Telomeres and life histories

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All's well that ends well?

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CHAPTER 1

TELOMERES AND LIFE HISTORIES: ALL'S WELL THAT ENDS WELL?

JELLE J. BOONEKAMP

INTRODUCTION

All life inevitably ends, either through internal or external causes. This simple law of nature seems inescapable to all organisms on our planet. Lifespan across the tree of life is highly variable, however, ranging from mere hours up to thousands of years in the case of Methuselah – a bristlecone pine tree that presumably is among the oldest organisms alive (~4845 years old; Brown 1996). Since Darwin it is known that natural selection favours individuals who are the most successful in passing on their genes to the next generation, and this imposes a paradox: Why did natural selection not result in unlimited lifespan and hence reproduction? Clearly, such a "Darwinian demon" would yield the highest fitness? Why is it then that almost all organisms show some degree of senescence – the decline in survival and / or reproduction with age? While it is apparently feasible to avoid internal ageing to a large extent, as is evident from species as the bristlecone pine.

The answer to these two questions is that all organisms experience some level of mortality that is not entirely intrinsically determined. For example, animals die because of predation and accidents and these are mostly extrinsic factors imposed by the environment in which they live. The greater the danger of such extrinsic factors, the shorter lifespan will be on average. This drives natural selection towards an early onset of reproduction, because individuals that start reproduction at an old(er) age are at risk of dying without having produced any progeny, and hence this strategy yields lower fitness. This mechanism has two evolutionary implications that apply almost universally: (i) natural selection prioritises early reproduction over late life effects, which allows that a mutation with negative effects specific to late life may persist or accumulate in the population. Such a mutation with negative effects specific to late life could in addition mitigate a positive effect early in life, i.e. antagonistic pleiotropy, which will be favoured by natural selection. And, (ii) since environmental resources are limited, reproductive investments are presumed to be costly and hence an early onset of reproduction that is favoured by natural selection imposes a greater cost relative to individuals that start reproducing at older age. Thus, senescence is an inevitable evolutionary outcome and the degree of senescence is expected to be optimised through adaptation to the local environment. In the rare case when extrinsic mortality is absent (or when extrinsic mortality declines with size by for example predation, and also with age in species with continuous growth) and when environmental resources are not limited, senescence may be avoided.

THE EVOLUTIONARY THEORY OF AGEING

Evolutionary theories of ageing can be divided into two types: (i) ageing emerges as an inevitable suboptimal phenomenon, and (ii) early- and late-life fitness components are balanced resulting in the evolution of ageing as part of the optimal strategy. Both depend on the fundamental process that, even without senescence, the strength of natural selection declines with age (Medawar 1952; Hamilton 1966; Fisher 1930). The driving force behind this phenomenon is the stochastic risk in nature to die from external causes, e.g. predation or accidents, which are not entirely genetically (intrinsically) determined. In an environment with high extrinsic mortality, only a few individuals grow old by chance, and as such, natural selection has a larger impact early in life. Given this so-called "selective shadow", (i) suboptimal mutations may accumulate if their negative effects become apparent only at old age (Medawar 1952). Although paradoxical at first glance, the evolution of senescence as suboptimal life history is inevitable. Not mutually exclusive, (ii) a mutation with late-life negative effects may be required for development, or impose positive effects early in life otherwise, and thereby become target of positive selection (Williams 1957). The benefits of a pleiotropic gene early in life outweighs the disadvantages later in life, simply because the strength of natural selection declines with age (Williams 1957). Hence, the evolution of ageing can be understood via two processes: (i) suboptimal passive mutation accumulation and (ii) natural selection for pleiotropic effects.

A number of selection experiments have tested the evolution theory of ageing. The general procedure of these experiments was to select each generation for either early or late life fitness advantages, and subsequently after a number of generations investigate the relationship between reproduction and lifespan (Wattiaux 1968; Sokal 1970; Mertz 1975; Rose 1984; Rose & Charlesworth 1980a; Rose & Charlesworth 1980b; Luckinbill *et al.* 1984). These selection experiments confirmed the principle of the evolutionary theories of ageing: selection for early reproduction resulted in shorter lifespans and selection for late reproduction resulted in longer lifespans. Also the selective pressure that exerts selection for early or late reproduction – extrinsic mortality – resulted when manipulated in the appearance across generations of reduced lifespan and high fecundity early in life (Stearns *et al.* 2000), in line with the hypothesis that (pleiotropic) genes cause high fecundity to be linked with accelerated ageing.

Although the evolutionary theories of ageing are conceptually attractive, the evidence that single genes are causing ageing is weak. Instead, comparative analyses suggest that reduced extrinsic mortality induced by captivity has little effect on lifespan in long-lived species (Ricklefs 1998; Ricklefs 2010a), while the

genetic theories of ageing predict that a reduction in extrinsic mortality leads to the evolution of prolonged lifespan. Furthermore, a comparative study on captive zoo populations showed that reproductive investment and lifespan were unrelated (Ricklefs & Cadena 2007). This body of evidence hints towards the hypothesis that ageing arises because of a physiological / ecological constraint, opposed to the theory that antagonistic pleiotropic genes or deleterious mutations cause ageing. Under this hypothesis the entire physiological and genetic machinery responsible for reproduction is expected to be optimised through natural selection to yield the highest lifetime reproductive output, as opposed to single genes causing ageing (Partridge & Barton 1993; Stearns 1992). A theory that rests on this principle is the disposable soma theory of ageing (Kirkwood 1977). In this theory, ageing is presumed to arise through the accumulation of somatic damage, making organisms more vulnerable to physiological and environmental challenges (Kirkwood & Rose 1991). Reproductive investments, assuming a limited resource budget, are made at the expense of somatic maintenance, causing ageing (Kirkwood 1977) (Fig.1.1). In essence, the disposable soma theory is a physiological embodiment of the genetic antagonistic pleiotropy, because individuals sacrifice late life survival and reproduction to gain reproductive success early in life. Therefore, the same evolutionary principles apply and natural selection is expected to optimise the balance between reproduction and somatic maintenance, according to the level of extrinsic mortality (Kirkwood & Rose 1991) (Fig. 1.1).

Reproductive effort, baseline mortality, and the rate of ageing

On a demographic level lifespan can be affected in different ways (i) via increased baseline mortality and (ii) via increased actuarial senescence – the rate with which mortality increases with age (Pletcher *et al.* 2000; Partridge *et al.* 2005) (Fig.1.2). In the example of figure 1.2, phenotypes A and B do not show any signs of ageing, because their mortality rates are independent of age. Yet, the phenotypes A and B have different lifespan due to their difference in baseline mortality (Fig.1.2). This example illustrates that variation in lifespan is not caused by variation in the rate of ageing *per se*, but rather that lifespan is determined by baseline mortality and actuarial senescence (C in figure 1.2). Establishing the relative contribution of actuarial senescence and baseline mortality to changes in lifespan is of interest because they are likely to represent different ecological and / or physiological mechanisms. Baseline mortality is often interpreted to reflect the intrinsic vulnerability to the process of damage accumulation with age, i.e. actuarial senescence (Pletcher *et al.* 2000; Partridge *et al.* 2005; Simons, Koch & Verhulst 2013). This idea is supported by experiments showing that, for example, dietary restriction reduces baseline mortality in fruit flies (Mair *et al.* 2003),

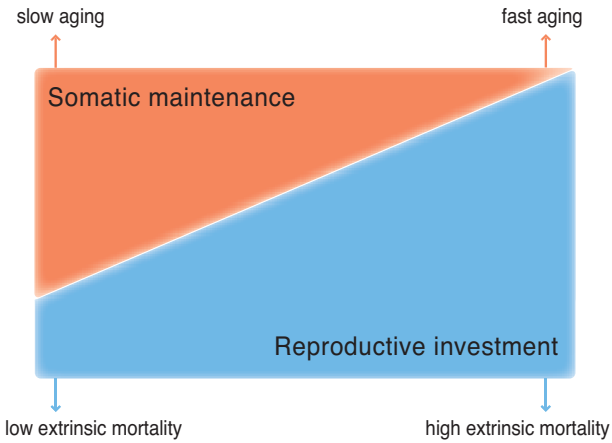

Figure 1.1 Antagonistic pleiotropy as predicted by the disposable soma theory of ageing (Kirkwood 1977). Reproductive investments are made at the expense of somatic maintenance or repair, accelerating ageing. High reproductive investment is expected under natural conditions aggravating extrinsic mortality.

because in this study the experimental effect on mortality was shown to be immediately reverted after switching treatment from high to low dietary restriction (and vice versa). This demonstrates that ageing did not cause these experimental effects, because if, e.g. manipulation of food intake reduced / increased the amount of damage accumulated up to the point of switching treatment, then this would have resulted either in a permanent effect, or a slower recovery (Partridge *et al.* 2005). Likewise, manipulations of reproductive effort have generally been shown to affect baseline mortality in captive invertebrates (Partridge & Andrews 1985; Hsin & Kenyon 1999; Flatt *et al.* 2008; Tatar *et al.* 1993), and in one of these studies it was shown that the effect on mortality rate was reversible, changing rapidly after reverting the manipulation of reproductive effort (Partridge & Andrews 1985). Therefore, these experiments challenge the hypothesis that reproductive investments are made at the expense of somatic maintenance and / or repair. However, since the evolution of ageing is only expected in the presence of extrinsic mortality (Medawar 1952; Williams 1957; Hamilton 1966), using inbred strains of invertebrates in captive conditions (where extrinsic mortality is virtually absent) may alter the predicted relationship between reproductive effort and actuarial senescence. This idea is supported by comparative analysis in vertebrates showing that reproductive output and lifespan are unrelated in captive zoo populations (Ricklefs & Cadena 2007). Comparative studies on free-living animals do find that reproductive output is positively correlated with actuarial senescence

(Ricklefs 2010b; Jones *et al.* 2008). Likewise, some longitudinal studies of free-living vertebrates report that high reproductive output early in life is associated with accelerated reproductive senescence within species (Reed *et al.* 2008; Bouwhuis *et al.* 2010; Robinson *et al.* 2012; McCleery *et al.* 1996; Reid *et al.* 2003). While these results support the evolution theory of ageing, they do not necessarily prove a causal relationship between reproduction and ageing. In the wild, experimental manipulation of reproductive effort has frequently been carried out, mainly in birds. A recent meta-analysis of these studies showed that there was no discernable effect of manipulation on adult survival when these studies were pooled (Santos & Nakagawa 2012), and also in free-living rodents experimental manipulation of reproductive effort yielded weak effects that were mixed between studies (Mappes *et al.* 1995; Koskela 1998; Koivula *et al.* 2003; Skibiel *et al.* 2013).

This overview of the literature suggests that there is little evidence that reproductive effort imposes a direct survival cost, challenging an important aspect of life history theory (Stearns 1992). However, in these studies survival was followed for a period of one year after treatment, and subject age was not taken into account. Only a few studies explored the effect of reproductive effort on subsequent survival over a longer period (2 or 3 years) (Reid 1987; Wheelwright *et al.* 1991; Erikstad *et al.* 2009), but subject age was not taken into account, and reproductive lifespans of these species were on average much longer. Hence, whether investment in reproduction accelerates actuarial senescence in free-living vertebrates is still an open question requiring long-term experimental data to resolve. To test the hypothesis that reproductive effort accelerates ageing I manipulated reproductive effort in free-living jackdaws by manipulation of brood size, and determined the effect of this manipulation on baseline mortality and actuarial senescence. I expect to find an effect on actuarial senescence, but not on baseline mortality, because the latter does not reflect the trade-off between reproduction and somatic maintenance / repair (Fig.1.1).

Jackdaw study population

The empirical work presented in this thesis is based on a population of jackdaws in the vicinity of Groningen, the Netherlands (53.1708° N, 6.6064° E), a semi-urban environment. Jackdaws (*Corvus monedula*) are sexually monogamous small semi-colonial corvids with bi-parental care and very low divorce rate (Lorenz 1931; Röell 1978). Natural breeding cavities are rare in the study area, and hence natural colonies did not exist at the start of the study. The first nest box colony was established in 1996. Since 2004, additional nest box colonies have been established in the vicinity and the data presented in this thesis is based on 7

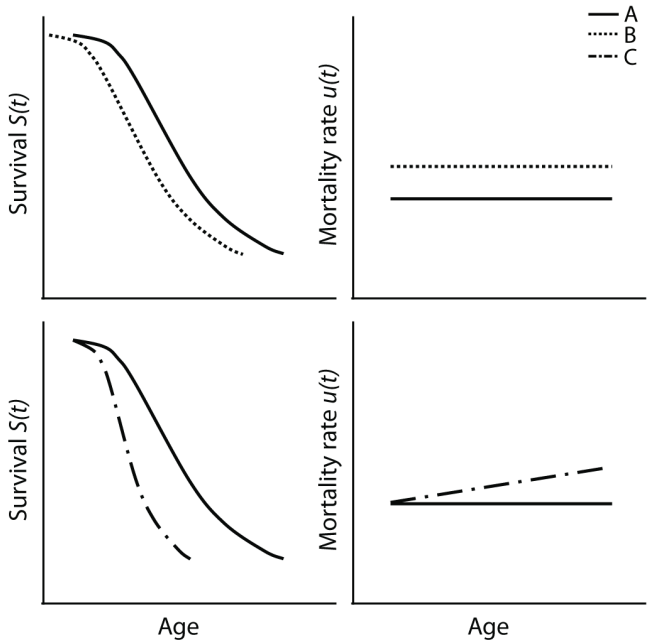

FIGURE 1.2 Three groups of individuals with different phenotypes (A, B, and C). Mortality is constant over time in A and B, implying that these phenotypes do not age, whereas mortality increases with age in C (actuarial senescence). Lifespan depends on baseline mortality (A versus B) and actuarial senescence (A versus C).

colonies. We caught birds in their nest box during the breeding season using remote controlled trapdoors. Upon first capture birds were marked with a unique combination of colour rings and a metal numbered ring, and in subsequent years individuals were identified either by re-trapping or by reading the colour rings. Since 1996, in total >1600 birds were caught and marked, and I used this population to study questions related to the evolution and the process of ageing.

