the genome, long telomeres are more informative than short telomeres with respect to fitness components in a long-lived seabird. Mol. Ecol. 23, 300–310. doi: 10.1111/mec.12602.

Bauch, C., Boonekamp, J. J., Korsten, P., Mulder, E., and Verhulst, S. (2022). High heritability of telomere length and low heritability of telomere shortening in wild birds. Mol. Ecol. 31, 6308. doi: 10.1111/mec.16183.

Beamer, E. M., and Sartori, J. C. (2000). Skagit Chinook Life History Study Progress Report Number 3. La Conner Skagit Syst. Coop.

Bejerano, G., Pheasant, M., Makunin, I., Stephen, S., Kent, W. J., Mattick, J. S., et al. (2004). Ultraconserved elements in the human genome. Science (80). 304, 1321–1325. doi: 10.1126/science.1098119.

Bekaert, S., Derradji, H., and Baatout, S. (2004). Telomere biology in mammalian germ cells and during development. Dev. Biol. 274, 15–30. doi: 10.1016/j.ydbio.2004.06.023.

Belmaker, A., Hallinger, K. K., Glynn, R. A., Winkler, D. W., and Haussmann, M. F. (2019). The environmental and genetic determinants of chick telomere length in Tree Swallows (Tachycineta bicolor). Ecol. Evol. 9, 8175–8186. doi: 10.1002/ece3.5386.

Bensch, S. (1987). Studies of the ecology of the Great Reed Warbler at lake Kvismaren, in Sweden. Var Fagelvarld 46, 89–92.

Bensch, S. (1993). Female Great Reed Warblers gain direct fitness compensation for the cost of polygyny. Disertation Lund Univ., 1–34.

Bensch, S. (1996). Female Mating Status and Reproductive Success in the Great Reed Warbler: Is there a Potential Cost of Polygyny that Requires Compensation. J. Anim. Ecol. 65, 283. doi: 10.2307/5875.

Bensch, S., and Hasselquist, D. (1991). Territory Infidelity in the Polygynous Great Reed Warbler Acrocephalus arundinaceus: The Effect of Variation in Territory Attractiveness. J. Anim. Ecol. 60, 857. doi: 10.2307/5418.

Bensch, S., and Hasselquist, D. (1992). Evidence for active female choice in a polygynous warbler. Anim. Behav. 44, 301–311.

Bensch, S., Hasselquist, D., Nielsen, B., and Hansson, B. (1998). Higher Fitness for Philopatric than for Immigrant Males in a Semi-Isolated Population of Great Reed Warblers. Evolution (N. Y). 52, 877. doi: 10.2307/2411282.

Bensch, S., Hellgren, O., and Pérez-Tris, J. (2009). MalAvi: A public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome b lineages. Mol. Ecol. Resour. 9, 1353–1358. doi: 10.1111/j.1755-0998.2009.02692.x.

Bensch, S., Stjernman, M., Hasselquist, D., Ostman, O., Hansson, B., Westerdahl, H., et al. (2000). Host specificity in avian blood parasites: A study of Plasmodium and Haemoproteus mitochondrial DNA amplified from birds. Proc. R. Soc. B Biol. Sci. 267, 1583–1589. doi: 10.1098/rspb.2000.1181.

Bensch, S., Waldenström, J., Jonzén, N., Westerdahl, H., Hansson, B., Sejberg, D., et al. (2007). Temporal dynamics and diversity of avian malaria parasites in a single host species. J. Anim. Ecol. 76, 112–122. doi: 10.1111/j.1365-2656.2006.01176.x.

Biro, P. A., and Stamps, J. A. (2008). Are animal personality traits linked to life-history productivity? Trends Ecol. Evol. 23, 361–368. doi: 10.1016/j.tree.2008.04.003.

Blackburn, E. H. (1991). Structure and function of telomeres. Nature 350, 569–573. doi: 10.1038/350569a0.

Blackburn, E. H. (2005). Telomeres and telomerase: Their mechanisms of action and the effects of altering their functions. FEBS Lett. 579, 859–862. doi: 10.1016/j.febslet.2004.11.036.

Bonneaud, C., Balenger, S. L., Zhang, J., Edwards, S. V., and Hill, G. E. (2012). Innate immunity and the evolution of resistance to an emerging infectious disease in a wild bird. Mol. Ecol. 21, 2628–2639. doi: 10.1111/j.1365-294X.2012.05551.x.

Bonneaud, C., Mazuc, J., Gonzalez, G., Haussy, C., Chastel, O., Faivre, B., et al. (2003). Assessing the cost of mounting an immune response. Am. Nat. 161, 367–379. doi: 10.1086/346134.

Boonekamp, J. J., Mulder, G. A., Salomons, H. M., Dijkstra, C., Verhulst, S., Mulder, G. A., et al. (2014). Nestling telomere shortening, but not telomere length, reflects developmental stress and predicts survival in wild birds. Proc. R. Soc. B Biol. Sci. 281. doi: 10.1098/rspb.2013.3287.

Bordes, F., and Morand, S. (2011). The impact of multiple infections on wild animal hosts: a review. Infect. Ecol. Epidemiol. 1, 7346. doi: 10.3402/iee.v1i0.7346.

Bourke, A. F. G. (2007). Kin selection and the evolutionary theory of aging. Annu. Rev. Ecol. Evol. Syst. 38, 103–128. doi: 10.1146/annurev.ecolsys.38.091206.095528.

Broer, L., Codd, V., Nyholt, D. R., Deelen, J., Mangino, M., Willemsen, G., et al. (2013). Meta-analysis of telomere length in 19 713 subjects reveals high heritability, stronger maternal inheritance and a paternal age effect. Eur. J. Hum. Genet. 21, 1163–1168. doi: 10.1038/ejhg.2012.303.

Brown, T. J., Spurgin, L. G., Dugdale, H. L., Komdeur, J., Burke, T., and Richardson, D. S. (2021). Causes and consequences of telomere lengthening in a wild vertebrate population. Mol. Ecol. doi: 10.1111/MEC.16059.

Calado, R. T., and Chen, J. (2006). Telomerase: Not just for the elongation of telomeres. BioEssays 28, 109–112. doi: 10.1002/bies.20365.

Campbell, R. D., Rosell, F., Newman, C., and Macdonald, D. W. (2017). Age-related changes in somatic condition and reproduction in the Eurasian beaver: Resource history influences onset of reproductive senescence. PLoS One 12. doi: 10.1371/journal.pone.0187484.

Canestrelli, D., Bisconti, R., Liparoto, A., Angelier, F., Ribout, C., Carere, C., et al. (2021). Biogeography of telomere dynamics in a vertebrate. Ecography (Cop.). 44, 453–455. doi: 10.1111/ecog.05286.

Capkova Frydrychova, R., Biessmann, H., and Mason, J. M. (2009). Regulation of telomere length in Drosophila. Cytogenet. Genome Res. 122, 356–364. doi: 10.1159/000167823.

Casagrande, S., and Hau, M. (2019). Telomere attrition: Metabolic regulation and signalling function? Biol. Lett. 15. doi: 10.1098/rsbl.2018.0885.

Catchpole, C. K. (1986). Song repertoires and reproductive success in the great reed warbler Acrocephalus arundinaceus. Behav. Ecol. Sociobiol. 19, 439–445. doi: 10.1007/BF00300547.

Caulin, A. F., and Maley, C. C. (2011). Peto's Paradox: Evolution's prescription for cancer prevention. Trends Ecol. Evol. 26, 175–182. doi: 10.1016/j.tree.2011.01.002.

Cawthon, R. M. (2002). Telomere measurement by quantitative PCR. Nucleic Acids Res. 30, 47e – 47. doi: 10.1093/nar/30.10.e47.

Cawthon, R. M. (2009). Telomere length measurement by a novel monochrome multiplex quantitative PCR method. Nucleic Acids Res. 37, e21. doi: 10.1093/nar/gkn1027.

Cawthon, R. M., Smith, K. R., O'Brien, E., Sivatchenko, A., and Kerber, R. A. (2003). Association between telomere length in blood and mortality in people aged 60 years or older. Lancet 361, 393–395. doi: 10.1016/S0140-6736(03)12384-7.

Cesare, A. J., and Reddel, R. R. (2010). Alternative lengthening of telomeres: Models, mechanisms and implications. Nat. Rev. Genet. 11, 319–330. doi: 10.1038/nrg2763.

Charmantier, A., Demeyrier, V., Lambrechts, M., Perret, S., and Grégoire, A. (2017). Urbanization is associated with divergence in pace-of-life in great tits. Front. Ecol. Evol. 5. doi: 10.3389/fevo.2017.00053.

Charmantier, A., Garant, D., and Kruuk, L. (2014). Quantitative Genetics in the Wild. doi: 10.1093/acprof:oso/9780199674237.001.0001.