DOES REPRODUCTIVE EFFORT ACCELERATES AGEING?

Brood size manipulation is known to affect parental effort, e.g. (Lessells 1993), and therefore directly manipulates the resources available for somatic maintenance (Kirkwood 1977) (Fig.1.1). Conceptually, I see this manipulation as an investigation of the mortality consequences of a hypothetical mutation that results in providing either increased or reduced parental care. Mutations are carried for life and in agreement with this perspective we subjected individuals to the same manipulation for life, and investigated the cumulative effect of the repeated

manipulations on survival. Thus, once an individual parent had been assigned to an experimental category (reduced or enlarged brood) it remained in that category for the duration of the study, receiving the same manipulation each year that it returned.

I studied survival using capture-mark-recapture analysis, which allows taking into account that a bird is not always observed, even when alive. For example, a bird may be overlooked or skip breeding, and these effects could lead to an underestimation of survival. The recapture rate estimate is based on the return rate of individuals that were not observed to return in the previous year(s). I performed these capture-mark-recapture analyses in a Bayesian framework, which allows to fit parametric density functions (Colchero *et al.* 2012). This is a relative new method (made publicly available in 2012) that enables to investigate various shapes of mortality in the data. I used the Gompertz equation (Gompertz 1825), because this model produced the best fit of the data. The Gompertz equation contains 2 parameters that respectively describe baseline mortality and actuarial senescence (Fig.1.2) (Partridge *et al.* 2005). To investigate whether reproductive effort increases actuarial senescence I quantified the effect of experimental manipulation of reproductive effort on both Gompertz parameters.

In chapter 2 my co-authors and I show that repeatedly raising enlarged broods shortened remaining life span by 34% on average, relative to birds in the reduced group. This effect was caused by a 3-fold difference in actuarial senescence between these two groups, while baseline mortality remained similar. This is the first experimental evidence demonstrating that reproductive effort accelerates actuarial senescence in vertebrates. These findings were robust in the sense that permanent emigration was unlikely to confound the effect of brood size manipulation on actuarial senescence, because the mean survival rate in our study was very close to the global survival rate of jackdaws estimated on the basis of ring recovery data (Dobson 1990). The disposable soma theory of ageing specifically predicts that longevity requires investments in somatic maintenance and repair that must compete against investments in reproduction among other fitness components (Kirkwood 2002). Our findings provide direct support for this theory, because I show that experimentally increased reproductive effort through manipulation of brood size reduced lifespan via an effect on actuarial senescence. I studied the effect of reproductive effort on survival, but ageing is also often shown in reproductive senescence. Preliminary analyses indicate that jackdaws show gradual senescence of clutch size after the age of 4, but more importantly, this pattern did not differ between manipulation categories. Altogether, these findings suggest that reproductive effort affects the rate of ageing through actuarial, but not reproductive senescence.

Behaviour is an integral part of the process of senescence

Key to the disposable soma theory of ageing is the assumption that resources are limited because this imposes a trade-off between reproductive investment and somatic maintenance or repair, causing senescence (Kirkwood & Rose 1991). Behaviour is generally not considered in studies on the process of senescence probably because most senescence research is carried out in model organisms in the laboratory, where individuals are often housed in single cages and where resources, e.g. food, are abundantly available. In the wild resources are scarce, leading to the evolution of competitive (and cognitive) behaviours that increase individual's resource holding potential (Parker 1974). The ability to secure resources inherently shapes the scope for allocation of resources between reproduction and somatic maintenance or repair, and therefore any behaviour that affects the ability to secure resources may be an important aspect of this process and thus the process of senescence. In my thesis I investigated social dominance, a behaviour hypothesised to be closely correlated to fitness and resource holding potential (Parker 1974) (chapter 5).

It has previously been shown that social dominance is positively correlated with age between differently aged subjects in birds, mammals, and insects (Arcese & Smith 1985; Henderson & Hart 1995; Weiss *et al.* 2011; Berdoy *et al.* 1995; Aujard & Perret 1998; Favre *et al.* 2008; McComb *et al.* 2001; McComb *et al.* 2011; Thouless & Guinness 1986; Bridge & Field 2007). On the basis of these observations it has been suggested that dominance increases with age within individuals due to experience or queuing, which would have implications for our understanding of the process of senescence. However, a positive correlation between individuals may also appear when subordinate individuals disappear / die when they are young, even when dominance is fixed within individuals (van de Pol & Verhulst 2006).

We studied social dominance over a period of 12 years in a jackdaw colony, allowing us to longitudinally record individual social careers. We found that social dominance increased with age within subjects, but steeply declined in the year preceding death (chapter 5). Social dominance, the ability to secure resources, and physiological state may positively affect each other, generating a positive reinforcement loop. Indeed, we found that dominance increased with age suggesting that this behaviour may delay the onset of senescence through positive effects on resource holding potential, mitigating somatic senescence. However, when physiological state starts to deteriorate due to senescence, the direction of events will be turned. High social rank is no longer adequately supported by the reduced physiological state, heralding a collapse. We therefore expect that under natural conditions behaviours affecting the ability to secure resources may delay

or accelerate the senescence process. I did not yet study the relationship between social dominance and (physiological) senescence, but I think that incorporating behaviour into senescence studies will prove novel insight in the senescence process.

How to detect ageing in the wild?

Ageing may take considerable time particular in vertebrates, which is a handicap when unravelling the causes of ageing. This makes biomarkers of ageing instrumental in ageing studies (Baker & Sprott 1988): when one or more biomarkers have been identified, new hypotheses can be developed using the biomarker as endpoint, before verifying the theory with more definitive but time-consuming endpoints such as (healthy) lifespan. Identifying biomarkers of ageing therefore constitutes a major step towards our understanding of ageing and specific mechanisms inherent of the ageing process underlying the biomarkers themselves.

Is telomere length a biomarker of ageing?

Telomeres are terminal DNA-protein complexes of the repeated base pair sequence TTAGGGn, which act as 'protective caps' of linear chromosomes (Blackburn 1991). Telomere length has recently emerged as a candidate biomarker of ageing – a research field that has been boosted by the finding that telomere length predicts remaining lifespan in humans (Cawthon *et al.* 2003). In the absence of telomerase, telomeres shorten due to incomplete end-replication with each cell division (Olovnikov 1973), erosion of the single strand overhang (Stewart *et al.* 2003), and oxidative damage (Zglinicki 2002), and when telomeres reach a critical length this induces cell cycle arrest or apoptosis (Allsopp & Harley 1995; Bodnar *et al.* 1998; Karlseder *et al.* 1999). Thus a potential explanation of why telomeres may be useful as biomarker of health and ageing is that their length reveals the cumulative oxidative stress an organism has experienced. Indeed, many stressors and ageing related diseases are associated with short telomeres, e.g. (Epel *et al.* 2004; Cherkas *et al.* 2006; Valdes *et al.* 2005). Moreover, the rate at which telomeres shorten is linked to life style (Epel *et al.* 2004; Monaghan & Haussmann 2005; Bakaysa *et al.* 2007), suggesting that environmental conditions and associated physiological stress may be reflected in telomere dynamics. Much remains unresolved however – largely because the epidemiological studies on humans are necessarily non-experimental, and because telomere shortening is slow, impeding longitudinal studies in long-lived species. For this reason, the study of telomere length as a biomarker of ageing in humans has been limited to

studying elderly subjects (>60 years) (Woo *et al.* 2008; Strandberg *et al.* 2011; Cawthon *et al.* 2003; Martin-Ruiz *et al.* 2005; Bischoff *et al.* 2006; Harris *et al.* 2006; Honig *et al.* 2006; Bakaysa *et al.* 2007; Kimura *et al.* 2008; Epel *et al.* 2009; Njajou *et al.* 2009; Willeit *et al.* 2010; Fitzpatrick *et al.* 2011; Houben *et al.* 2011; Martin-Ruiz *et al.* 2011; Zekry *et al.* 2011) and only 4 out of these 16 studies found a significant association between telomere length and mortality risk. Using meta-regression analysis I showed that (i) telomere length is a significant predictor of mortality when studies were pooled, and (ii) that this association diminished with subject age (chapter 4). The latter finding suggests that telomere length is a strong predictor of lifespan early in life, but unfortunately no data on humans is available to test this exciting prediction.

In the wild, most birds have a relatively short lifespan of several years, facilitating studying the association between telomere length and mortality over a large range of their natural lifespan. Bird studies show that there is a very strong association between telomere length and subsequent survival (Angelier *et al.* 2013; Barrett *et al.* 2013; Bauch *et al.* 2014; Bize *et al.* 2009; Foote, Daunt, *et al.* 2011a; Haussmann *et al.* 2005; Pauliny *et al.* 2006; Salomons *et al.* 2009). Furthermore, in captive zebra finches telomere length in the nestling phase is a predictor of lifespan (Heidinger *et al.* 2012), but see (Caprioli *et al.* 2013). Altogether, these studies provide substantial evidence that telomere length is useful as a biomarker of ageing. However, simultaneously, large variation in telomere length between same aged subjects remains unexplained (Monaghan & Haussmann 2005) raising the question what causes this variation. Investigation of the physiological mechanisms that modulate telomere attrition in vertebrates is usually hampered by the long time it takes for noticeable telomere attrition to occur. Nestling birds provide a unique opportunity in this respect, because they lose a substantial number of base pairs in the (short) nestling period (Salomons *et al.* 2009; Foote, Gault, *et al.* 2011b) and nestlings are readily accessible for repeated sampling.

We measured telomere dynamics in jackdaw nestlings that were reared in the context of the brood size manipulation experiment that was designed to study the effects of manipulated reproductive effort on the rate of ageing in the parents. Although parents adjust their reproductive effort in response to manipulated brood sizes, they usually do not fully compensate the increased demands of enlarged broods (for a meta-analysis see (Werf 1992)). This results in a manipulation of both the parental effort and the developmental conditions of nestlings, which provided me with the unique possibility to test the effects of developmental conditions on telomere dynamics and their relationship to long-term survival. This is of particular interest because developmental conditions are known to have

long-term effects on fitness, e.g. (Lindström 1999; Lummaa & Clutton-Brock 2002), but the underlying mechanisms in free-living vertebrates remain poorly understood. Observational studies reported positive correlations between adult body size and telomere length (Caprioli *et al.* 2013; Hall *et al.* 2004), and therefore telomere dynamics may provide a link between developmental conditions and adult performance.

In chapter 3 my co-authors and I show that adverse rearing conditions accelerated telomere attrition. Low telomere shortening rate in the nestling phase was associated with high adult survival rate, and this effect was independent of brood size manipulation and fledgling mass. Therefore, telomere dynamics link developmental conditions to fitness prospects, but whether telomeres are causally involved in this relationship remains subject to further study.

Is telomere length causally involved in the process of ageing?

The brood size manipulation experiment in chapter 3 did not affect telomere length at the age of fledging and likewise we did not find a relationship between absolute nestling telomere length and adult survival, but we do find a relationship with telomere shortening rate. This is in contrast with previous findings that absolute telomere length is positively associated with survival in adult birds (Angelier *et al.* 2013; Barrett *et al.* 2013; Bauch *et al.* 2014; Bize *et al.* 2009; Foote, Daunt, *et al.* 2011a; Haussmann *et al.* 2005; Salomons *et al.* 2009), raising the question why nestlings would differ from adults in their relationship between telomere length and survival. One explanation for this may be that telomere length is not causal to senescence, but rather serves as readout of the accumulation of experienced stress or damage. If the accumulated damage increases with age, this could result in larger detectable differences in telomere length through damage with increasing age. This mechanism would explain that the accuracy of telomere length as a biomarker of ageing increases with age, as opposed to the pattern observed in humans (chapter 4). The causal agent of mortality however is the accumulated damage, and this is merely reflected in telomere length. Important supporting evidence for the hypothesis that telomere length is not causal in the process of senescence is that in adult jackdaws the shortest observed telomere length (> 4100 base pairs; (Salomons *et al.* 2009)) is far above the critical limit of 78 base pairs that causes cell senescence in vitro (Capper *et al.* 2007). Although this critical limit was determined in human cells, it is probably not much different in birds. This suggests that telomere length is not a limiting factor for lifespan in vivo, but rather that telomere length is correlated to the process of ageing.

In general, the accuracy of telomere length as biomarker of ageing probably depends on the proportion of variance in initial telomere length relative to the

amount that is lost over time, which reflects the amount of oxidative stress and other ageing related processes (Epel *et al.* 2004; Cherkas *et al.* 2006; Valdes *et al.* 2005; Zglinicki 2002). For example, if there would be no variance in initial telomere length, then telomere length at later ages conveys precisely the amount of telomere shortening. Hence, in this example telomere length and shortening rate would be surrogate biomarkers of ageing. The observed variation in initial telomere length is often very large, which was also the case in my study (chapter 3), and heritable (Broer *et al.* 2013). Variation in initial telomere length thereby reduces the accuracy of telomere length as biomarker of ageing, because this variation is not caused by or related to the process of ageing.

SOMATIC REDUNDANCY AS MECHANISM OF AGEING

Descriptive models of ageing, e.g. the Gompertz and Weibull equations (Gompertz 1825; Weibull 1951), are often used to determine the onset and the shape of senescence (Ricklefs & Scheuerlein 2002; Gavrilov & Gavrilova 1991). These models consist of an age independent mortality component, reflecting baseline mortality, and an age dependent mortality component, reflecting ageing (Partridge *et al.* 2005), but do not explain how ageing arises itself. The redundancy model is different in this respect, because it is a mechanistic model of ageing, grounded in reliability theory (Gavrilov & Gavrilova 2001). In this model it is assumed that individuals consist of multiple elements, which are identical in function, causing redundancy. Redundancy generates damage tolerance because the entire system does not fail until the last remaining redundancy elements fail (Gavrilov & Gavrilova 2001). Essential in this model is that the redundancy elements do not age themselves, i.e. they have a constant failure rate over time. The risk of failure of the entire system nonetheless increases over time, due to the fact that the number of intact redundancy elements decreases (Gavrilov & Gavrilova 2001).

The redundancy model explains two characteristics of ageing that are observed in humans and other species, which are not captured with the Gompertz and Weibull equations. These characteristics are: (i) the exponential increase in mortality rate with age does not continue at high age, but levels off to a lower than exponential increase (Gavrilov & Gavrilova 1991), and (ii) different mortality rates converge to the same mortality rate at high age (Gavrilov & Gavrilova 1991). Both of these features are implicitly part of the redundancy model, because when element failure rate is constant over time the number of elements determines how many elements fail per unit of time. Consequently, the

rate at which elements are lost decelerates, matching mortality deceleration. Regardless of the initial number of redundancy elements, ultimately redundancy systems converge to the same state where only a single redundancy element remains. System failure rate therefore converges to the failure rate of the last remaining element, which is in agreement with the observed convergence of mortality rate in humans and other species (Gavrilov & Gavrilova 1991).

In chapter 4 I considered telomere length as a measure of somatic redundancy, i.e. the number of redundancy elements. This is compatible with the observation that long telomeres shorten faster than short telomeres (Buijs *et al.* 2004; Salomons *et al.* 2009; Grasman *et al.* 2011; Verhulst *et al.* 2013), because when there are more redundancy elements also more are lost per unit of time. My co-authors and I found that the relationship between telomere length and mortality diminished with age in a way that closely resembled the observed pattern from the meta-regression analysis (chapter 4). The reduction in redundancy variance between individuals renders telomere length less important, but element failure rate increasingly important with increasing age in predicting mortality. This model feature is supported by recent studies showing that at old age the variation in telomere length is reduced (Halaschek-Wiener *et al.* 2008), and that at old age telomere shortening rate predicts mortality more accurately than telomere length (Epel *et al.* 2009). Our finding that telomere length behaves as a biomarker of somatic redundancy is an indication that organisms may age through exhaustion of the system's redundancy. However, the same pattern may emerge because telomeres share features of redundancy systems without redundancy being causal to senescence. In this respect it is of interest that the association with mortality is also observed to diminish in other well-studied ageing biomarkers, such as body-mass-index, cholesterol, and blood pressure, while in my view these are not directly related to somatic redundancy. This suggests that somatic redundancy is causal to ageing, because this would explain that diminishing redundancy is apparent in other, if not all, biomarkers of ageing. To my best knowledge no example exists of a biomarker where this diminishing relationship with mortality is not apparent, and therefore I think that this pattern is likely to be a general feature of biomarkers of ageing. Little attention has been given in the literature to this phenomenon, while in my view further research on this subject may reveal important information to the extent in which organisms behave as redundancy systems.

The redundancy model predicts that the association between telomere length and remaining lifespan is strongest early in life, and this prediction is supported by one study in captive zebra finches, showing that telomere length in nestlings was better associated with remaining lifespan than telomere length at older ages

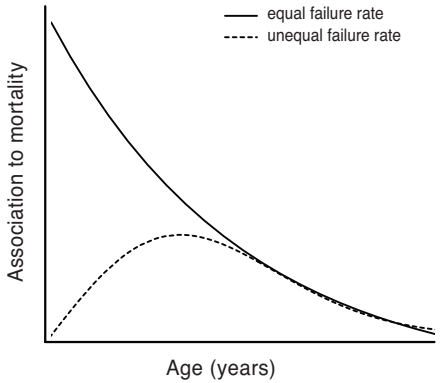

FIGURE 1.3 The association between telomere length and mortality is variable with age, depending on the degree in which redundancy element failure rate is fixed or variable between subjects. Lines are drawn on the basis of simulated data using the redundancy model with fixed or heterogeneous element failure rate in combination with heterogeneous number of redundancy elements (see chapter 4 for simulation procedures).

(Heidinger *et al.* 2012). In chapter 3 my co-authors and I show that telomere shortening in nestlings, but not telomere length, predicts post-fledging survival, which raises the question how this finding fits in the context of the redundancy model of ageing. In chapter 4 I assumed that redundancy elements of different individuals are subject to equal failure rates. Under this assumption, redundancy at birth best predicts age at death, because at birth the variation in redundancy between individuals is largest. However, element failure rate is likely to vary between individuals due to variation in life style and genetic background as has been shown for telomere shortening (Chen *et al.* 2011). In this case variation in redundancy between individuals initially increases with age, up to a maximum, after which it decreases (Fig.1.3). Consequently, when element failure rate is heterogeneous, I predict that the association between telomere length and mortality first increases with age before decreasing with age as observed in humans (chapter 4). Thus, a potential explanation for the observation that nestling telomere length did not predict survival in my study could be that redundancy element failure rate is heterogeneous in jackdaw nestlings. Indeed, the difference in nestling telomere length between post-fledging survivors and non-survivors is larger when they are 30 days old, compared with when they are 5 days old (chapter 3), suggesting that the accuracy of telomere length as biomarker of ageing increases with jackdaw age. Lifelong data is required to investigate to what extent biomarkers of the number of redundancy elements and

the failure rate of these elements are informative biomarkers of morality at different age classes.

Manipulation of reproductive effort in jackdaws did not affect parental survival to the next year (chapter 2) in agreement with previous findings in birds (Santos & Nakagawa 2012), suggesting that birds are restrained from maximal reproductive effort. The redundancy model may explain such a buffer in the effect of reproductive effort on mortality because when reproductive effort increases the failure rate of redundancy elements, this increases mortality, but only in the long run when redundancy levels are approaching critical levels. Redundancy capacity diminishes over time due to the accumulation of ageing related damage, and if reproductive effort is increased at the life stage when redundancy is almost depleted, this could lead to a fatal collapse. This idea is supported by one correlational study showing that reproductive effort late in life is stronger (negatively) associated with subsequent survival relative to reproductive effort earlier in life (Descamps *et al.* 2009).

Terminal declines have been reported in various traits (Coulson & Fairweather 2001; Rattiste 2004; Reed *et al.* 2008; Nussey *et al.* 2011), including our finding that dominance decreased in the year before disappearance / death (chapter 5). These findings may be caused by a physiological collapse when death comes near.

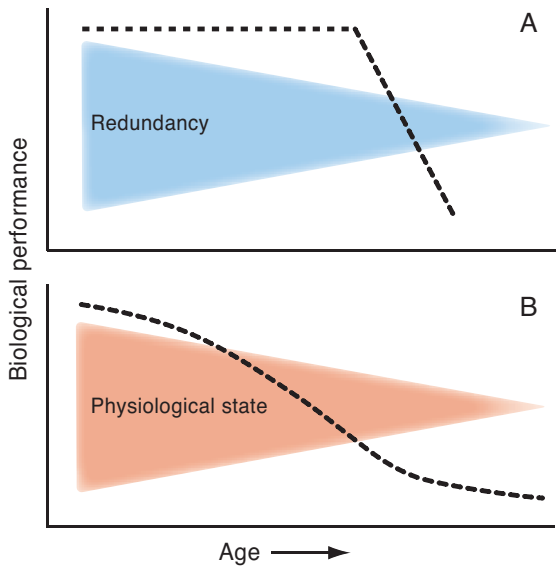

Figure 1.4 A terminal decline (A) and gradual senescence (B) and their conceptual relationships to somatic redundancy and physiological state respectively.

Alternatively, physiological senescence is a gradual process that may cause a terminal decline in a certain trait when the physiological state can no longer support high expression level of that trait. The redundancy model may help to explain a terminal decline, because this model predicts that a system's performance is maintained until redundancy reaches critically low levels (Fig.1.4A). Gradual senescence is also observed, e.g. (Dugdale *et al.* 2011; Bouwhuis *et al.* 2009; McCleery *et al.* 2008), suggesting that some traits may not depend on somatic redundancy, or that redundancy elements do not completely cover each other's function. Biological performance of these traits may therefore be proportional to physiological state (Fig.1.4B). Another explanation why some traits gradually decline with age may be that reduction in the expression level of a trait may yield benefits in the long run (Stearns 1992). Particularly in long-lived iteroparous breeders the cost of reproduction is more often expressed in terms of skipped reproduction in later years, or of gradual reproductive senescence, than in survival reduction (Nussey *et al.* 2013). To skip reproduction may be optimal when the environmental circumstances are poor, because this strategy may maintain the physiological state until the environmental conditions improve. Lastly, the redundancy model assumes that element failure rate remains constant over time, and as I explained earlier this results in a mortality demography that matches the observed mortality patters of several species (Gavrilov & Gavrilova 1991). Failure rate may not be constant however because in jackdaws telomere shortening rate is elevated in the year preceding death (Salomons *et al.* 2009). If redundancy element failure rate increases shortly prior to death then this would further explain a terminal decline. Excitingly, the available data on senescence patterns of many traits is rapidly growing (Nussey *et al.* 2013), which enables us to investigate to what extent somatic redundancy underlies these patterns.

CHAPTER 2

REPRODUCTIVE EFFORT ACCELERATES ACTUARIAL SENESCENCE IN WILD BIRDS: AN EXPERIMENTAL STUDY

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Abstract

Optimality theories of aging predict that the balance between reproductive effort and somatic maintenance determines the rate of aging. Laboratory studies find that increased reproductive effort shortens lifespan, but through increased short-term mortality rather than aging. In contrast, high fecundity in early-life is associated with accelerated senescence in free-living vertebrates, but these studies are non-experimental. We performed lifelong brood size manipulation in free-living jackdaws. Actuarial senescence – the increase of mortality rate with age – was 3-fold higher in birds rearing enlarged- compared to reduced broods, confirming a key prediction of the optimality theory of aging. Our findings contrast with the results of single year brood size manipulation studies carried out in many species, in which there was no overall discernible manipulation effect on mortality. We suggest that our and previous findings are in agreement with predictions based on the reliability theory of aging and propose further tests of this proposition.

Introduction

Actuarial senescence – the increase of mortality rate with age – is thought to arise through the accumulation of somatic damage, making organisms more vulnerable to physiological and environmental challenges (Kirkwood & Rose 1991). The rate of aging, or the rate of actuarial senescence, is generally assumed to depend on resource allocation towards reproduction, at the expense of somatic repair (Kirkwood 1977; Kirkwood & Rose 1991; Partridge & Barton 1993; McNamara *et al.* 2009). However, empirical tests of the effect of reproductive effort on the rate of senescence have yielded mixed results.

Variation in mortality trajectories and lifespan can arise through changes in the rate of aging, but also through age-independent changes in baseline mortality (Pletcher *et al.* 2000; Partridge *et al.* 2005). For instance, human mortality risk may be reduced at old age by the use of a walker, but removal of the walker reverses the effect, which shows that aging is not affected by this intervention. Both kind of effects have been found in response to interventions that affect lifespan (Pletcher *et al.* 2000; Mair *et al.* 2003; Simons *et al.* 2013). Establishing the relative contribution of actuarial senescence and baseline mortality to changes in lifespan is of interest because they are likely to represent different ecological and / or physiological mechanisms. Moreover, life history evolution of iteroparous species will depend on whether fitness costs of reproduction are due to increased baseline mortality, and hence no longer play a role when a reproductive bout is completed, or due to actuarial senescence, in which case they are carried through to later bouts of reproduction.

Studies in which reproductive effort was manipulated in captive invertebrates generally found that increased reproductive effort shortened lifespan, but this effect was due to increased baseline mortality rate, without affecting actuarial senescence (Partridge & Andrews 1985; Tatar *et al.* 1993; Hsin & Kenyon 1999; Flatt *et al.* 2008). This may be due to the fact that the invertebrate studies were carried out in captivity, because actuarial senescence is the outcome of an increase in susceptibility to environmental and physiological challenges with age (Medawar 1952) and it seems plausible that removal of most environmental challenges will affect the pattern that is observed. Comparative studies do find that fecundity correlates positively with actuarial senescence between species (Jones *et al.* 2008; Ricklefs 2010). Likewise, some long-term studies of free-living vertebrates report that high reproductive output early in life is associated with accelerated reproductive senescence within species (McCleery *et al.* 1996; Reid *et al.* 2003; Reed *et al.* 2008; Bouwhuis *et al.* 2010; Robinson *et al.* 2012). However, these studies used natural variation in reproductive output and it is difficult to

ascertain that the accelerated senescence can be attributed to increased reproductive effort, as opposed to some unidentified confounding variable. Such confounds may be unexpected and have large effects. For example, reproductive senescence in great tits distinctly differed between locally born and immigrant birds (Bouwhuis *et al.* 2010). Manipulating reproductive effort largely resolves this issue (Gustafsson & Sutherland 1988) and in the wild such experiments have frequently been carried out, mainly in birds. Although this work has yielded some convincing examples of survival costs of reproduction, (Reid 1987; Daan *et al.* 1996; Verhulst 1998), a recent meta-analysis showed no overall effect across bird studies (Santos & Nakagawa 2012). Similar experiments in rodents have also yielded mixed results (Mappes *et al.* 1995; Koskela 1998; Koivula *et al.* 2003; Skibiel *et al.* 2013). More importantly, with respect to actuarial senescence, experimental effects on survival were studied for only one year after the manipulation (but see Reid 1987; Wheelwright *et al.* 1991; Erikstad *et al.* 2009), and without considering age, precluding estimates of the manipulation on actuarial senescence. Hence, whether investment in reproduction accelerates actuarial senescence in free-living vertebrates is still an open question.