Charmantier, A., Perrins, C., McCleery, R. H., and Sheldon, B. C. (2006). Quantitative genetics of age at reproduction in wild swans: Support for antagonistic pleiotropy models of senescence. Proc. Natl. Acad. Sci. U. S. A. 103, 6587–6592. doi: 10.1073/pnas.0511123103.

Chavatte, J.-M., Grès, V., Snounou, G., Chabaud, A., and Landau, I. (2009). Plasmodium (Apicomplexa) of the skylark (Alauda arvensis). Zoosystema 31, 369–383. doi: 10.5252/z2009n2a8.

Chen, W., Kimura, M., Kim, S., Cao, X., Srinivasan, S. R., Berenson, G. S., et al. (2011). Longitudinal versus cross-sectional evaluations of leukocyte telomere length dynamics: Age-dependent telomere shortening is the rule. Journals Gerontol. - Ser. A Biol. Sci. Med. Sci. 66 A, 312–319. doi: 10.1093/gerona/glq223.

Chow, T. T., Zhao, Y., Mak, S. S., Shay, J. W., and Wright, W. E. (2012). Early and late steps in telomere overhang processing in normal human cells: The position of the final RNA primer drives telomere shortening. Genes Dev. 26, 1167–1178.

Ciloglu, A., Ellis, V. A., Bernotienė, R., Valkiūnas, G., and Bensch, S. (2019). A new one-step multiplex PCR assay for simultaneous detection and identification of avian haemosporidian parasites. Parasitol. Res. 118, 191–201. doi: 10.1007/s00436-018-6153-7.

Clinchy, M., Sheriff, M. J., and Zanette, L. Y. (2013). Predator-induced stress and the ecology of fear. Funct. Ecol. 27, 56–65. doi: 10.1111/1365-2435.12007.

Cooper, E. B., and Kruuk, L. E. B. (2018). Ageing with a silver-spoon: A meta-analysis of the effect of developmental environment on senescence. Evol. Lett., 460–471. doi: 10.1002/evl3.79.

Cotto, O., and Ronce, O. (2014). Maladaptation as a source of senescence in habitats variable in space and time. Evolution (N. Y). 68, 2481–2493. doi: 10.1111/evo.12462.

Cramp, S., and Perrins, C. M. (1994). Handbook of the Birds of the Western Palearctic.

Criscuolo, F., Bize, P., Nasir, L., Metcalfe, N. B., Foote, C. G., Griffiths, K., et al. (2009). Real-time quantitative PCR assay for measurement of avian telomeres. J. Avian Biol. 40, 342–347. doi: 10.1111/j.1600-048X.2008.04623.x.

Cumming, G., Fidler, F., and Vaux, D. L. (2007). Error bars in experimental biology. J. Cell Biol. 177, 7–11. doi: 10.1083/jcb.200611141.

Dammhahn, M., Dingemanse, N. J., Niemelä, P. T., and Réale, D. (2018). Pace-of-life syndromes: a framework for the adaptive integration of behaviour, physiology and life history. Behav. Ecol. Sociobiol. 72, 62. doi: 10.1007/s00265-018-2473-y.

Darling, E. S., Alvarez-Filip, L., Oliver, T. A., Mcclanahan, T. R., and Côté, I. M. (2012). Evaluating life-history strategies of reef corals from species traits. Ecol. Lett. 15, 1378–1386. doi: 10.1111/j.1461-0248.2012.01861.x.

Darwin, C. (1859). On the origin of species by means of natural selection, or, the preservation of favoured races in the struggle for life. London: J. Murray.

De Lange, T., and Jacks, T. (1999). For better or worse? Telomerase inhibition and cancer. Cell 98, 273–275. doi: 10.1016/S0092-8674(00)81955-8.

Delany, M. E., Daniels, L. M., Swanberg, S. E., and Taylor, H. A. (2003). Telomeres in the chicken: Genome stability and chromosome ends. Poult. Sci. 82, 917–926. doi: 10.1093/ps/82.6.917.

Delibes-Mateos, M., Delibes, M., Ferreras, P., and Villafuerte, R. (2008). Key role of European rabbits in the conservation of the western Mediterranean Basin hotspot. Conserv. Biol. 22, 1106–1117. doi: 10.1111/j.1523-1739.2008.00993.x.

Delibes-Mateos, M., Ferreira, C., Carro, F., Escudero, M. A., and Gortázar, C. (2014). Ecosystem effects of variant rabbit hemorrhagic disease virus, Iberian Peninsula. Emerg. Infect. Dis. 20, 2166–2168. doi: 10.3201/eid2012.140517.

Dingemanse, N. J., and De Goede, P. (2004). The relation between dominance and exploratory behavior is context-dependent in wild great tits. Behav. Ecol. 15, 1023–1030. doi: 10.1093/beheco/arh115.

Douhard, M., Loe, L. E., Stien, A., Bonenfant, C., Justin Irvine, R., Veiberg, V., et al. (2016). The influence of weather conditions during gestation on life histories in a wild Arctic ungulate. Proc. R. Soc. B Biol. Sci. 283. doi: 10.1098/rspb.2016.1760.

Dyrcz, A. (2020). Great Reed Warbler (Acrocephalus arundinaceus), version 1.0. In Birds of the World (J. del Hoyo, A. Elliott, J. Sargatal, D. A. Christie, and E. de Juana, Editors). Cornell Lab of Ornithology, Ithaca, NY, USA. https://doi.org/10.2173/bow.grrwar1.01.

Eastwood, J. R., Hall, M. L., Teunissen, N., Kingma, S. A., Hidalgo Aranzamendi, N., Fan, M., et al. (2019). Early-life telomere length predicts lifespan and lifetime reproductive success in a wild bird. Mol. Ecol. 28, 1127–1137. doi: 10.1111/mec.15002.

Ebert, D., Lipsitch, M., and Mangin, K. L. (2000). The effect of parasites on host population density and extinction: Experimental epidemiology with Daphnia and six microparasites. Am. Nat. 156, 459–477. doi: 10.1086/303404.

Effros, R. B. (2011). Telomere/telomerase dynamics within the human immune system: Effect of chronic infection and stress. Exp. Gerontol. 46, 135–140. doi: 10.1016/j.exger.2010.08.027.

Eisenberg, D. T. A. (2011). An evolutionary review of human telomere biology: The thrifty telomere hypothesis and notes on potential adaptive paternal effects. Am. J. Hum. Biol. 23, 149–167. doi: 10.1002/ajhb.21127.

Ejiri, H., Sato, Y., Kim, K. S., Tsuda, Y., Murata, K., Saito, K., et al. (2011). Blood meal identification and prevalence of Avian Malaria parasite in mosquitoes collected at Kushiro Wetland, A Subarctic Zone of Japan. J. Med. Entomol. 48, 904–908. doi: 10.1603/ME11053.

Ellis, V. A., Kunkel, M. R., and Ricklefs, R. E. (2014). The ecology of host immune responses to chronic avian haemosporidian infection. Oecologia 176, 729–737. doi: 10.1007/s00442-014-3048-x.

Elmore, L. W., Norris, M. W., Sircar, S., Bright, A. T., McChesney, P. A., Winn, R. N., et al. (2008). Upregulation of telomerase function during tissue regeneration. Exp. Biol. Med. 233, 958–967. doi: 10.3181/0712-RM-345.

Endler, J. A. (1986). Natural selection in the wild. Princeton University Press.

Entringer, S., de Punder, K., Buss, C., and Wadhwa, P. D. (2018). The fetal programming of telomere biology hypothesis: An update. Philos. Trans. R. Soc. B Biol. Sci. 373. doi: 10.1098/rstb.2017.0151.

Epel, E. S., Blackburn, E. H., Lin, J., Dhabhar, F. S., Adler, N. E., Morrow, J. D., et al. (2004). Accelerated telomere shortening in response to life stress. PNAS 101, 17312–17315. doi: 10.1073/pnas.0407162101.

Ezaki, Y. (1987). Male Time Budgets and Recovery of Singing Rate after Pairing in Polygamous Great Reed Warblers. Japanese J. Ornithol. 36, 1–11. doi: 10.3838/jjo.36.1.

Factor-Litvak, P., Susser, E., Kezios, K., McKeague, I., Kark, J. D., Hoffman, M., et al. (2016). Leukocyte telomere length in newborns: Implications for the role of telomeres in human disease. Pediatrics 137. doi: 10.1542/peds.2015-3927.

Fairlie, J., Holland, R., Pilkington, J. G., Pemberton, J. M., Harrington, L., and Nussey, D. H. (2016). Lifelong leukocyte telomere dynamics and survival in a free-living mammal. Aging Cell 15, 140–148. doi: 10.1111/acel.12417.

Flanary, B. E., and Kletetschka, G. (2005). Analysis of telomere length and telomerase activity in tree species of various life-spans, and with age in the bristlecone pine Pinus longaeva. Biogerontology 6, 101–111. doi: 10.1007/s10522-005-3484-4.