Here, we manipulated brood size in a population of free-living jackdaws *Corvus monedula* to test the hypothesis that investment in reproduction increases actuarial senescence. This manipulation successfully modifies the reproductive effort by the parents (Lessells 1993; Santos & Nakagawa 2012), and hence their remaining resources for somatic maintenance and repair. Conceptually, we see our manipulation as an investigation of the mortality consequences of a hypothetical mutation that results in females producing either more or fewer offspring than they would otherwise do. Mutations are carried for life and in agreement with this perspective we subjected individuals to the same manipulation for life, and investigated the cumulative effect of the repeated manipulations. We show that experimentally increasing reproductive effort increased actuarial senescence in a wild bird population without affecting baseline mortality.

Material and methods

Study system and manipulation of reproductive effort

Jackdaws are sexually monogamous small semi-colonial corvids with bi-parental care and very low divorce rate that produce on average 4.5 eggs per year in a single clutch (Röell 1978). We studied jackdaws in 7 nest box colonies in the vicinity of Groningen, the Netherlands (53.1708° N, 6.6064° E) in the period 2004–2012. Laying date of the first egg, clutch size and hatch date were estab-

lished by regular nest checks (see Salomons *et al.* 2008 for details). We caught birds in their nest box during the breeding season using remote controlled trapdoors. Upon first capture birds were marked with a unique combination of color rings and a metal numbered ring, and in subsequent years individuals were identified either by re-trapping or by reading the color rings.

We manipulated brood size by net +2 or –2 nestlings (we had no control group to increase statistical power). Whenever possible, 3 nestlings were moved to the brood that was designated to be enlarged, and one nestling from this enlarged brood was relocated to the matched reduced brood. Manipulated broods were equally distributed over the colonies (Table S2.2) and matched by hatch date. Nestlings were relocated when the oldest nestling was 4 days old. Relocated nestlings were randomly chosen using first a laptop and later on a smart phone app that simulated dice with a numeric range that was set to be equal to the number of nestlings. When a brood that was designated to be reduced contained 2 nestlings, we reduced brood size by one nestling, and broods containing 1 nestling (0.3% of broods; excluded from analysis) were not further reduced to avoid nest desertion. Likewise, some broods were enlarged with one nestling in case the matched reduced brood contained only 2 nestlings. Broods that could not be enlarged were excluded from analysis. Once an individual parent had been assigned to an experimental category (reduced or enlarged brood) it remained in that category for the duration of the study, and received the same manipulation each year that it returned. In newly formed pairs it occasionally happened that the two pair-members had received different manipulations in a previous year (<5%), and in these cases we assigned the pair to the manipulation category of the female. Survival of the corresponding males was censored at the moment of switching treatments, i.e. all survival data after switch of experimental treatment were omitted from the analysis and the fate of these individuals was designated to be "unknown".

EXPERIMENTAL DATA

In total 186 individual parents were manipulated since 2005. Of these individuals, year of birth was known for 30 individuals (18 ringed as nestling, and 12 as yearling, which can be distinguished by their brown plumage coloration). The exact year of death was known for only 2 individuals. Of the 186 individuals that were manipulated at least once, 101 individuals were manipulated two times or more, and 36 three times or more (maximum = 5).

Nestlings were weighed at manipulation and subsequently when 10, 20 and 30 days old (they fledge when ±35 days old). Cumulative brood mass gain after manipulation was calculated by summing the last mass measurement of nestlings

that died before fledging with the mass of surviving nestlings at fledging, subtracting the brood mass immediately after the brood size manipulation. We take cumulative brood mass gain to be a proxy for the amount of food provisioned by the parents. Cumulative brood mass gain (gram) was substantially higher in enlarged broods (513.69 ± S.E. 28.05) when compared to reduced broods (398.34 ± 32.03; $P < 0.001$), and we take this effect as evidence that our manipulation successfully altered reproductive effort. This agrees with what is generally found in response to brood size manipulation when parental care is observed directly (Lessells 1993; Santos & Nakagawa 2012).

Bayesian survival trajectory analysis

Our capture mark recapture (CMR) data are based on the repeated sampling of individuals that we first marked and released, and at each subsequent year were either observed or missed or recovered dead. With respect to the estimation of age specific mortality rate two challenges need to be solved; (i) because individuals may be alive while not being observed, the proportion of returning individuals underestimates the survival probability. Furthermore (ii), our data are left truncated (a number of individuals were born before the start of the study), and both left and right censored (years of birth and, or, death are unknown for a number of individuals). To solve (i) we performed CMR analysis using the "Bayesian Survival Trajectory Analysis" (BaSTA) package in R (Colchero *et al.* 2012), which yields survival estimates that are corrected for the probability of recapture. In our data, recapture probability was 85% (CI 79–90%). More importantly, recapture rate was similar across manipulation groups (reduced 86% (CI 80–92%), enlarged 82% (CI 71-90%)), and this finding was also supported by multistate capture-recapture analysis performed with E-SURGE (see S1 in the supporting information), allowing for robust comparison of survival between groups. To solve (ii) we used BaSTA (Colchero *et al.* 2012) to perform CMR analysis within a Bayesian hierarchical framework, which allowed us to fit parametric survival functions. With this procedure, missing times of birth and death are imputed from the population means, and based on the survival function, actuarial senescence can be quantified (Colchero & Clark 2011). Parametric survival functions are optimized using a Markov chain Monte Carlo (MCMC) simulation procedure. Actuarial senescence has been described with a number of mathematical functions (Gavrilov & Gavrilova 1991), of which the Gompertz and the Weibull functions are used most frequently. The Weibull function assumes independence of extrinsic/baseline and intrinsic/aging mortality (Ricklefs & Scheuerlein 2002), while in the Gompertz function actuarial senescence is an age-dependent multiplier of baseline mortality rate. We used the Gompertz function because it best fitted our observed survival

trajectories (see model selection below). Instantaneous mortality rate $(u)x$ is determined by

$$(1) \quad u(x) = \exp(b0 + b1 * x + c)$$

where *b0* is the baseline mortality, and *b1* the dependency of mortality on age (x). We included the age at first manipulation as proportional hazard covariate (c = coefficient*mean age at first manipulation) to control for variation in the age at which individuals were enrolled in treatment. Mean age of first manipulation was 2.04 years and, probably due to the limited range, did not significantly affect mortality (log hazard ratio = –0.257; CI –0.70, 0.10). BaSTA estimates *b0* and *b1* for each level of categorical covariate included in the analysis, thus we obtained separate estimates of *b0* and *b1* for individuals rearing reduced or enlarged broods. Remaining life span was calculated using the life table produced by BaSTA.

MCMC optimization was done using 4 parallel simulations with eight hundred thousand iterations, one hundred thousand burn in period and 2000 interval sampling each (see Fig.S2.2 for trace plots). Model parameters converged appropriately, serial autocorrelations where low (<0.05), and the resulting posterior distributions of *b0* and *b1* (n = 1400 each) allowed for robust comparison between manipulation groups. After optimization we described the posterior distribution divergence of $b0_{reduced}$ vs. $b0_{enlarged}$ and $b1_{reduced}$ vs. $b1_{enlarged}$ using the Kullback-Leibler divergence calibration (KLDC) (McCulloch 1989) that is included in BaSTA. KLDC values can be interpreted as the probability between 0.5 and 1 of values drawn from one distribution being from the other distribution. If the KLDC = 0.5, this signifies that the distributions are identical, if the KLDC = 1, this signifies that the distributions are completely non-overlapping. Following general convention, we determined probabilities of >95% to indicate a significant difference.

MODELS AND MODEL SELECTION

To verify which function (Gompertz or Weibull) was the most appropriate to use we compared the fit of these two functions to our data. We performed model selection based on the lowest deviance information criterion (DIC) (Millar 2009). The Gompertz had the lowest DIC value relative to the Weibull function (DIC values were 1048 and 1066, respectively), but we note that the results with respect to the manipulation effect are similar for both models.

Results

Individuals rearing enlarged broods (EB) had lower annual mean local survival rate (59%) compared with individuals rearing reduced broods (RB; 72%, Fig.2.1). This difference in mean survival rate gradually increased when sub setting the data for individuals which received >1 (37% vs. 67% survival) and >2 (20% vs. 61%) manipulations (Fig.2.1). Survival decreased with increasing number of consecutive manipulations, which is likely to be partly due to decreasing age-specific survival. Survival declined more in EB individuals, which is in qualitative agreement with increased actuarial senescence, but the same pattern could arise if reproductive effort increases baseline mortality rate. These differences in the raw data are supported by multistate capture-recapture analysis (see S1 in the supporting information), which showed that the best fitting models included manipulation dependent survival probabilities, and furthermore, that there was a negligible (~1%) difference in recapture probability between manipulation groups (Fig.S2.1). Models allowing the recapture rate to vary simultaneously across time and manipulation groups were not supported (Table S2.1), showing that reproductive effort affected survival, but not the probability of recapture.

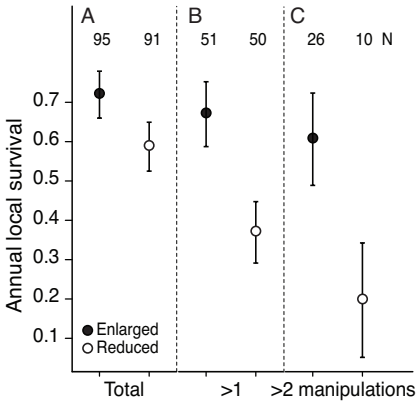

Figure 2.1 Mean annual local survival rate of individuals rearing reduced or enlarged broods (±S.E.). Values on the y-axis represent the raw data, i.e. probability to return, corrected for the probability of recapture as estimated by BaSTA (using the population mean of 85% because capture probability was independent of manipulation; see also S1). On the x-axis data is shown of all individuals (A), and subsets of individuals that received more than 1 (B), or more than 2 (C) consecutive brood size manipulations. The survival differences between manipulation groups are supported by multistate capture-recapture models (see supporting information S2.1).

We used Bayesian survival trajectory analysis (Colchero *et al.* 2012) to test the hypothesis that reproductive effort significantly affects actuarial senescence. BaSTA models that included experimental treatment yielded a lower DIC (delta DIC = 32.72). EB individuals showed a three-fold increase in actuarial senescence relative to RB individuals (mean posterior $b1_{EB}$ = 0.50; 95% CI 0.19, 0.83 vs. $b1_{RB}$ = 0.15; 95% CI –0.04, 0.37; KLDC = 0.96; Fig.2.2A), while baseline mortality was indistinguishable between treatment groups (mean posterior $b0_{EB}$ = –1.59; 95% CI –2.34, –0.87 vs. $b0_{RB}$ = –1.25; 95% CI –1.88, –0.60; KLDC = 0.69; Fig.2.2A). This resulted in 34% lower mean remaining life span of EB individuals at the age of 2 (remaining lifespan = 1.73 versus 2.64 years). Female and male mortality patterns were indistinguishable, also within brood size manipulation categories, showing that reproductive effort affected mortality of the sexes similarly (see table S2.3). There was a weak trend for RB male baseline mortality to be somewhat higher than EB baseline mortality, for which we have no particular explanation.

Our estimates refer to local survival, in that permanent dispersal cannot be distinguished from death. Thus if our manipulation affected dispersal rate this would bias our estimate of the experimental effect on survival. However, very few jackdaws dispersed between colonies and the likelihood of dispersal was independent of manipulation direction (5.5 versus 6.3%). Because natural cavities suitable for breeding are scarce in our study area, there is also little opportunity for birds to move elsewhere. Furthermore, the annual probability to return is 66% on average (range = 56–81% between years), which overlaps with a survival estimate of 65–69% based on ring recovery data of dead birds (Dobson 1990), which method yields global survival estimates that are not confounded by dispersal. This also suggests that permanent dispersal from our study area is very low and unlikely to bias our estimates.

We note however that there is likely to be some heterogeneity with respect to dispersal propensity within our population. Jackdaw colonies consist of a majority of resident birds that return each year, and a minority of intruding non-residents that may or may not return to breed in the same colony in later years (Röell 1978). Such heterogeneity may distort in particular the estimates of the first part of the survival trajectory, because emigration of intruders will be translated into higher mortality than the actual mortality. We therefore repeated the MCMC optimization using the same model, but with the subset of resident individuals, defined as individuals that were present in a colony for at least 2 years (n = 115). In this subset, mortality rate accelerated almost twice as fast with age in EB individuals (mean posterior $b1_{EB}$ = 1.08; 95% CI 0.65, 1.56 vs. $b1_{RB}$ = 0.60; 95% CI 0.25, 0.99; KLDC = 0.96; Fig.2.2B), while baseline mortality was again

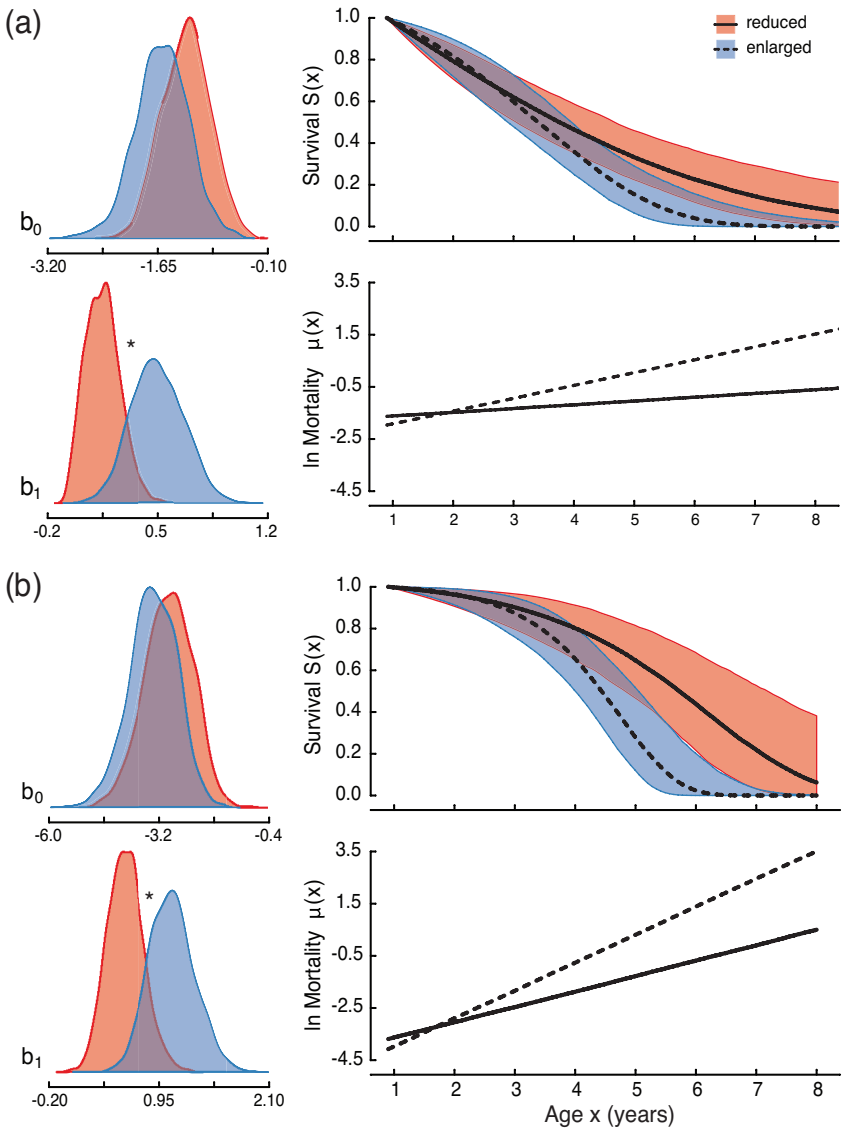

Figure 2.2 Age specific survival and mortality rate of (A) the total number of individuals and (B) the subset of resident birds, rearing reduced or enlarged broods. The colored areas in the survival graphs represent the 95% confidence intervals. Posterior distributions of b0 and b1 are shown, where b0 determines the intercept and b1 the slope of the natural logarithm (ln) of age dependent mortality rate ($(u)x$), which is plotted on the right side. Posterior distributions with a divergence of > 95% are highlighted with an asterisk (*).

indistinguishable between treatments (mean posterior $b0_{EB}$ = –3.35; 95% CI –4.60, –2.23 vs. $b0_{RB}$ = –2.96; 95% CI –4.21, –1.85; KLDC = 0.59; Fig.2.2B). The difference in *b1* resulted in a 64% lower mean remaining life span of EB individuals at the age of 3 (remaining lifespan = 0.69 versus 1.90 years). We conclude (i) that brood size manipulation increased actuarial senescence, without affecting baseline mortality and (ii) that the consequences for remaining lifespan are more pronounced when the analysis is restricted to resident birds.

Discussion

We experimentally tested the prediction that investment in reproduction accelerates actuarial senescence in a wild bird population. Our main finding is that repeatedly raising enlarged broods shortens remaining life span by 34–64% on average, depending on the exact data selection. Furthermore, this effect arises through an increase in actuarial senescence (b1 in the Gompertz equation), with no discernible effect on baseline mortality rate (b0). Although this manipulation effect is considerable, it probably underestimates the effects of a natural change in clutch size. This is because we manipulated the number of young when broods were four days old, thereby excluding the costs associated with egg production and incubation. Investment up to hatching can be substantial (Monaghan & Nager 1997; Visser & Lessells 2001; de Heij *et al.* 2006), and we therefore predict that a natural change in the number of offspring would yield an even larger difference in actuarial senescence.

The fitness costs of reproduction and other fitness enhancing resource drains (e.g. sexual signaling) are a major component of the contemporary evolutionary ecology framework, and a large effort has gone into testing for such trade-offs using brood size manipulation in birds. It is striking therefore that this collective research effort has not yielded a convincing verdict on the importance of the fitness costs of reproduction (Santos & Nakagawa 2012), and we see understanding this result as a major challenge. In our view, the way forward is to on the one hand generate replicates of our study to ascertain the generality of our results. On the other hand, identifying the mechanistic basis is another major challenge, and we see a prominent role for longitudinal sampling of physiological parameters in the wild in an experimental setting. Identifying the mechanistic basis of our results may provide a key to understanding why single-year brood size manipulations have revealed little effect on parental survival.

It is generally assumed that the effects of reproductive effort on actuarial senescence arise as a consequence of increased somatic damage accumulation

with age (Kirkwood 1977). Alternatively however, reproductive effort has a larger impact on mortality at old age compared to young age without direct effects on aging (Partridge & Andrews 1985). This could for instance arise if mortality risk by predation increases with age, independent of the manipulation, and when at the same time parents rearing enlarged broods spend more time foraging, thereby being more exposed to predators. Thus increased actuarial senescence may be observed in response to a manipulation, without accelerated physiological aging as the underlying cause. A way to resolve whether treatment effects are caused by accelerated physiological aging, as opposed to a change in age dependent susceptibility to the manipulation, is switching the manipulation from enlarged to reduced effort and vice versa. In invertebrates such switching experiments have demonstrated that reproductive effort increases short-term mortality, but not the rate of aging (Partridge & Andrews 1985; Tatar *et al.* 1993; Hsin & Kenyon 1999; Flatt *et al.* 2008), because after the switch subjects quickly adopted the instantaneous mortality rate that matched their current treatment. When the manipulation of effort had affected physiological aging, there would have been a lagging effect of the previous treatment on mortality rate. Unfortunately, our current data set includes too few individuals that switched treatment (n=9, excluded from the analysis from the switch onward) to use this approach, and obtaining sufficient numbers of birds that were manipulated for a number of years before and after a treatment switch would be a challenge. Instead, to resolve this issue in future studies we propose to measure physiological aging more directly in the context of our experimental design, using biomarkers such as telomere length for which we have previously identified an association with mortality in our study species (Salomons *et al.* 2009).

Previous studies that manipulated avian reproductive effort in the wild found mixed results for survival rate and, when studies were pooled in a meta-analysis, the overall effect was not significant despite a clear manipulation effect on reproductive effort (Santos & Nakagawa 2012). At first sight, this contrasts with our study, where manipulation of brood size had a strong effect on remaining lifespan. However, earlier studies estimated effects of a single brood manipulation on survival over only one year, and also in our data set there is no manipulation effect on mortality at young age after the first manipulation (Fig.2.2). Thus, we see our findings as being consistent with the meta-analysis results of Santos & Nakagawa (2012).

Why survival is unaffected after the first year of manipulation is a question that still needs to be resolved, because it suggests that parents could make a larger reproductive effort than they do, without paying a price in terms of reduced survival. Our findings, together with the meta-analysis results (Santos and

Nakagawa 2012), suggest that birds can apparently cope with the increased effort for one year without paying an immediate survival cost. Restraining from maximal reproductive effort may be optimal, because this enables birds to cope with unpredictable adverse circumstances during breeding. This could be beneficial, because high reproductive output relative to the population level in a bad year likely outweighs equal effort in a good year (Fisher 1930). Apparently, this buffer is exhausted at some point after the second manipulation, leading to a large survival cost in later years.

Conceptually, a buffer as outlined above is reminiscent of the reliability theory of aging (Gavrilov & Gavrilova 2001; Boonekamp *et al.* 2013), although we recognize that there are likely to be several models that would fit our results. In brief, reliability models of aging assume that a system (organism) consists of multiple elements that can replace each other, and that the system collapses at the demise of the last element. The failure rate of redundancy elements is synonym to the rate of damage accumulation. Element failure rate is generally assumed to be age independent, but such models nevertheless predict an exponential increase in mortality, much like the pattern in natural populations. High reproductive effort may be interpreted to increase the failure rate of redundancy elements resulting in accelerated aging. When animals are young, and hence redundancy is not yet approaching critical levels, an increase in element failure rate would increase mortality rate but only in the long term, advancing the moment that redundancy reaches a critical level. Thus, such a model would explain why there is no effect of a single year of manipulation, in particular when animals are first manipulated when they are young, as in our study. According to this theory, one would predict that a brood size manipulation carried out in late life would have stronger effects on survival to the next year, compared to the effect of the same manipulation early in life. Unfortunately, we do not have the data to test this, but correlational studies indeed suggest that the costs of reproduction may vary with age (Proaktor *et al.* 2007; Descamps *et al.* 2009). We see testing this hypothesis as an important subject of future studies, to further evaluate the theoretical setting in which best to understand our results and the lack of results from single year brood size manipulation studies.

Acknowledgements

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Supporting information

S2.1 E-SURGE: Multistate capture-recapture analysis

Methods

For capture-recapture survival analyses we constructed encounter histories, which noted for each of the 9 breeding seasons in our dataset (2004–2012) whether birds were observed breeding (1) or not observed breeding (0). Based on the experimental treatment birds were subjected to, we defined the following three states: (i) alive as a bird with enlarged broods, (ii) alive as a bird with reduced broods, or (iii) dead, which could probabilistically be inferred from three events: observed as a bird with enlarged broods, observed as a bird with reduced broods, or not observed. Transition was only allowed between being alive and dead, but not between having enlarged or reduced broods, since birds were subjected to the same experimental treatment throughout life. We considered two subsets of data. The first data subset noted encounters from the first manipulation onwards, while the second subset noted encounters from the second manipulation onwards to assess cumulative manipulation effects. Figure 2.1 in the main text also considers a subset with three or more manipulations, but low sample sizes of this subset hindered model convergence in the present analyses.

Goodness-of-fit tests on raw encounter histories were performed to test for transience (i.e., single capture because of nomadic behaviour or a short life span) and trap dependence (i.e., variation in recapture probability depending on the trapping history), using U-CARE 2.3 (Choquet *et al.* 2009*a*). The transience tests, assessing the null hypotheses of (i) previously and newly ringed birds being equally likely to be reencountered (called 3.SR: $\chi^2_6 = 4.994$, $P = 0.545$) and (ii) the time until reencounter not being different for previously and newly ringed birds (called 3.SM: $\chi^2_3 = 2.562$, $P = 0.464$), were not statistically significant. Furthermore, the tests of trap dependence assessing the null hypotheses of (i) no difference in reencounter probability between birds trapped and not trapped at a previous occasion (given that they are alive) (called 2.CT: $\chi^2_2 = 1.566$, $P = 0.457$), and (ii) no difference in the time until reencounter between birds trapped and not trapped at a previous occasion (called 2.CL: $\chi^2_1 = 0.000$, $P = 1.000$) also showed no statistical differences. The variance in our data was therefore not larger (overdispersed, captured in an overdispersion parameter $\hat{c} > 1$) or smaller (underdispersed, captured in an overdispersion parameter $\hat{c} < 1$) than predicted by a simple Cormack-Jolly-Seber model, which allows only for year-specific esti-

mates of recapture and survival probability, and we used this model as a starting point for model selection (Lebreton *et al.* 2009) and assigned a value of 1 to ĉ.

We analysed the data using multistate capture-recapture models (Clobert *et al.* 1987; Lebreton *et al.* 2009) implemented in the programme E-SURGE 1.6.0 (Choquet *et al.* 2009*b*). We modelled recapture probability (*p*) and survival probability (Φ) by allowing them to be constant or to vary with experimental treatment and/or time (table S2.1). Models were compared and ranked based on a small sample unbiased quasi-Akaike's information criterion (Burnham and Anderson 2002), calculated as qAICc = ((deviance/ĉ) + ((2*number of parameters estimated)*(sample size/(sample size – number of parameters estimated – 1))). All models within 2 qAICc points from the model with the lowest qAICc score were used to obtain a weighted average of the model-specific estimates of recapture and survival probability based on the Akaike weight.

RESULTS

From the first manipulation onwards, the three best models included constant or manipulation-dependent recapture probabilities and survival probabilities that depended on time and manipulation (table S2.1). Model-averaged values, however, showed extremely small manipulation effects of a 1% and 2% lower probability for enlarged versus reduced-brood birds, for recapture and survival probabilities, respectively (figure S2.1).

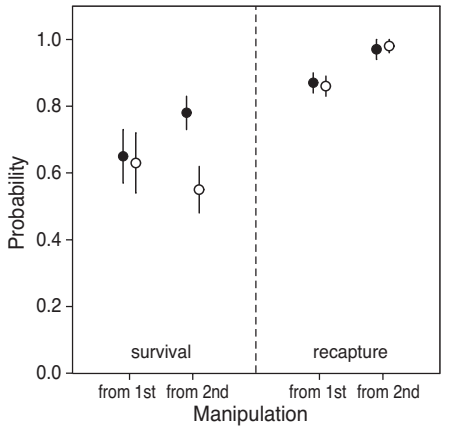

FIGURE S2.1 Survival and recapture probabilities (± s.e.) of jackdaws with experimentally enlarged (white circles) or reduced (black circles) broods, from the first or second year of manipulation onwards.