Flatt, T., and Heyland, A. (2011). Mechanisms of life history evolution: the genetics and physiology of life history traits and trade-offs. Oxford University Press.

Foley, N. M., Hughes, G. M., Huang, Z., Clarke, M., Jebb, D., Whelan, C. V., et al. (2018). Growing old, yet staying young: The role of telomeres in bats' exceptional longevity. Sci. Adv. 4. doi: 10.1126/sciadv.aao0926.

Folstad, I., and Karter, A. J. (1992). Parasites, bright males, and the immunocompetence handicap. Am. Nat. 139, 603–622. doi: 10.1086/285346.

Garamszegi, L. Z. (2011). Climate change increases the risk of malaria in birds. Glob. Chang. Biol. 17, 1751–1759. doi: 10.1111/j.1365-2486.2010.02346.x.

Garnham, P. C. C. (1966). Malaria Parasites and other Haemosporidia. Malar. Parasites other Haemosporidia.

Giraudeau, M., Angelier, F., and Sepp, T. (2019). Do Telomeres Influence Pace-of-Life-Strategies in Response to Environmental Conditions Over a Lifetime and Between Generations? BioEssays 41, 1–6. doi: 10.1002/bies.201800162.

Gomes, N. M. V., Ryder, O. A., Houck, M. L., Charter, S. J., Walker, W., Forsyth, N. R., et al. (2011). Comparative biology of mammalian telomeres: Hypotheses on ancestral states and the roles of telomeres in longevity determination. Aging Cell 10, 761–768. doi: 10.1111/J.1474-9726.2011.00718.X.

Gomes, N. M. V., Shay, J. W., and Wright, W. E. (2010). Telomere biology in Metazoa. FEBS Lett. 584, 3741–3751. doi: 10.1016/j.febslet.2010.07.031.

Gómez-Blanco, D., Santoro, S., Borrás, A., Cabrera, J., Senar, J. C., and Edelaar, P. (2019). Beak morphology predicts apparent survival of crossbills: due to selective survival or selective dispersal? J. Avian Biol. 50, 1–10. doi: 10.1111/jav.02107.

Goodson, J. L. (2005). The vertebrate social behavior network: Evolutionary themes and variations. Horm. Behav. 48, 11–22. doi: 10.1016/j.yhbeh.2005.02.003.

Gorbunova, V., and Seluanov, A. (2009). Coevolution of telomerase activity and body mass in mammals: From mice to beavers. Mech. Ageing Dev. 130, 3–9. doi: 10.1016/j.mad.2008.02.008.

Griffith, J. D., Comeau, L., Rosenfield, S., Stansel, R. M., Bianchi, A., Moss, H., et al. (1999). Mammalian telomeres end in a large duplex loop. Cell 97, 503–514. doi: 10.1016/S0092-8674(00)80760-6.

Hammers, M., Richardson, D. S., Burke, T., and Komdeur, J. (2013). The impact of reproductive investment and early-life environmental conditions on senescence: Support for the disposable soma hypothesis. J. Evol. Biol. 26, 1999–2007. doi: 10.1111/jeb.12204.

Hansson, B., Bensch, S., and Hasselquist, D. (2002a). Predictors of natal dispersal in great reed warblers: Results from small and large census areas. J. Avian Biol. 33, 311–314. doi: 10.1034/j.1600-048X.2002.330314.x.

Hansson, B., Bensch, S., Hasselquist, D., and Nielsen, B. (2002b). Restricted dispersal in a long-distance migrant bird with patchy distribution, the great reed warbler. Oecologia 130, 536–542. doi: 10.1007/s00442-001-0831-2.

Harley, C. B., Futcher, A. B., and Greider, C. W. (1990). Telomeres shorten during ageing of human fibroblasts. Nature 345, 458–460. doi: 10.1038/345458a0.

Hasselquist, D. (1994). Male attractiveness, mating tactics and realized fitness in the polygynous Great Reed Warbler. Disertation Lund Univ.

Hasselquist, D. (1995). Demography and lifetime reproductive success in the polygynous great reed warbler. Japanese J. Ornithol. 44, 181–194.

Hasselquist, D. (1998). Polygyny in Great Reed Warblers : A Long-Term Study of Factors Contributing to Male Fitness. Ecology 79, 2376–2390. doi: 10.1890/0012-9658(1998)079[2376:PIGRWA]2.0.CO;2.

Hasselquist, D., and Bensch, S. (1991). Trade-off between mate guarding and mate attraction in the polygynous great reed warbler. Behav. Ecol. Sociobiol. 28, 187–193. doi: 10.1007/BF00172170.

Hasselquist, D., and Bensch, S. (2008). Daily energy expenditure of singing great reed warblers acrocephalus arundinaceus. J. Avian Biol. 39, 384–388. doi: 10.1111/j.0908-8857.2008.04427.x.

Hasselquist, D., Östman, Ö., Waldenström, J., and Bensch, S. (2007). Temporal patterns of occurrence and transmission of the blood parasite Haemoproteus payevskyi in the great reed warbler Acrocephalus arundinaceus. J. Ornithol. 148, 401–409. doi: 10.1007/s10336-007-0144-2.

Hasselquist, D., and Tobler, M. (2021). The Accumulating Costs Hypothesis—to Better Understand Delayed “Hidden” Costs of Seemingly Mild Disease and Other Moderate Stressors. Front. Ecol. Evol. 0, 489. doi: 10.3389/FEVO.2021.685057.

Haussmann, M. F., and Mauck, R. A. (2008a). New strategies for telomere-based age estimation. Mol. Ecol. Resour. 8, 264–274. doi: 10.1111/j.1471-8286.2007.01973.x.

Haussmann, M. F., and Mauck, R. A. (2008b). Telomeres and longevity: Testing an evolutionary hypothesis. Mol. Biol. Evol. 25, 220–228. doi: 10.1093/molbev/msm244.

Haussmann, M. F., Winkler, D. W., Huntington, C. E., Nisbet, I. C. T., and Vleck, C. M. (2007). Telomerase activity is maintained throughout the lifespan of long-lived birds. Exp. Gerontol. 42, 610–618. doi: 10.1016/j.exger.2007.03.004.

Haussmann, M. F., Winkler, D. W., O'Reilly, K. M., Huntington, C. E., Nisbet, I. C. T., and Vleck, C. M. (2003). Telomeres shorten more slowly in long-lived birds and mammals than in short-lived ones. Proc. R. Soc. B Biol. Sci. 270, 1387–1392. doi: 10.1098/rspb.2003.2385.

Haussmann, M. F., Winkler, D. W., and Vleck, C. M. (2005). Longer telomeres associated with higher survival in birds. Biol. Lett. 1, 212–214. doi: 10.1098/rsbl.2005.0301.

Hedenström, A. (2008). Adaptations to migration in birds: Behavioural strategies, morphology and scaling effects. Philos. Trans. R. Soc. B Biol. Sci. 363, 287–299. doi: 10.1098/rstb.2007.2140.

Heidinger, B. J., Blount, J. D., Boner, W., Griffiths, K., Metcalfe, N. B., and Monaghan, P. (2012). Telomere length in early life predicts lifespan. Proc. Natl. Acad. Sci. 109, 1743–1748. doi: 10.1073/pnas.1113306109.

Hellgren, O., Atkinson, C. T., Bensch, S., Albayrak, T., Dimitrov, D., Ewen, J. G., et al. (2015). Global phylogeography of the avian malaria pathogen Plasmodium relictum based on MSP1 allelic diversity. Ecography (Cop.). 38, 842–850. doi: 10.1111/ecog.01158.

Hellgren, O., Waldenström, J., and Bensch, S. (2004). A new PCR assay for simultaneous studies of Leucocytozoon, Plasmodium, and Haemoproteus from avian blood. J. Parasitol. 90, 797–802. doi: 10.1645/GE-184R1.

Herborn, K. A., Heidinger, B. J., Boner, W., Noguera, J. C., Adam, A., Daunt, F., et al. (2014). Stress exposure in early post-natal life reduces telomere length: An experimental demonstration in a long-lived seabird. Proc. R. Soc. B Biol. Sci. 281. doi: 10.1098/rspb.2013.3151.

Hoelzl, F., Cornils, J. S., Smith, S., Moodley, Y., and Ruf, T. (2016). Telomere dynamics in free-living edible dormice (Glis glis): The impact of hibernation and food supply. J. Exp. Biol. 219, 2469–2474. doi: 10.1242/jeb.140871.

Holt, S. E., Shay, J. W., and Wright, W. E. (1996). Refining the telomere-telomerase hypothesis of aging and cancer. Nat. Biotechnol. 14, 836–839. doi: 10.1038/nbt0796-836.

Hooper, A. K., Spagopoulou, F., Wylde, Z., Maklakov, A. A., and Bonduriansky, R. (2017). Ontogenetic timing as a condition-dependent life history trait: High-condition males develop quickly, peak early, and age fast. Evolution (N. Y). 71, 671–685. doi: 10.1111/evo.13172.

Hornsby, P. J. (2007). Telomerase and the aging process. Exp. Gerontol. 42, 575–581. doi: 10.1016/j.exger.2007.03.007.

Houle, D. (2001). The character problem in life history evolution. Character Concept Evol. Biol. Acad. Press. San Diego, 109–140.

Hudson, P. J. P., Rizzoli, A. P., Grenfell, B., Heesterbeek, H., Dobson, A. P., and Heesterbeek, J. (2002). Ecology of wildlife diseases. Oxford Biol.

Huzen, J., Wong, L. S. M., van Veldhuisen, D. J., Samani, N. J., Zwinderman, A. H., Codd, V., et al. (2014). Telomere length loss due to smoking and metabolic traits. J. Intern. Med. 275, 155–163. doi: 10.1111/joim.12149.

Ilmonen, E., Hämäläinen, A., Schuett, W., and Tarka, M. (2018). Evolution of sex-specific pace-of-life syndromes: genetic architecture and physiological mechanisms. Behav. Ecol. Sociobiol. 72, 60. doi: 10.1007/s00265-018-2462-1.

Ilmonen, P., Kotrschal, A., and Penn, D. J. (2008). Telomere attrition due to infection. PLoS One 3, e2143. doi: 10.1371/journal.pone.0002143.

Jones, O. R., Scheuerlein, A., Salguero-Gómez, R., Camarda, C. G., Schaible, R., Casper, B. B., et al. (2014). Diversity of ageing across the tree of life. Nature 505, 169–173. doi: 10.1038/nature12789.

Karell, P., Bensch, S., Ahola, K., and Asghar, M. (2017). Pale and dark morphs of tawny owls show different patterns of telomere dynamics in relation to disease status. Proc. R. Soc. B Biol. Sci. 284. doi: 10.1098/rspb.2017.1127.

Kirkwood, T. B. L., and Holliday, R. (1979). The evolution of ageing and longevity. Proc. R. Soc. London - Biol. Sci. 205, 531–546. doi: 10.1098/rspb.1979.0083.

Kirkwood, T. B. L., and Rose, M. R. (1991). Evolution of senescence: late survival sacrificed for reproduction. Philos. Trans. - R. Soc. London, B 332, 15–24. doi: 10.1098/rstb.1991.0028.

Klein, S. L. (2003). Parasite manipulation of the proximate mechanisms that mediate social behavior in vertebrates. Physiol. Behav. 79, 441–449. doi: 10.1016/S0031-9384(03)00163-X.

Klomp, H. (2002). The Determination of Clutch-Size in Birds a Review. Ardea 38–90, 1–124. doi: 10.5253/arde.v58.p1.

Kotrschal, A., Ilmonen, P., and Penn, D. J. (2007). Stress impacts telomere dynamics. Biol. Lett. 3, 128–130. doi: 10.1098/rsbl.2006.0594.

Lack, D. (1947). The Significance of Clutch-size. Ibis (Lond. 1859). 89, 302–352. doi: 10.1111/j.1474-919X.1947.tb04155.x.

Lack, D. L. (1966). Population studies of birds. Clarendon P.

Lafferty, K. D., Dobson, A. P., and Kuris, A. M. (2006). Parasites dominate food web links. Proc. Natl. Acad. Sci. U. S. A. 103, 11211–11216. doi: 10.1073/pnas.0604755103.

Lai, T. P., Wright, W. E., and Shay, J. W. (2018). Comparison of telomere length measurement methods. Philos. Trans. R. Soc. B Biol. Sci. 373. doi: 10.1098/rstb.2016.0451.

Latifovic, L., Peacock, S. D., Massey, T. E., and King, W. D. (2016). The influence of alcohol consumption, cigarette smoking, and physical activity on leukocyte telomere length. Cancer Epidemiol. Biomarkers Prev. 25, 374–380. doi: 10.1158/1055-9965.EPI-14-1364.

Lemaître, J. F., and Gaillard, J. M. (2017). Reproductive senescence: new perspectives in the wild. Biol. Rev. 92, 2182–2199. doi: 10.1111/brv.12328.

Lemaître, J. F., Gaillard, J. M., Pemberton, J. M., Clutton-Brock, T. H., and Nussey, D. H. (2014). Early life expenditure in sexual competition is associated with increased reproductive senescence in male red deer. Proc. R. Soc. B Biol. Sci. 281. doi: 10.1098/rspb.2014.0792.

Lemke, H. W., Tarka, M., Klaassen, R. H. G., Åkesson, M., Bensch, S., Hasselquist, D., et al. (2013). Annual Cycle and Migration Strategies of a Trans-Saharan Migratory Songbird: A Geolocator Study in the Great Reed Warbler. PLoS One 8, e79209. doi: 10.1371/journal.pone.0079209.

Leroi, A. M., Bartke, A., Benedictis, G. De, Franceschi, C., Gartner, A., Gonos, E., et al. (2005). What evidence is there for the existence of individual genes with antagonistic pleiotropic effects? Mech. Ageing Dev. 126, 421–429. doi: 10.1016/j.mad.2004.07.012.

Lessios, H. A. (1988). Mass Mortality of Diadema Antillarum in the Caribbean: What Have We Learned? Annu. Rev. Ecol. Syst. 19, 371–393. doi: 10.1146/annurev.es.19.110188.002103.

Lin, J., Smith, D. L., Esteves, K., and Drury, S. (2019). Telomere length measurement by qPCR – Summary of critical factors and recommendations for assay design. Psychoneuroendocrinology 99, 271–278. doi: 10.1016/j.psyneuen.2018.10.005.

Lindrose, A. R., McLester-Davis, L. W. Y., Tristano, R. I., Kataria, L., Gadalla, S. M., Eisenberg, D. T. A., et al. (2021). Method comparison studies of telomere length measurement using qPCR approaches: A critical appraisal of the literature. PLoS One 16. doi: 10.1371/journal.pone.0245582.

López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., and Kroemer, G. (2013). The hallmarks of aging. Cell 153, 1194. doi: 10.1016/j.cell.2013.05.039.

Losos, J. B. (2014). The Princeton guide to evolution. Choice Rev. Online 51, 51-5585-51–5585. doi: 10.5860/choice.51-5585.

Lyon, B. E., and Montgomerie, R. (2012). Sexual selection is a form of social selection. Philos. Trans. R. Soc. B Biol. Sci. 367, 2266–2273. doi: 10.1098/RSTB.2012.0012.

Malaquin, N., Martinez, A., and Rodier, F. (2016). Keeping the senescence secretome under control: Molecular reins on the senescence-associated secretory phenotype. Exp. Gerontol. 82, 39–49. doi: 10.1016/j.exger.2016.05.010.

Malmiga, G., Tarka, M., Alerstam, T., Hansson, B., and Hasselquist, D. (2021). Individual and sex-related patterns of prolonged flights during both day and night by great reed warblers crossing the Mediterranean Sea and Sahara Desert. J. Avian Biol. 52, 2549. doi: 10.1111/jav.02549.

Martin-Ruiz, C. M., Baird, D., Roger, L., Boukamp, P., Krunic, D., Cawthon, R., et al. (2015). Reproducibility of telomere length assessment: An international collaborative study. Int. J. Epidemiol. 44, 1673–1683. doi: 10.1093/ije/dyu191.

Martin, L. B., Hasselquist, D., and Wikelski, M. (2006). Investment in immune defense is linked to pace of life in house sparrows. Oecologia 147, 565–575. doi: 10.1007/s00442-005-0314-y.

Martin, L. B., Weil, Z. M., and Nelson, R. J. (2007). Immune defense and reproductive pace of life in Peromyscus mice. Ecology 88, 2516–2528. doi: 10.1890/07-0060.1.

Mathur, M. B., Epel, E., Kind, S., Desai, M., Parks, C. G., Sandler, D. P., et al. (2016). Perceived stress and telomere length: A systematic review, meta-analysis, and methodologic considerations for advancing the field. Brain. Behav. Immun. 54, 158–169. doi: 10.1016/j.bbi.2016.02.002.

Medawar, P. B. (1952). An unsolved problem of biology. H. K. Lewis, London.

Meyne, J., Ratliff, R. L., and Moyzis, R. K. (1989). Conservation of the human telomere sequence (TTAGGG)(n) among vertebrates. Proc. Natl. Acad. Sci. U. S. A. 86, 7049–7053. doi: 10.1073/pnas.86.18.7049.

Mizutani, Y., Tomita, N., Niizuma, Y., and Yoda, K. (2013). Environmental perturbations influence telomere dynamics in long-lived birds in their natural habitat. Biol. Lett. 9, 20130511. doi: 10.1098/rsbl.2013.0511.