TABLE S2.1 Model selection results for time and manipulation effects on local survival (Φ) and recapture probability (*p*) in jackdaws.

data subset	Φ	*p*	np	deviance	ΔqAICc
from first manipulation onwards	***t***	***i***	**10**	**781.726**	**0.000**
	f+t	***i***	**11**	**780.298**	**0.686**
	t	***f***	**11**	**781.208**	**1.596**
	f+t	*f*	12	780.168	2.680
	i	*i*	4	797.124	2.939
	f	*i*	5	795.721	3.587
	i	*f*	5	796.535	4.401
	t	*t*	15	775.570	4.520
	f	*f*	6	795.520	5.447
	i	*t*	10	788.306	6.580
	f	*t*	11	786.950	7.337
	f+t	*t*	17	774.145	7.442
	i	*f+t*	11	787.442	7.829
	t	*f+t*	17	774.590	7.887
	f	*f+t*	12	786.495	9.007
	f+t	*f+t*	18	773.660	9.148
from second manipulation onwards	***f***	***i***	**5**	**311.916**	**0.000**
	f	***f***	**6**	**311.335**	**1.551**
	f+t	*i*	10	303.441	2.418
	f+t	*f*	11	302.792	4.020
	i	*i*	4	319.656	5.631
	i	*f*	5	319.246	7.330
	f	*t*	10	308.843	7.820
	t	*i*	9	311.543	8.293
	f+t	*t*	14	300.910	9.044
	f	*f+t*	11	307.882	9.110
	f+t	*f+t*	15	298.919	9.408
	t	*f*	10	311.116	10.093
	t	*t*	11	309.298	10.526
	i	*f+t*	10	314.140	13.117
	i	*t*	9	316.552	13.302
	t	*f+t*	14	305.406	13.540

Note: Best-supported models are presented in bold (models within 2 qAIC of the best supported model). For each model, the number of estimated parameters (np) is shown, along with the deviance and the difference in small sample unbiased quasi-Akaike's information criterion (qAICc) between that model and the best-supported model. In the model description, *i* indicates constant recapture or survival, *f* manipulation category (i.e., enlarged versus reduced broods), and *t* time (i.e., year effects).

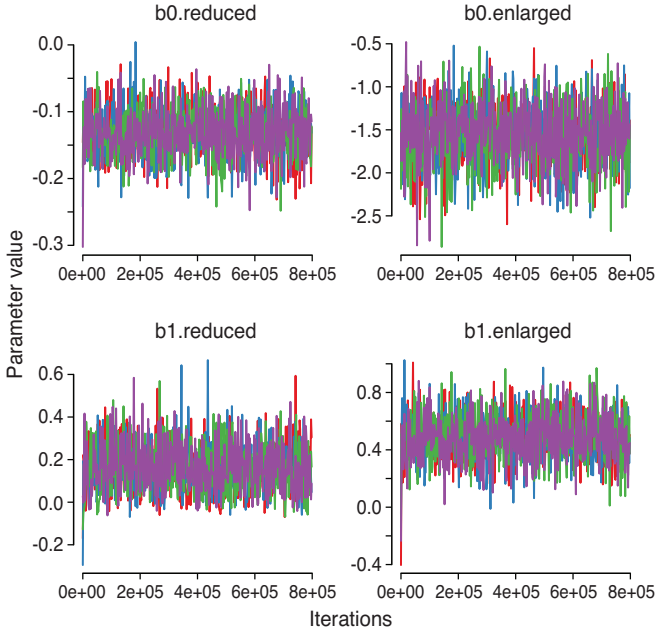

FIGURE S2.2 Parameter trace of the MCMC optimization. Settings were 800.000 iterations with 100.000 burn in and 2.000 thinning times 4 parallel simulations.

From the second manipulation onwards, a manipulation effect appeared and became substantial. The two best models included constant or manipulation-dependent recapture probabilities and survival probabilities that depended on manipulation (table S2.1). Model-averaged values showed a 1% higher recapture probability for enlarged versus reduced-brood birds, but a 23% lower survival probability (figure S2.1). Recapture probabilities in the set from second manipulation onwards was higher than in the complete set (from the first manipulation onwards) in line with our assumption that the reduced set contained only resident birds (see main text for further details).

Table S2.2 Number of manipulations per colony. Manipulation types (reduced, enlarged) were equally distributed across colonies ($X^2 = 9.0$, d.f. = 6, P = 0.2).

Colony	N enlarged	N reduced
BC	32	39
TY	20	18
LW	32	26
SW	2	10
ZW	2	1
BW	2	0
BS	1	1
Total	91	95

Table S2.3 Sex specific estimates and 95% confidence limits of baseline (b0) and age-dependent (b1) mortality rate of individuals rearing reduced (RB) or enlarged (EB) broods. There were no sex differences in either parameter for either experimental treatment as indicated by the Kullback-Leibler divergence criterion (KLDC; considered significant when >0.95).

	b0			b1		
	female	*male*	KLDC	*female*	*Male*	KLDC
RB	-1.96 (-2.90, -1.04)	-1.47 (-2.29, -0.56)	0.71	0.26 (0.06, 0.54)	0.14 (-0.06, 0.41)	0.66
EB	-1.80 (-2.79, -0.73)	-2.19 (-3.46, -1.23)	0.61	0.64 (0.11, 1.17)	0.68 (0.18, 1.27)	0.51

PHOTO: HANS REITSMA

CHAPTER 3

NESTLING TELOMERE SHORTENING, BUT NOT TELOMERE LENGTH, REFLECTS DEVELOPMENTAL STRESS AND PREDICTS SURVIVAL IN WILD BIRDS

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Abstract

Developmental stressors often have long-term fitness consequences, but linking offspring traits to fitness prospects has remained a challenge. Telomere length predicts mortality in adult birds, but whether telomere dynamics also provide a link between developmental conditions and fitness prospects is not known. Here, we examine the effects of manipulated brood size on growth, telomere dynamics, and post-fledging survival in free-living jackdaws. Nestlings in enlarged broods achieved lower mass and lost 21% more telomere repeats relative to nestlings in reduced broods, showing that developmental stress accelerates telomere shortening. Adult telomere length was positively correlated with their telomere length as nestling ($r = 0.83$). Thus, an advantage of long telomeres in nestlings is carried through to adulthood. Nestling telomere shortening predicted post-fledging survival and recruitment independent of manipulation and fledgling mass. This effect was strong, with a three-fold difference in recruitment probability over the telomere shortening range. In contrast, absolute telomere length was neither affected by brood size manipulation, nor related to survival. We conclude that telomere loss, but not absolute telomere length, links developmental conditions to subsequent survival, and suggest that telomere shortening may provide a key to unravelling the physiological causes of developmental effects on fitness.

Introduction

Poor nutritional conditions during development impair subsequent fitness prospects in many species (Lindström 1999), including humans (Lummaa & Clutton-Brock 2002). Stress research usually takes place in the laboratory, but fitness can only be measured in the wild, where some level of developmental stress is likely to be the rule rather than the exception. Little is known about the physiological mechanisms mediating effects of developmental conditions on fitness prospects and life histories. Here, we investigate whether telomere dynamics link developmental conditions to subsequent fitness prospects in free-living jackdaws, *Corvus monedula*.

Telomeres are DNA-protein complexes that protect the chromosome ends from fusing, but, when in shortened state, induce apoptosis or replicative senescence (Armanios & Blackburn 2012) (and references therein). In the absence of telomerase, telomeres shorten with each cell division due to incomplete end-replication (Olovnikov 1973), erosion of the single strand overhang (Stewart *et al.* 2003), and oxidative damage (Zglinicki 2002). Accordingly, telomere length has been shown to shorten with age and reflects remaining lifespan across species in nematodes (Joeng *et al.* 2004), birds (Haussmann *et al.* 2005; Bize *et al.* 2009; Salomons *et al.* 2009; Heidinger *et al.* 2012; Angelier *et al.* 2013; Barrett *et al.* 2013), and humans (Boonekamp *et al.* 2013). Furthermore, in captive zebra finches early-life telomere length predicted subsequent lifespan (Heidinger *et al.* 2012), but in free-living barn swallows no such relationship was found (Caprioli *et al.* 2013). Moreover, the rate at which telomeres shorten is linked to life style (Epel *et al.* 2004; Monaghan & Haussmann 2005; Bakaysa *et al.* 2007), suggesting that environmental conditions and associated physiological stress may be reflected in telomere dynamics.

Telomeres shorten at much higher rate during development compared to adult life (Salomons *et al.* 2009; Pauliny *et al.* 2011), which has been attributed to the rapid cell proliferation that accompanies growth (Frenck *et al.* 1998; Rufer *et al.* 1999; Zeichner *et al.* 1999). Prenatal environmental conditions have been shown to be correlated with telomere length at young adulthood in humans (Entringer *et al.* 2011; Haussmann *et al.* 2012). Furthermore, poor growth is associated with increased oxidative stress and accelerated telomere shortening in wild birds (Geiger *et al.* 2012; Caprioli *et al.* 2013; Hall *et al.* 2004). However, these relationships are correlational and whether they are caused by the focal environmental conditions or an unidentified confound cannot be determined. A manipulation of environmental conditions largely solves the problem of unknown confounding variables. We are aware of only one experimental study of rearing

environment and telomere dynamics, and in this study the manipulation had no effect on nestling telomere shortening rate (Voillemot *et al.* 2012). Thus, whether early-life telomere dynamics links developmental conditions to subsequent fitness prospects remains an unresolved issue.

We manipulated the developmental conditions of jackdaw nestlings *Corvus monedula* by modification of natal brood size, to experimentally study the effects of rearing environment on growth and telomere dynamics. We measured telomere length at the ages of 5 and 30 days, and at the adult age in recruits (nestlings that returned as breeding bird). Thus we could investigate the effects of manipulated brood size on both telomere length and shortening rate and relate these to survival until adulthood. We also investigated whether individuals with long telomeres as nestlings also had long telomeres as adult. This is of interest, because we have previously shown that adult telomere length is a predictor of survival in our study species [10], and thus it is possible that nestlings with long telomeres carry this advantage through to adulthood.

Methods

Study system

We studied jackdaw life histories in 7 different nest box colonies in the vicinity of Groningen, the Netherlands (53.1708° N, 6.6064° E) in the period 2005-2013. Regular nest checks were performed to determine laying date, clutch size, and hatch date as previously described (Salomons *et al.* 2008). Nestling blood samples were collected (± 50 μl from the inner metatarsal vein at day 5 and brachial vein at day 30) for telomere measurement between 2005–2010 at ages 5 and 30 days old. Nestlings were ringed when 30 days old with a combination of colour bands and a metal numbered ring that was unique for each individual bird, enabling follow-up measurements of survival through observation (until 2013) without the necessity of recapture.

We manipulated brood size by net +2 or –2 nestlings as follows: 3 nestlings were moved to the brood that was enlarged, and one of the original nestlings from this enlarged brood was relocated to the matched reduced brood. We chose either to reduce or enlarge all broods, and thus have no unmanipulated broods, to increase statistical power with respect to the manipulation effects. Pairs of broods were matched by hatch date and the manipulation was carried out when the oldest nestling was 5 days old (day of hatching was day one). Translocated nestlings were randomly chosen using random numbers generated by a computer or smartphone. When a brood that was to be reduced contained only 2 nestlings,

we reduced brood size with one nestling. As a consequence, some broods were enlarged with only one nestling in cases where the matched reduced brood contained only 2 nestlings. Date that the first egg of a clutch was laid, clutch size, brood size, nestling mass and telomere length did not differ significantly between manipulation groups before the experimental exchange of the nestlings (Table S3.1).

Our telomere assays are labour intensive, and we therefore made a selection of the samples to be analysed that included all nestlings from manipulation dyads from which at least one fledgling survived beyond March 1st of the next year. This yields higher statistical power than randomly selecting nestlings, because it provides a better control for rearing and genetic background. Recruitment rate will by definition be higher in our sample than in the population at large, but the estimated effects of nestling traits on recruitment will not be biased. In total 54 broods were selected (26 reduced, 28 enlarged), with 50 and 107 fledglings from reduced and enlarged broods respectively. With respect to the survival analysis we omitted offspring from 2 colonies (n = 3 broods) because survival was not recorded in these very small colonies (we removed the nest boxes because of low occupation). Hence for the survival analysis the sample size was 152 experimental fledglings from 51 broods.

Some individuals, those that fledged in 2010 in particular (the last year for which we measured telomere length), may yet recruit while being falsely coded as "not returned" in this analysis, causing bias in the estimate of survival and recruitment. However, based on the observed distribution of age at recruitment of earlier years the expected frequency of such cases is less than 5% and therefore unlikely to bias our analysis. Furthermore, including the year 2010 as additional factor in the analysis did not change the model fit (data not shown), suggesting that the potential problem of censored cases biasing our analysis is negligible.

TELOMERE LENGTH ASSAY

Telomere length was determined in erythrocytes using pulsed-field gel electrophoresis as previously described (Salomons *et al.* 2009). In short, DNA was extracted from erythrocyte nuclei using the CHEF Genomic DNA Plug kit (Bio-Rad, Hercules, CA, USA). Digested DNA from each sample was separated by pulsed-field gel electrophoresis at 14°C for 24h (3V/cm, initial switch time 0.5s, final switch time 7.0s). Dried gels (Bio-Rad model 538) were hybridized overnight using a ^{32}P-endlabelled oligo $(5'\text{-CCCTAA-}3')_4$ that binds to the 3' end-cap telomere overhang. Note that because of the latter no oligos bind to the interstitial telomeric repeats, because these are double stranded, and hence interstitial telomeric repeats are not included in the signal (this contrasts with telomere

measurements using e.g. qPCR which technique does not distinguish between different telomere types). Subsequently, the radioactive signal was determined (Cyclone™ Storage Phosphor System, PerkinElmer) resulting in a gel picture with a distribution of grey values (smear) reflecting the distribution of telomere lengths in a sample. Individual telomere length size distributions were quantified through densitometry using ImageJ v. 1.38x as previously described (Salomons *et al.* 2009), and we used the mean values for further analyses.

STATISTICAL ANALYSES

We analysed the effects of the brood size manipulation (reduced versus enlarged) on nestling growth and telomere length using mixed effects models. We included birth nest as random term to account for the dependence of nestlings that shared the same genetic ancestry and pre-manipulation environment. Rearing nest was not included as a random term in the models, due to limited degrees of freedom, but this term also had a negligible effect on the estimated fixed effects.

We used logistic regression to study the effects of fledgling mass, brood size manipulation, and telomere dynamics on survival and recruitment. We tested the effect of birth or rearing nest as random terms, but since most survivors originated from different broods (34 survivors out of 27 broods), birth and / or rearing nest had a negligible effect on the fixed effects estimates and significance levels, and were therefore excluded from the model. To account for possible spatial and temporal variation in telomere length independent of the manipulation we included birth year and colony as fixed factors. All analyses were performed in R using the lme4 and MASS packages.

RESULTS

Fledgling mass was lower in birds reared in enlarged broods (Table 3.1, model 1, and Fig.3.1A). Telomere length at ages 5 and 30 days were strongly correlated ($r = 0.95$; $P < 0.001$; Fig.3.2A) and average telomere loss over this 25-day period was 266 base pairs (s.e. = 14.44). Telomere length at the end of the rearing period (age 30 days) was shorter in nestlings in enlarged broods, but this difference was not statistically significant (Table 3.1, model 2). However, this analysis does not control for the large variation in initial telomere length (Fig.3.2A), and when we include the initial telomere length (day 5) into the model we find a significantly lower day 30 telomere length in enlarged broods compared to reduced broods (Table 3.1, model 3, and Fig.3.1B). Thus, adverse rearing conditions accelerated telomere attrition.

We further examined the association between growth and telomere attrition to investigate whether telomere attrition yielded information on development over and above the information contained in fledgling mass. When pooling all broods, fledgling mass was negatively correlated with telomere attrition (Table 3.1, model 4). We chose to use mass as dependent variable because it allows us to control for other factors affecting mass such as offspring sex. Further analysis showed that this correlation was present among nestlings in enlarged broods only (Table 3.1, model 5, interaction term: Delta TL*Manip P = 0.008; Fig.3.1C). Thus, variation in growth was not related with telomere shortening *per se*, but only when experimental rearing conditions are poor.

TABLE 3.1 Effects of brood size manipulation on fledgling mass and telomere shortening. Birth nest was included as random term in each model. The estimates of the fixed effects were calculated after rejection of non-significant terms. The covariate "brood size" denotes the brood size at day 5 before the manipulation. n = 157 nestlings in 54 broods. Significant P-values (< 0.05) are shown in bold.

model	fixed effect	rejected term(s)	estimate (s.e.)	P-value
1. fledgling mass	manipulation		–13.57 (3.50)	**<0.001**
		brood size	2.31 (2.00)	0.245
2. TL day 30		brood size	–10.55 (49.77)	0.830
		manipulation	–155.03 (84.16)	0.068
3. TL day 30	manipulation		–67.71 (30.87)	**0.034**
	TL5		0.98 (0.027)	**<0.001**
		TL5*manipulation	–0.09 (0.05)	0.061
		brood size	–17.30 (18.17)	0.335
4. fledging mass	delta TL[i]		27.66 (9.23)	**0.003**
		brood size	2.88 (2.47)	0.240
5. fledgling mass	manipulation		–2.40 (4.18)	1
	delta TL		–5.38 (10.52)	1
	delta TL*manip[ii]		34.64 (13.22)	**0.008**
	sex		9.59 (3.13)	**0.002**
	tarsus		5.98 (1.17)	**<0.001**
		brood size	1.17 (1.52)	0.433

[i] delta TL is telomere length difference between day 5 minus day 30
[ii] The slopes of the relation between delta TL and fledgling mass per manipulation category were 0.008 ±0.009 (reduced) and 0.025 ±0.008 (enlarged) g/bp.

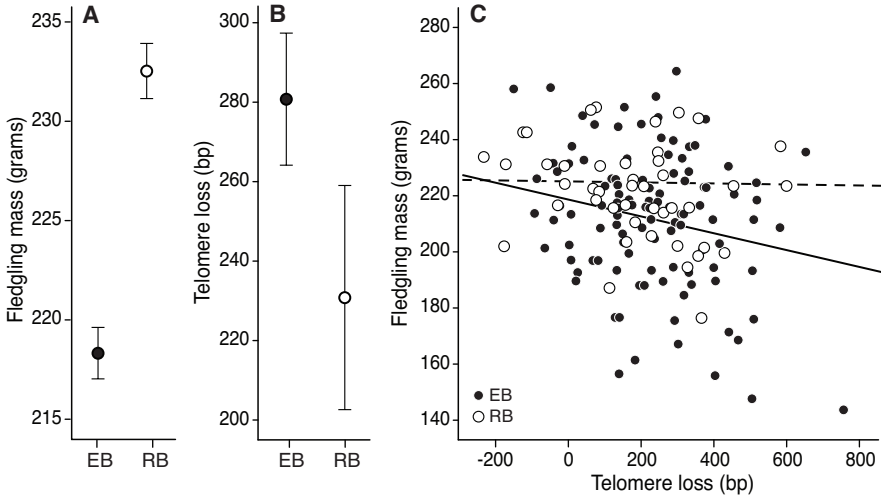

FIGURE 3.1 Fledgling mass and telomere shortening (± s.e.) in relation to brood size manipulation (EB = enlarged, RB = reduced broods). A. Fledgling mass. B. Telomere loss. C. Telomere loss in relation to fledging mass. See Table 3.1 for statistical details.

Adult telomere length (average age at sampling±s.e.: 2.77± 0.16) was highly correlated with telomere length of that same individual when it was a fledgling (r = 0.83; P < 0.001; Fig.3.2B). This correlation opens the possibility that any advantageous effect of telomere length is carried for life.

In total 34 out of 152 fledglings were observed to have survived beyond March 1st of the year after fledging, and 30 of these 34 birds recruited into our nest box colonies. The effects of the manipulation and telomere dynamics on survival were indistinguishable between survivors and recruits (Table 3.2) and we here only discuss the results on fledgling survival. Reduced and enlarged broods produced 8 and 26 survivors respectively (18% and 24% N.S., Table 3.2, model 2). Nestling telomere length at either day 5 or day 30 was not associated with survival (Table 3.2, models 3 and 4), but telomere attrition between day 5 and 30 was significantly lower in survivors compared to non-survivors (Table 3.2, model 5, and Fig.3.3). We tested for a quadratic effect of telomere shortening on survival, but this term was not significant (P = 0.72), indicating that the relationship between telomere shortening rate and survival is approximately linear (Fig.3.4).

Some fledglings may disperse outside our study area, and hence not be recorded as survivor, which would confound our analysis when the likelihood of dispersal depends on telomere attrition. To test this hypothesis, we compared

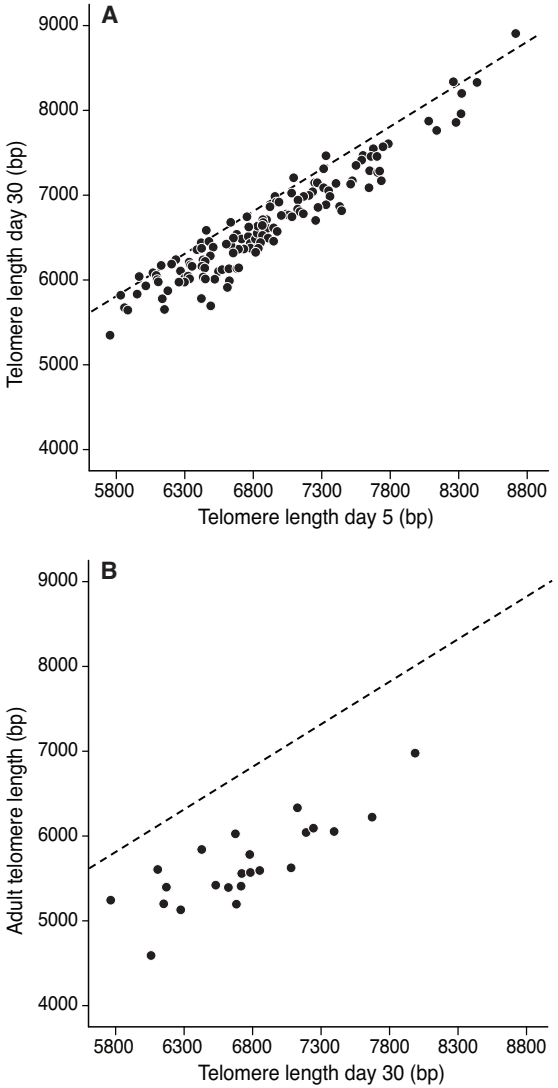

FIGURE 3.2 Correlations within individuals between telomere lengths measured at different ages. A. Telomere length at day 30 against telomere length at day 5, and B. Telomere length in adulthood against telomere length at fledging (day 30). The dashed line describes equal values of x and y, thus the vertical distance to this line is indicates the amount of telomere shortening. Note that we succeeded in sampling only 23 out of 30 recruits for telomere length.

telomere attrition between dispersers and non-dispersers making use of the fact that we studied multiple colonies, and hence our sample included known dispersers (n = 5 out of 30 recruits). Telomere attrition did not differ between dispersing and philopatric fledglings (difference = –4.56 ± 85.80 bp; P = 0.99). We recognize that a larger data set is required to detect subtle associations between telomere attrition and dispersal, but for now we conclude that a potential bias caused by dispersal is negligible.

Mean age at recruitment was 2.77 (s.e. = 0.16) years, ranged from 1-4 years old, and did not differ between fledglings from reduced and enlarged broods. Telomere length and attrition were not significantly associated with age of recruitment, or reproductive success of recruits (results not shown), but we note that sample sizes for these tests were small (n = 23 sampled recruits).

Fledgling mass has previously been shown to be a predictor of survival and recruitment in wild birds (Perrins 1965) including jackdaws (Verhulst & Salomons 2004). However, we found only a weak association between fledging mass and survival in the present dataset (Table 3.2, model 1). Jackdaws are sexually dimorphic in size, but correcting mass for sex and size did not significantly improve the model fit. More importantly, the change in the estimate of the effect of telomere attrition on survival after including fledgling mass in the model was negligible (Table 3.2, model 6), indicating that these effects are additive rather than that one variable can be substituted by the other.

TABLE 3.2 Survival and recruitment (in parentheses) rates by logistic regression. The factors "birth year" and "rear colony" were included as fixed effects in each model, to account for temporal and spatial variation in survival and recruitment. Sample sizes are 34 survivors (30 recruits) from 152 fledglings. P-values <0.05 are shown in bold.

model	fixed effect	estimate	st. error	P-value
1	fledgling mass	0.01 (0.02)	0.01 (0.01)	0.241 (0.087)
2	manipulation	0.54 (0.72)	0.48 (0.54)	0.393 (0.179)
3	TL day5	-0.16 (0.006)	0.35 (0.36)	0.639 (0.986)
4	TL day30	0.05 (0.25)	0.33 (0.34)	0.970 (0.515)
5	delta TL	2.49 (2.73)	1.20 (1.28)	**0.037 (0.033)**
6	delta TL	2.28 (2.30)	1.24 (1.32)	0.066 (0.080)
	fledgling mass	0.006 (0.01)	0.01 (0.01)	0.530 (0.223)

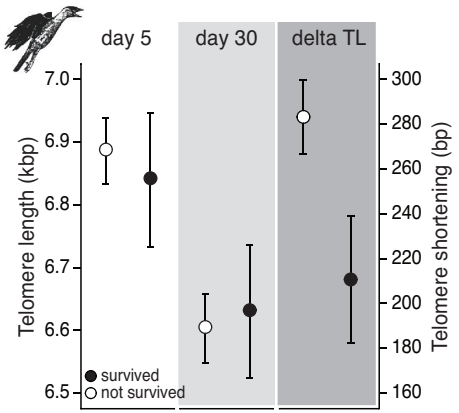

FIGURE 3.3 Telomere length and telomere shortening (± s.e.) in relation to survival. Panels from left to right show telomere length at ages 5 days, 30 days, and the change in telomere length in the nestling phase (day 5 – day 30) for survivors and non-survivors separately. See table 3.2 for statistical details.