Monaghan, P. (2010). Telomeres and life histories: The long and the short of it. Ann. N. Y. Acad. Sci. 1206, 130–142. doi: 10.1111/j.1749-6632.2010.05705.x.

Monaghan, P. (2014). Organismal stress, telomeres and life histories. J. Exp. Biol. 217, 57–66. doi: 10.1242/jeb.090043.

Monaghan, P., Eisenberg, D. T. A., Harrington, L., and Nussey, D. (2018). Understanding diversity in telomere dynamics. Philos. Trans. R. Soc. B Biol. Sci. 373. doi: 10.1098/rstb.2016.0435.

Monaghan, P., and Haussmann, M. F. (2006). Do telomere dynamics link lifestyle and lifespan? Trends Ecol. Evol. 21, 47–53. doi: 10.1016/j.tree.2005.11.007.

Monaghan, P., and Ozanne, S. E. (2018). Somatic growth and telomere dynamics in vertebrates: Relationships, mechanisms and consequences. Philos. Trans. R. Soc. B Biol. Sci. 373. doi: 10.1098/rstb.2016.0446.

Montiglio, P. O., Dammhahn, M., Dubuc Messier, G., and Réale, D. (2018). The pace-of-life syndrome revisited: the role of ecological conditions and natural history on the slow-fast continuum. Behav. Ecol. Sociobiol. 72. doi: 10.1007/s00265-018-2526-2.

Montpetit, A. J., Alhareeri, A. A., Montpetit, M., Starkweather, A. R., Elmore, L. W., Filler, K., et al. (2014). Telomere length: A review of methods for measurement. Nurs. Res. 63, 289–299.

Moyzis, R. K., Buckingham, J. M., Cram, L. S., Dani, M., Deaven, L. L., Jones, M. D., et al. (1988). A highly conserved repetitive DNA sequence, (TTAGGG)(n), present at the telomeres of human chromosomes. Proc. Natl. Acad. Sci. U. S. A. 85, 6622–6626. doi: 10.1073/pnas.85.18.6622.

Muller, M. N., Enigk, D. K., Fox, S. A., Lucore, J., Machanda, Z. P., Wrangham, R. W., et al. (2021). Aggression, glucocorticoids, and the chronic costs of status competition for wild male chimpanzees. Horm. Behav. 130, 104965. doi: 10.1016/j.yhbeh.2021.104965.

Muraki, K., Nyhan, K., Han, L., and Murnane, J. P. (2012). Mechanisms of telomere loss and their consequences for chromosome instability. Front. Oncol. 2, 135. doi: 10.3389/fonc.2012.00135.

Murnane, J. P., Sabatier, L., Marder, B. A., and Morgan, W. F. (1994). Telomere dynamics in an immortal human cell line. EMBO J. 13, 4953–4962. doi: 10.1006/excr.1998.4293.

Nakagawa, S., Gemmell, N. J., and Burke, T. (2004). Measuring vertebrate telomeres: Applications and limitations. Mol. Ecol. 13, 2523–2533. doi: 10.1111/j.1365-294X.2004.02291.x.

Nakamura, A. J., Redon, C. E., Bonner, W. M., and Sedelnikova, O. A. (2009). Telomere-dependent and telomere-independent origins of endogenous DNA damage in tumor cells. Aging (Albany. NY). 1, 212–218. doi: 10.18632/aging.100019.

Nanda, I., Schrama, D., Feichtinger, W., Haaf, T., Schartl, M., and Schmid, M. (2002). Distribution of telomeric (TTAGGG)n sequences in avian chromosomes. Chromosoma 111, 215–227. doi: 10.1007/s00412-002-0206-4.

Nettle, D., Monaghan, P., Gillespie, R., Brilot, B., Bedford, T., and Bateson, M. (2015). An experimental demonstration that early-life competitive disadvantage accelerates telomere loss. Proc. R. Soc. B Biol. Sci. 282. doi: 10.1098/rspb.2014.1610.

Nettle, D., Seeker, L., Nussey, D., Froy, H., and Bateson, M. (2019). Consequences of measurement error in qPCR telomere data: A simulation study. PLoS One 14, e0216118. doi: 10.1371/journal.pone.0216118.

Nordfjäll, K., Svenson, U., Norrback, K. F., Adolfsson, R., Lenner, P., and Roos, G. (2009). The individual blood cell telomere attrition rate is telomere length dependent. PLoS Genet. 5, e1000375. doi: 10.1371/journal.pgen.1000375.

Noreen, E., Bourgeon, S., and Bech, C. (2011). Growing old with the immune system: A study of immunosenescence in the zebra finch (Taeniopygia guttata). J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. 181, 649–656. doi: 10.1007/s00360-011-0553-7.

Nowicki, S., and Searcy, W. A. (2004). Song function and the evolution of female preferences: Why birds sing, why brains matter. Ann. N. Y. Acad. Sci. 1016, 704–723. doi: 10.1196/annals.1298.012.

Nunes, M. C. P., Guimarães, M. H., Diamantino, A. C., Gelape, C. L., and Ferrari, T. C. A. (2017). Cardiac manifestations of parasitic diseases. Heart 103, 651–658. doi: 10.1136/heartjnl-2016-309870.

Nussey, D. H., Baird, D., Barrett, E., Boner, W., Fairlie, J., Gemmell, N., et al. (2014). Measuring telomere length and telomere dynamics in evolutionary biology and ecology. Methods Ecol. Evol. 5, 299–310. doi: 10.1111/2041-210X.12161.

Nussey, D. H., Froy, H., Lemaitre, J. F., Gaillard, J. M., and Austad, S. N. (2013). Senescence in natural populations of animals: Widespread evidence and its implications for bio-gerontology. Ageing Res. Rev. 12, 214–225. doi: 10.1016/j.arr.2012.07.004.

Nussey, D. H., Kruuk, L. E. B., Donald, A., Fowlie, M., and Clutton-Brock, T. H. (2006). The rate of senescence in maternal performance increases with early-life fecundity in red deer. Ecol. Lett. 9, 1342–1350. doi: 10.1111/j.1461-0248.2006.00989.x.

Olsson, M. (2016). Lewerhulme Telomere Conference. in, ed. P. Monaghan (Glasgow, Scottland).

Olsson, M., Pauliny, A., Wapstra, E., Uller, T., Schwartz, T., and Blomqvist, D. (2011). Sex differences in sand lizard telomere inheritance: Paternal epigenetic effects increases telomere heritability and offspring survival. PLoS One 6, e17473. doi: 10.1371/journal.pone.0017473.

Olsson, M., Wapstra, E., and Friesen, C. R. (2018). Evolutionary ecology of telomeres: a review. Ann. N. Y. Acad. Sci. 1422, 5–28. doi: 10.1111/nyas.13443.

Owen, J. P., Nelson, A. C., and Clayton, D. H. (2010). Ecological immunology of bird-ectoparasite systems. Trends Parasitol. 26, 530–539. doi: 10.1016/J.PT.2010.06.005.

Paperna, I., Yosef, R., and Landau, I. (2007). Plasmodium spp. in raptors on the Eurasian-African migration route. Parasite 14, 313–322. doi: 10.1051/parasite/2007144313.

Partridge, L., and Gems, D. (2007). Benchmarks for ageing studies. Nature 450, 165–167. doi: 10.1038/450165a.

Pauliny, A., Wagner, R. H., Augustin, J., Szép, T., and Blomqvist, D. (2006). Age-independent telomere length predicts fitness in two bird species. Mol. Ecol. 15, 1681–1687. doi: 10.1111/j.1365-294X.2006.02862.x.

Pedersen, A. B., and Babayan, S. A. (2011). Wild immunology. Mol. Ecol. 20, 872–880. doi: 10.1111/j.1365-294X.2010.04938.x.

Pepper, G. V., Bateson, M., and Nettle, D. (2018). Telomeres as integrative markers of exposure to stress and adversity: A systematic review and meta-analysis. R. Soc. Open Sci. 5. doi: 10.1098/rsos.180744.

Pfeiffer, V., and Lingner, J. (2013). Replication of Telomeres and the Regulation of Telomerase. Cold Spring Harb. Perspect. Biol. 5, 10405–10406. doi: 10.1101/CSHPERSPECT.A010405.

Pianka, E. R. (1970). On r- and K-Selection. Am. Nat. 104, 592–597.

Pianka, E. R. (1974). Niche overlap and diffuse competition. Proc. Natl. Acad. Sci. U. S. A. 71, 2141–2145. doi: 10.1073/pnas.71.5.2141.

Price, P. W., Westoby, M., Rice, B., Atsatt, P. R., Fritz, R. S., Thompson, J. N., et al. (1986). Parasite Mediation in Ecological Interactions. Annu. Rev. Ecol. Syst. 17, 487–505. doi: 10.1146/annurev.es.17.110186.002415.

Réale, D., Garant, D., Humphries, M. M., Bergeron, P., Careau, V., and Montiglio, P. O. (2010). Personality and the emergence of the pace-of-life syndrome concept at the population level. Philos. Trans. R. Soc. B Biol. Sci. 365, 4051–4063. doi: 10.1098/rstb.2010.0208.

Reed, T. E., Kruuk, L. E. B., Wanless, S., Frederiksen, M., Cunningham, E. J. A., and Harris, M. P. (2008). Reproductive senescence in a long-lived seabird: Rates of decline in late-life performance are associated with varying costs of early reproduction. Am. Nat. 171, 89–101. doi: 10.1086/524957.

Reichert, S., Rojas, E. R., Zahn, S., Robin, J. P., Criscuolo, F., and Massemin, S. (2015). Maternal telomere length inheritance in the king penguin. Heredity (Edinb). 114, 10–16. doi: 10.1038/hdy.2014.60.

Reid, J. M., Bignal, E. M., Bignal, S., McCracken, D. I., and Monaghan, P. (2003). Environmental variability, life-history covariation and cohort effects in the red-billed chough Pyrrhocorax pyrrhocorax. J. Anim. Ecol. 72, 36–46. doi: 10.1046/j.1365-2656.2003.00673.x.

Reznick, D. (1985). Costs of Reproduction: An Evaluation of the Empirical Evidence. Oikos 44, 257. doi: 10.2307/3544698.

Reznick, D. (2014). III. 11 Evolution of life-histories. The Princeton Guide to Evolution. , eds. J. B. Losos, D. A. Baum, D. J. Futuyma, H. E. Hoekstra, and R. E. Lenski Princeton & Oxford: Princeton University Press.

Reznick, D. N., Butler IV, M. J., Rodd, F. H., and Ross, P. (1996). Life-history evolution in guppies (Poecilia reticulata) 6. Differential mortality as a mechanism for natural selection. Evolution (N. Y). 50, 1651–1660. doi: 10.1111/j.1558-5646.1996.tb03937.x.

Ricklefs, R. E. (2010). Insights from comparative analyses of aging in birds and mammals. Aging Cell 9, 273–284. doi: 10.1111/j.1474-9726.2009.00542.x.

Ricklefs, R. E., Swanson, B. L., Fallon, S. M., Martínez-Abraín, A., Scheuerlein, A., Gray, J., et al. (2005). Community relationships of avian malaria parasites in southern Missouri. Ecol. Monogr. 75, 543–559. doi: 10.1890/04-1820.

Ricklefs, R., and Wikelski, M. (2002). The physiology/life-history nexus. Trends Ecol. Evol. 17, 462–468.

Roff, D. (1993). Evolution of life histories: theory and analysis. Springer Science & Business Media.

Roff, D. A., and Fairbairn, D. J. (2007). The evolution of trade-offs: Where are we? J. Evol. Biol. 20, 433–447. doi: 10.1111/j.1420-9101.2006.01255.x.

Rosner, B. (2015). Fundamentals of biostatistics. 8th ed. Harvard University.

Roved, J., Westerdahl, H., and Hasselquist, D. (2017). Sex differences in immune responses: Hormonal effects, antagonistic selection, and evolutionary consequences. Horm. Behav. 88, 95–105. doi: 10.1016/j.yhbeh.2016.11.017.

Ruiz-Herrera, A., Nergadze, S. G., Santagostino, M., and Giulotto, E. (2009). Telomeric repeats far from the ends: Mechanisms of origin and role in evolution. Cytogenet. Genome Res. 122, 219–228. doi: 10.1159/000167807.

Salmón, P., Millet, C., Selman, C., and Monaghan, P. (2021). Growth acceleration results in faster telomere shortening later in life. Proc. R. Soc. B Biol. Sci. 288. doi: 10.1098/rspb.2021.1118.

Salomons, H. M., Mulder, G. A., Van De Zande, L., Haussmann, M. F., Linskens, M. H. K., and Verhulst, S. (2009). Telomere shortening and survival in free-living corvids. Proc. R. Soc. B Biol. Sci. 276, 3157–3165. doi: 10.1098/rspb.2009.0517.

Samani, N. J., Boultby, R., Butler, R., Thompson, J. R., and Goodall, A. H. (2001). Telomere shortening in atherosclerosis. Lancet 358, 472–473. doi: 10.1016/S0140-6736(01)05633-1.

Sambrook, J., Fritsch, E., and Maniatis, T. (1989). Molecular cloning: a laboratory manual. CSHL Press.

Sampson, M. J., and Hughes, D. A. (2006). Chromosomal telomere attrition as a mechanism for the increased risk of epithelial cancers and senescent phenotypes in type 2 diabetes. Diabetologia 49, 1726–1731. doi: 10.1007/s00125-006-0322-4.

Santiago-Alarcon, D., Palinauskas, V., and Schaefer, H. M. (2012). Diptera vectors of avian Haemosporidian parasites: Untangling parasite life cycles and their taxonomy. Biol. Rev. 87, 928–964. doi: 10.1111/j.1469-185X.2012.00234.x.

Sarma, S. S. S., Nandini, S., and Gulati, R. D. (2006). “Life history strategies of cladocerans: comparisons of tropical and temperate taxa,” in Aquatic Biodiversity II (Springer-Verlag), 315–333. doi: 10.1007/1-4020-4111-x_29.

Savage, A. F., Robert, V., Goodman, S. M., Raharimanga, V., Raherilalao, M. J., Andrianarimisa, A., et al. (2009). Blood parasites in birds from madagascar. J. Wildl. Dis. 45, 907–920. doi: 10.7589/0090-3558-45.4.907.

Scheiner, S. M., and Stearns, S. C. (1992). The evolution of life histories. Oxford University Press.

Seeker, L. A., Underwood, S. L., Wilbourn, R. V., Dorrens, J., Froy, H., Holland, R., et al. (2021). Telomere attrition rates are associated with weather conditions and predict productive lifespan in dairy cattle. Sci. Rep. 11, 1–11. doi: 10.1038/s41598-021-84984-2.

Sejberg, D., Bensch, S., and Hasselquist, D. (2000). Nestling provisioning in polygynous great reed warblers (Acrocephalus arundinaceus): Do males bring larger prey to compensate for fewer nest visits? Behav. Ecol. Sociobiol. 47, 213–219. doi: 10.1007/s002650050658.

Seluanov, A., Chen, Z., Hine, C., Sasahara, T. H. C., Ribeiro, A. A. C. M., Catania, K. C., et al. (2007). Telomerase activity coevolves with body mass not lifespan. Aging Cell 6, 45–52. doi: 10.1111/j.1474-9726.2006.00262.x.

Sepp, T., McGraw, K. J., Kaasik, A., and Giraudeau, M. (2018). A review of urban impacts on avian life-history evolution: Does city living lead to slower pace of life? Glob. Chang. Biol. 24, 1452–1469. doi: 10.1111/gcb.13969.

Seress, G., Sándor, K., Evans, K. L., and Liker, A. (2020). Food availability limits avian reproduction in the city: An experimental study on great tits Parus major. J. Anim. Ecol. 89, 1570–1580. doi: 10.1111/1365-2656.13211.

Seward, A. M., Beale, C. M., Gilbert, L., Jones, T. H., and Thomas, R. J. (2014). The impact of increased food availability on reproduction in a long-distance migratory songbird: Implications for environmental change? PLoS One 9, 111180. doi: 10.1371/journal.pone.0111180.

Shalev, I., Entringer, S., Wadhwa, P. D., Wolkowitz, O. M., Puterman, E., Lin, J., et al. (2013). Stress and telomere biology: A lifespan perspective. Psychoneuroendocrinology 38, 1835–1842. doi: 10.1016/j.psyneuen.2013.03.010.

Shammas, M. A. (2011). Telomeres, lifestyle, cancer, and aging. Curr. Opin. Clin. Nutr. Metab. Care 14, 28–34. doi: 10.1097/MCO.0b013e32834121b1.

Shay, J. W. (2016). Role of telomeres and telomerase in aging and cancer. Cancer Discov. 6, 584–593. doi: 10.1158/2159-8290.CD-16-0062.

Shay, J. W. (2018). Telomeres and aging. Curr. Opin. Cell Biol. 52, 1–7. doi: 10.1016/J.CEB.2017.12.001.

Shay, J. W., and Wright, W. E. (2007). Hallmarks of telomeres in ageing research. J. Pathol. 211, 114–123. doi: 10.1002/path.2090.

Shine, R. (2003). Reproductive strategies in snakes. Proc. R. Soc. B Biol. Sci. 270, 995–1004. doi: 10.1098/rspb.2002.2307.

Sih, A., Bell, A. M., Johnson, J. C., and Ziemba, R. E. (2004). Behavioral syndromes: An integrative overview. Q. Rev. Biol. 79, 241–277. doi: 10.1086/422893.

Sjöberg, S., Malmiga, G., Nord, A., Andersson, A., Bäckman, J., Tarka, M., et al. (2021). Extreme altitudes during diurnal flights in a nocturnal songbird migrant. Science (80-. ). 372, 646–648. doi: 10.1126/science.abe7291.

Smith, C. C., and Fretwell, S. D. (1974). The Optimal Balance between Size and Number of Offspring. Am. Nat. 108, 499–506. doi: 10.1086/282929.

Smith, S., Turbill, C., and Penn, D. J. (2011). Chasing telomeres, not red herrings, in evolutionary ecology. Heredity (Edinb). 107, 372–373. doi: 10.1038/hdy.2011.14.

Sokal, R., and Rohlf, F. (1973). Introduction to biostatistics. San Francisco: Freeman, WH.

Sokolovskis, K., Lundberg, M., Åkesson, S., Willemoes, M., Zhao, T., Caballero-Lopez, V., et al. (2023). Migration direction in a songbird explained by two loci. Nat. Commun. 14, 1–6. doi: 10.1038/s41467-023-35788-7.

Spurgin, L. G., Bebbington, K., Fairfield, E. A., Hammers, M., Komdeur, J., Burke, T., et al. (2018). Spatio-temporal variation in lifelong telomere dynamics in a long-term ecological study. J. Anim. Ecol. 87, 187–198. doi: 10.1111/1365-2656.12741.

Stamm, D. D., Davis, D. E., and Robbins, C. S. (1960). A Method of Studying Wild Bird Populations by Mist-Netting and Banding. Bird-Banding 31, 115. doi: 10.2307/4510816.

Steenstrup, T., Hjelmborg, J. V. B., Kark, J. D., Christensen, K., and Aviv, A. (2013a). The telomere lengthening conundrum - Artifact or biology? Nucleic Acids Res. 41. doi: 10.1093/nar/gkt370.

Steenstrup, T., Hjelmborg, J. V. B., Mortensen, L. H., Kimura, M., Christensen, K., and Aviv, A. (2013b). Leukocyte telomere dynamics in the elderly. Eur. J. Epidemiol. 28, 181–187. doi: 10.1007/s10654-013-9780-4.

Stewart, S. A., and Weinberg, R. A. (2000). Telomerase and human tumorigenesis. Semin. Cancer Biol. 10, 399–406. doi: 10.1006/scbi.2000.0339.

Stigler, S. M. (1997). Regression towards the mean, historically considered. Stat. Methods Med. Res. 6, 103–114. doi: 10.1177/096228029700600202.

Sudyka, J. (2019). Does Reproduction Shorten Telomeres? Towards Integrating Individual Quality with Life-History Strategies in Telomere Biology. BioEssays 41. doi: 10.1002/bies.201900095.

Sudyka, J., Arct, A., Drobniak, S., Gustafsson, L., and Cichoń, M. (2016). Longitudinal studies confirm faster telomere erosion in short-lived bird species. J. Ornithol. 157, 373–375. doi: 10.1007/s10336-015-1304-4.

Svenson, U., Nordfjäll, K., Baird, D., Roger, L., Osterman, P., Hellenius, M. L., et al. (2011). Blood cell telomere length is a dynamic feature. PLoS One 6, e21485. doi: 10.1371/journal.pone.0021485.

Tarik, M., Ramakrishnan, L., Sachdev, H. S., Tandon, N., Roy, A., Bhargava, S. K., et al. (2018). Validation of quantitative polymerase chain reaction with Southern blot method for telomere length analysis. Futur. Sci. OA 4. doi: 10.4155/fsoa-2017-0115.

Tarka, M., Åkesson, M., Beraldi, D., Hernández-Sánchez, J., Hasselquist, D., Bensch, S., et al. (2010). A strong quantitative trait locus for wing length on chromosome 2 in a wild population of great reed warblers. Proc. R. Soc. B Biol. Sci. 277, 2361–2369. doi: 10.1098/rspb.2010.0033.

Tarka, M., Guenther, A., Niemelä, P. T., Nakagawa, S., and Noble, D. W. A. (2018). Sex differences in life history, behavior, and physiology along a slow-fast continuum: a meta-analysis. Behav. Ecol. Sociobiol. 72, 1–13. doi: 10.1007/s00265-018-2534-2.

Taylor, H. A., and Delany, M. E. (2000). Ontogeny of telomerase in chicken: Impact of downregulation on pre- and postnatal telomere length in vivo. Dev. Growth Differ. 42, 613–621. doi: 10.1046/j.1440-169X.2000.00540.x.

Tobler, M., Gómez-Blanco, D., Hegemann, A., Lapa, M., Neto, J. M., Tarka, M., et al. (2022). Telomeres in ecology and evolution: A review and classification of hypotheses. Mol. Ecol. doi: 10.1111/MEC.16308.

Tompkins, D. M., White, A. R., and Boots, M. (2003). Ecological replacement of native red squirrels by invasive greys driven by disease. Ecol. Lett. 6, 189–196. doi: 10.1046/j.1461-0248.2003.00417.x.

Tricola, G. M., Simons, M. J. P., Atema, E., Boughton, R. K., Brown, J. L., Dearborn, D. C., et al. (2018). The rate of telomere loss is related to maximum lifespan in birds. Philos. Trans. R. Soc. B Biol. Sci. 373. doi: 10.1098/rstb.2016.0445.

Valera-Gran, D., Prieto-Botella, D., Hurtado-Pomares, M., Baladia, E., Petermann-Rocha, F., Sánchez-Pérez, A., et al. (2022). The Impact of Foods, Nutrients, or Dietary Patterns on Telomere Length in Childhood and Adolescence: A Systematic Review. Nutrients 14, 3885. doi: 10.3390/NU14193885/S1.

Valkiunas, G. (2005). Avian Malaria Parasites and other Haemosporidia. 1st ed. CRC Press doi: 10.1201/9780203643792.

Valkiunas, G., Bensch, S., Iezhova, T. A., Križanauskiene, A., Hellgren, O., and Bolshakov, C. V. (2006). Nested cytochrome b polymerase chain reaction diagnostics underestimate mixed infections of avian blood haemmosporidian parasites: Microscopy is still essential. J. Parasitol. 92, 418–422. doi: 10.1645/GE-3547RN.1.

Valkiunas, G., Iezhova, T. A., Križanauskiene, A., Palinauskas, V., Sehgal, R. N. M., and Bensch, S. (2008). A comparative analysis of microscopy and PCR-based detection methods for blood parasites. J. Parasitol. 94, 1395–1401. doi: 10.1645/GE-1570.1.

van Lieshout, S. H. J., Bretman, A., Newman, C., Buesching, C. D., Macdonald, D. W., and Dugdale, H. L. (2019). Individual variation in early-life telomere length and survival in a wild mammal. Mol. Ecol. 28, 4152–4165. doi: 10.1111/MEC.15212.

Vega, L. R., Mateyak, M. K., and Zakian, V. A. (2003). Getting to the end: Telomerase access in yeast and humans. Nat. Rev. Mol. Cell Biol. 4, 948–959. doi: 10.1038/nrm1256.

Velando, A., Drummond, H., and Torres, R. (2006). Senescent birds redouble reproductive effort when ill: Confirmation of the terminal investment hypothesis. Proc. R. Soc. B Biol. Sci. 273, 1443–1448. doi: 10.1098/rspb.2006.3480.

Vergara, P., I. Aguirre, J., and Fernández-Cruz, M. (2007). Arrival date, age and breeding success in white stork Ciconia ciconia. J. Avian Biol. 38, 573–579. doi: 10.1111/j.2007.0908-8857.03983.x.

Verhulst, S., Aviv, A., Benetos, A., Berenson, G. S., and Kark, J. D. (2013). Do leukocyte telomere length dynamics depend on baseline telomere length? An analysis that corrects for "regression to the mean." Eur. J. Epidemiol. 28, 859–866. doi: 10.1007/s10654-013-9845-4.

Verhulst, S., Susser, E., Factor-Litvak, P. R., Simons, M. J. P., Benetos, A., Steenstrup, T., et al. (2015). Commentary: The reliability of telomere length measurements. Int. J. Epidemiol. 44, 1683–1686. doi: 10.1093/ije/dyv166.

Viney, M. E., Riley, E. M., and Buchanan, K. L. (2005). Optimal immune responses: Immunocompetence revisited. Trends Ecol. Evol. 20, 665–669. doi: 10.1016/j.tree.2005.10.003.

Von Zglinicki, T. (2002). Oxidative stress shortens telomeres. Trends Biochem. Sci. 27, 339–344. doi: 10.1016/S0968-0004(02)02110-2.

Waldenström, J., Bensch, S., Hasselquist, D., and Östman, Ö. (2004). A New Nested Polymerase Chain Reaction Method Very Efficient in Detecting Plasmodium and Haemoproteus infections from Avian Blood. J. Parasitol. 90, 191–194. doi: 10.1645/GE-3221RN.

Wallace, A. R. (1865). "The action of natural in producing old age, decay and death," in Weissman, A. ed. Essays upon heredity and kindred biological problems (Oxford: Clarendon Press).

Watson, J. D. (1972). Origin of concatemeric T7 DNA. Nat. New Biol. 239, 197–201. doi: 10.1038/newbio239197a0.

Watson, R. L., Bird, E. J., Underwood, S., Wilbourn, R. V., Fairlie, J., Watt, K., et al. (2017). Sex differences in leucocyte telomere length in a free-living mammal. Mol. Ecol. 26, 3230–3240. doi: 10.1111/mec.13992.

Weathers, W. W., and Sullivan, K. A. (1989). Juvenile foraging proficiency, parental effort, and avian reproductive success. Ecol. Monogr. 59, 223–246. doi: 10.2307/1942600.

Weismann, A. (1889). Essays upon heredity and kindred biological problems. Oxford: Clarendon Press.

Westerdahl, H., Bensch, S., Hansson, B., Hasselquist, D., and Von Schantz, T. (2000). Brood sex ratios, female harem status and resources for nestling provisioning in the great reed warbler (Acrocephalus arundinaceus). Behav. Ecol. Sociobiol. 47, 312–318. doi: 10.1007/s002650050671.

Westerdahl, H., Waldenström, J., Hansson, B., Hasselquist, D., von Schantz, T., and Bensch, S. (2005). Associations between malaria and MHC genes in a migratory songbird. Proc. R. Soc. B Biol. Sci. 272, 1511–1518. doi: 10.1098/rspb.2005.3113.

Whittemore, K., Vera, E., Martínez-Nevado, E., Sanpera, C., and Blasco, M. A. (2019). Telomere shortening rate predicts species life span. Proc. Natl. Acad. Sci. U. S. A. 116, 15122–15127. doi: 10.1073/pnas.1902452116.

Wiersma, P., Muñoz-Garcia, A., Walker, A., and Williams, J. B. (2007). Tropical birds have a slow pace of life. Proc. Natl. Acad. Sci. U. S. A. 104, 9340–9345. doi: 10.1073/pnas.0702212104.

Wikelski, M., Spinney, L., Schelsky, W., Scheuerlein, A., and Gwinner, E. (2003). Slow pace of life in tropical sedentary birds: A common-garden experiment on four stonechat populations from different latitudes. Proc. R. Soc. B Biol. Sci. 270, 2383–2388. doi: 10.1098/rspb.2003.2500.

Williams, G. C. (1957). Pleiotropy, Natural Selection, and the Evolution of Senescence. Evolution (N. Y). 11, 398. doi: 10.2307/2406060.

Williams, G. C. (1966). Natural Selection, the Costs of Reproduction, and a Refinement of Lack's Principle. Am. Nat. 100, 687–690.

Williams, N. M., Crone, E. E., Roulston, T. H., Minckley, R. L., Packer, L., and Potts, S. G. (2010). Ecological and life-history traits predict bee species responses to environmental disturbances. Biol. Conserv. 143, 2280–2291.

Wright, W. E., Piatyszek, M. A., Rainey, W. E., Byrd, W., and Shay, J. W. (1996). Telomerase activity in human germline and embryonic tissues and cells. Dev. Genet. 18, 173–179. doi: 10.1002/(SICI)1520-6408(1996)18:2<173::AID-DVG10>3.0.CO;2-3.

Xia, W., Grueter, C. C., Ren, B., Zhang, D., Yuan, X., and Li, D. (2021). Determinants of harem size in a polygynous primate: Reproductive success and social benefits. Animals 11, 2915. doi: 10.3390/ani11102915.

Young, A. J. (2018). The role of telomeres in the mechanisms and evolution of life-history trade-offs and ageing. Philos. Trans. R. Soc. B Biol. Sci. 373. doi: 10.1098/rstb.2016.0452.

Young, R. C., Kitaysky, A. S., Haussmann, M. F., Descamps, S., Orben, R. A., Elliott, K. H., et al. (2013). Age, Sex, and Telomere Dynamics in a Long-Lived Seabird with Male-Biased Parental Care. PLoS One 8, e74931. doi: 10.1371/journal.pone.0074931.

Yudkin, P. L., and Stratton, I. M. (1996). How to deal with regression to the mean in intervention studies. Lancet 347, 241–243. doi: 10.1016/S0140-6736(96)90410-9.

Zehtindjiev, P., Ilieva, M., Westerdahl, H., Hansson, B., Valkiunas, G., and Bensch, S. (2008). Dynamics of parasitemia of malaria parasites in a naturally and experimentally infected migratory songbird, the great reed warbler Acrocephalus arundinaceus. Exp. Parasitol. 119, 99–110. doi: 10.1016/j.exppara.2007.12.018.

Zhang, J., Rane, G., Dai, X., Shanmugam, M. K., Arfuso, F., Samy, R. P., et al. (2016). Ageing and the telomere connection: An intimate relationship with inflammation. Ageing Res. Rev. 25, 55–69. doi: 10.1016/j.arr.2015.11.006.

Zimmerman, M. S., Kinsel, C., Beamer, E., Connor, E. J., and Pflug, D. E. (2015). Abundance, Survival, and Life History Strategies of Juvenile Chinook Salmon in the Skagit River, Washington. Trans. Am. Fish. Soc. 144, 627–641. doi: 10.1080/00028487.2015.1017658.

Zuk, M., and Stoehr, A. M. (2002). Immune defense and host life history. Am. Nat. 160. doi: 10.1086/342131.

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Me gustaría tomarme un momento para honrar y expresar mi gratitud a mis abuelos, quienes lamentablemente fallecieron antes de que pudieran presenciar la culminación de mi tesis. Aunque ya no están con nosotros, sé que habrían estado orgullosos de mis logros y del esfuerzo que he hecho para poder llegar hasta aquí. Estoy seguro de que me miran desde donde quieran que estén, me animan y comparten mi alegría. Siempre tendré su amor y guía cerca de mi corazón, y espero haberlos enorgullecido con mi ejemplo de vida.

Finally, I believe that the person who deserves the biggest thanks for this thesis is… **me**! I mean, let's face it – I left Spain for the first time in my life, with a mediocre English level, none of Swedish, and unclear ideas of what I wanted to do in life. However, I persevered through the hard work in the lab, searching for samples in random boxes and cupboards, adjusting protocols that never seemed to work... and in the fieldwork, almost new to ringing and blood sampling, struggling at first to distinguish between the songs and looks of a great reed warbler compared to a never-ending list of warblers in Kvismaren, walking in waders in deep mud, finding my first nest (which was H9-81a in Nyängen) and many other after that,… I had to rediscover new ways of doing research: statistics, R, ggplot, the writing process, working at the weekends, the late nights, the stress-eating… all of that while dealing with the impostor syndrome, believing that I am not good enough. So definitely without my huge effort to complete this thesis, who knows where I would be?

probably still staring at a blank Word document titled “Connexions between telomeres and …” wondering if pursuing a PhD was a good idea. Therefore, I believe it is important I thank myself for my work, acknowledge my commitment, dedication and my determination to never give up and achieve this thesis successfully. I also want to boost my self-esteem and motivate me to reflect on my past and how I learned from both positive and negative experiences necessary for my growth. I believe, and I would improve, in practising self-care and self-love, focussing on my own needs first. This is essential for maintaining a healthy and positive mindset. I realized that “everything happens because it had to happen”. So, congratulations to myself! ;-)

- Male great reed warbler singing in the reed -
Gift from Ana Abellanas

## Poetic summary of my doctoral studies

David, now PhD scientist of great renown,
Studied telomeres and malaria, with research quite profound.
The great reed warbler, a subject of his study,
Revealed telomere length, a sign of health or worry.

Short telomeres, an indicator of ageing,
A gradual decline, a process engaging,
The passage of time takes its evil toll,
With each day passing, the DNA strands unroll.

Natural selection, a force to be accounted,
A process that leaves only the strong, undaunted.
And now, it seems, birds with short telomeres,
Are being eliminated, as nature interferes.

David found that those with shorter strands,
Were elongating to fight off life-history demands,
Those with parasites plaguing their body,
Couldn't live a life with more glory.

If you're a male with telomeres intact,
You'll have no trouble attracting a mate, that's a fact,
For your strength and vigour, they'll be on full display,
And a large harem to breed will come your way.

But if you are a female, the pattern's not so clear,
The effects of malaria can bring about fear,
Larger telomere shortening may cause love to fade,
And finding a mate may be a difficult crusade.

David sought to tell his PhD quest,
Uncovering new hypotheses, and putting them to test,
And this modest research summary, that you can peer,
Tells the wonders of great reed warbler, malaria, and telomeres.