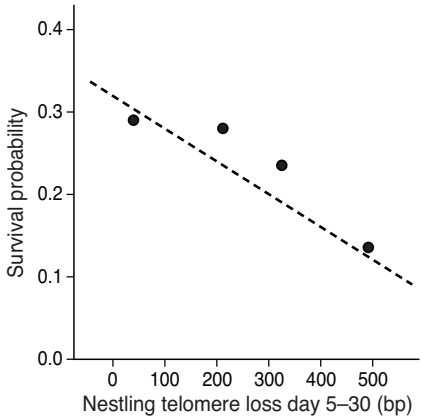

FIGURE 3.4 Telomere loss and survival probability. The four markers show the average survival rates for the telomere loss quartiles, and the line shows the logistic regression (Table 3.2).

Discussion

Developmental conditions can have strong effects on fitness prospects in humans and other species, but in particular in free-living animals little is known of the underlying mechanisms. We manipulated developmental conditions in jackdaw nestlings, and found that adverse rearing conditions accelerate telomere shortening (Fig.3.1). This experimental result is in agreement with observational studies that reported positive correlations between body size and telomere length (Caprioli *et al.* 2013; Hall *et al.* 2004). A low rate of telomere shortening in the nestling phase resulted in high survival until adulthood (Fig.3.3), independent of brood size manipulation and fledgling mass. The effect on survival is substantial because survival probability was three-fold higher for fledglings that lost the fewest base pairs compared to fledglings at the opposite end of the telomere loss range (Fig.3.4). These findings together suggest that the relationship between developmental conditions and fitness prospects is linked by telomere shortening rate.

We previously showed that long telomeres provide adult jackdaws with a survival advantage (Salomons *et al.* 2009), as in other avian species where this was investigated (Angelier *et al.* 2013; Barrett *et al.* 2013; Bauch *et al.* 2014; Bize *et al.* 2009; Foote, Daunt, *et al.* 2011a; Haussmann *et al.* 2005). It is of relevance therefore that fledglings with long telomeres also had long telomeres in adulthood (Fig.3.2B), because this suggests that advantageous effects of long telomeres, in part due to benign developmental conditions, are carried for life. However, more study years are required to verify whether individuals that fledge with long telomeres do indeed enjoy higher survival rates in adulthood.

We found that telomere shortening rather than telomere length predicted survival until adulthood (Fig.3.3), and likewise the brood size manipulation affected telomere shortening without significantly affecting fledgling telomere length. In principle, when there is an association of a factor with telomere shortening one would also expect an association of that factor with absolute telomere length. However, absolute telomere length at a given age is the outcome of initial telomere length and subsequent attrition, and hence variation in initial telomere length induced by genetic and other parental factors may reduce the accuracy of telomere length as a biomarker later in life. In particular genetic effects are likely to be important in this context, because reported heritabilities of telomere length are high (Broer *et al.* 2013; Olsson *et al.* 2011; Horn *et al.* 2011), but see (Voillemot *et al.* 2012). When variation in initial telomere length is large relative to the telomere shortening, as is the case in our study (Fig.3.2A), the association between telomere shortening and final length may be weak (in our study,

$R^2 = 0.02$ for the correlation between telomere shortening and telomere length at day 30; R^2 was corrected for regression to the mean following Verhulst *et al.* 2013 [36]). In a similar vein, when telomere shortening rather than telomere length is the primary variable that contains information, it is not surprising when absolute telomere length predicts survival (e.g. (Heidinger *et al.* 2012)); we interpret this as an indication that telomere attrition was a relatively important source of variation in absolute telomere length when compared to the contribution of initial telomere length. We do however predict that in such a situation the telomere loss would be an even better predictor of survival.

Associations between telomere length and fitness proxies may be due to a direct (causal) effect of telomere length on for example survival, when animals die because their telomeres have reached a critical length. Alternatively, telomere length is a biomarker that reflects various forms of cumulative (DNA) damage and for that reason alone is a predictor of fitness proxies. Critically short telomere lengths can have direct detrimental effects, as illustrated by the human disease dyskeratosis in which very short telomeres result in an early death (Batista *et al.* 2011), or telomerase deficient mice, that have a very short lifespan only when the effect of telomerase deficiency on telomere length is accumulated over a number of generations (Choudhury *et al.* 2006). However, telomeres in these two examples are substantially shorter than those found in the general population, which by itself suggests that observed associations between telomere length and fitness proxies that are observed in the general population do not reflect direct effects. We see our result that telomere shortening predicted survival, while absolute length did not, as support for the hypothesis that telomeres predict fitness proxies in the general population because they are a biomarker for phenotypic state, as opposed to the hypothesis that telomere length directly affects fitness. This view is further supported by the observation that the shortest telomere lengths in (old) adult jackdaws (Salomons *et al.* 2009) were substantially longer (>4100 base pairs) than the critical limit that causes cell senescence in human cells in vitro which is 78 base pairs (Capper *et al.* 2007). At the same time, we are aware that we may not be able to measure telomeres that are less than a few hundred base pairs long when they occur on just one or a few chromosomes, because in our measurements many cells and chromosomes are pooled. Thus we cannot yet rule out the possibility that individuals with high rates of telomere shortening that did not survive their first winter also had one or more telomeres of a critical length, causing their death directly.

While our manipulation of developmental conditions has the advantage of generating a natural stressor (many siblings), unravelling the mechanism underlying the manipulation effects is difficult because the manipulation changes devel-

opmental conditions in multiple dimensions. For example, nestlings in larger broods are likely to receive less food, face more competition when the parents bring food, and require less energy for thermoregulation, and these factors will each trigger a physiological response (Naguib *et al.* 2004; Verhulst *et al.* 2006; Metcalfe & Monaghan 2001) (and references therein). Of interest in this context is that the association between growth and telomere shortening was apparent in enlarged broods, with high telomere loss being associated with low growth, but not in reduced broods (Fig.3.1C). This suggests that low growth rate per se does not accelerate telomere attrition, but that it depends on the context whether telomeres are also affected. Individuals that grew less well presumably obtained less food, but the effect of low per capita provisioning rate may be restricted to low growth when brood size is small and competition is therefore low, while low provisioning rate in combination with high competition may cause high telomere shortening in addition to low growth.

ACKNOWLEDGEMENTS

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TABLE S3.1 Pre-manipulation characteristics (mean ± standard deviation) of broods that were subsequently reduced or enlarged. Laying dates refer to days in April. Sample sizes are n = 157 nestlings in 54 broods. Statistical results are from a model including birth nest as random effect.

	reduced	enlarged	P-value
laying date	14.96 ± 3.33	14.83 ± 3.05	1
clutch size	5.02 ± 0.65	4.75 ± 0.85	0.43
brood size (day 5)	4.68 ± 0.87	4.08 ± 0.97	1
mass (day 5)	40.87 ± 13.63	44.18 ± 12.72	0.24
TL (day 5)	6914 ± 606	6855 ± 564	0.39

CHAPTER 4

TELOMERE LENGTH BEHAVES AS BIOMARKER OF SOMATIC REDUNDANCY RATHER THAN BIOLOGICAL AGE

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Abstract

Biomarkers of aging are essential to predict mortality and aging related diseases. Paradoxically, age itself imposes a limitation on the use of known biomarkers of aging, because their associations with mortality generally diminish with age. How this pattern arises is however not understood. With meta-analysis we show that human leucocyte telomere length (TL) predicts mortality, and that this mortality association diminishes with age, as found for other biomarkers of aging. Subsequently, we demonstrate with simulation models that this observation cannot be reconciled with the popular hypothesis that TL is proportional to biological age. Using the reliability theory of aging we instead propose that TL is a biomarker of somatic redundancy, the body's capacity to absorb damage, which fits the observed pattern well. We discuss to what extent diminishing redundancy with age may also explain the observed diminishing mortality modulation with age of other biomarkers of aging. Considering diminishing somatic redundancy as the causal agent of aging may critically advance our understanding of the aging process, and improve predictions of life expectancy and vulnerability to aging-related diseases.

Biomarkers are used to assess health, risk of aging related diseases and remaining lifespan. However, the association with mortality of well-studied biomarkers, such as blood-pressure (BP), cholesterol (CHOL) and body-mass-index (BMI) diminishes with age (Collaboration 2002; Collaboration 2007; Collaboration 2009), indicating that they provide less information in old compared to young subjects. How this pattern arises is not yet understood, despite its relevance for understanding and predicting aging. We investigated this phenomenon using data on telomere length (TL). Telomeres are terminal DNA-protein complexes that protect chromosomes, but shorten with age (Armanios & Blackburn 2012). TL is a candidate biomarker of aging, but studies linking TL and mortality have yielded inconsistent results. Weak relationships were found in the oldest cohorts, suggesting that the association of TL and mortality diminishes with age (Martin-Ruiz *et al.* 2005; Bischoff *et al.* 2006). However, whether sampling age explains the observed study heterogeneity has not been quantitatively tested. We carried out meta-analyses to i) test whether TL predicts mortality and ii) test whether the association of TL and mortality diminishes with age.

Literature search yielded 16 eligible studies (SI–I.A) comprising 10,157 individuals, with an average follow up of 7.9 years during which 36% died. Effect sizes were expressed as hazard ratios (HR), the change in mortality risk associated with a decrease of 1 kilo base pairs in TL. Across studies, the natural log (ln) of

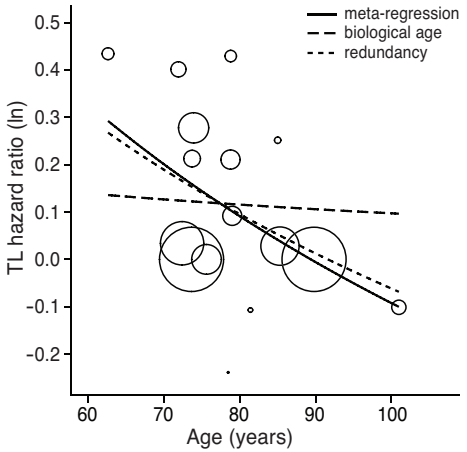

FIGURE 4.1 Meta-regression analysis of the association between mortality predicted by TL and ln age (continuous line). Bubble area is proportional to weight in the analysis ($1/s.e.^2$). Dashed lines depict the simulated mortality association of TL according to biological age (long dash), and redundancy (short dash).

the HR of TL was larger than zero (lnHR = 0.112; P = 0.007), indicating that longer TL was associated with lower mortality risk (SI–I). As hypothesized, lnHR of TL diminished with sampling age (Fig.4.1; slope for ln age = –0.822; 95% CI –1.556, –0.088; P = 0.028), from lnHR = 0.29 at age 63 to a negligible level (lnHR<0.05) at age ≥ 85. We conclude therefore that TL predicts mortality, but this association diminishes with age.

This pattern of diminishing mortality modulation (DMM; Fig.4.1) raises fundamental questions about the relationship between age, TL, and mortality. We tested two different models of this relationship using simulation models. Our first model was based on the popular perception of TL as indicator of biological age (Aviv 2002) in the sense that, for example, 70-year-old individuals with a TL of the average 60-year-old individual will experience the mortality risk of someone 10 years younger. More complex links between a biomarker and biological age can be envisaged, but in our perception this is the most common and simplest way that a biomarker is interpreted as indicator of biological age. We simulated mortality data using the Weibull distribution, and subsequently analysed these data on the association of TL, age and mortality using meta-regression analysis (see SI–II and SI–III.A for details on general simulation procedures and the biological age model respectively). HR of TL declined with subject age, but in the best fitting simulation results the slope was only ~10% of the observed slope (slope = –0.082 vs. –0.822; Fig.4.1). Repeating this analysis using the Gompertz distribution yielded the same result (SI–III.B; Fig. S4.2).

Our second model assumed TL to be a measure of somatic redundancy. It has been hypothesized that organisms consist of redundancy elements that can functionally replace each other, allowing for damage to accumulate until the last element fails, causing death. The redundancy elements themselves are assumed to be non-aging in that they have a constant failure rate over time. The resulting redundancy exhaustion generates mortality trajectories with age that resemble observed mortality patterns (Gavrilov & Gavrilova 2001). Treating TL as measure of redundancy is at least superficially compatible with the observation that long telomeres shorten faster than short telomeres (Grasman *et al.* 2011), and references therein), because with more redundancy elements also more are lost per unit of time. Furthermore, this approach is compatible with the observation that telomere shortening does not influence cell performance until a threshold limit is reached inducing cell cycle arrest (Armanios & Blackburn 2012). Thus, although we do not suggest that TL is a direct measure of somatic redundancy, we do consider that telomeres share critical features with redundancy elements. We considered TL as index of the number of redundancy elements, and simulated mortality data using this model (SI–IV). HR of TL declined with subject age in the

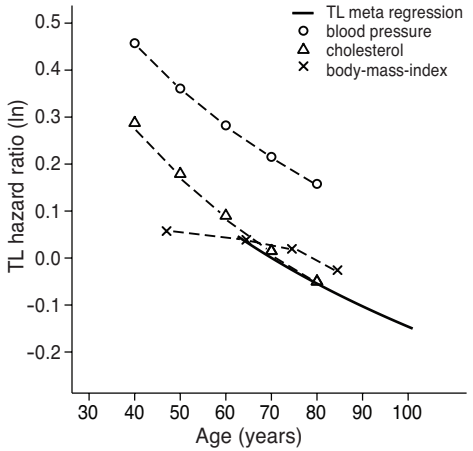

FIGURE 4.2 DMM of BP (○), CHOL (Δ), and BMI (×). The meta-regression line of TL is shown as a reference (solid line). HR values of BP, CHOL, and BMI were obtained from Prospective Studies Collaboration, 2002; 2007; 2009.

simulated data with a slope close to the observed pattern (Fig.4.1; slope –0.704 vs. –0.822). The redundancy model was substantially better than the biological age model in generating data that resembled the observations (ΔAIC = 4.0; SI–II.C) and we therefore conclude that the redundancy model best describes DMM of TL with age.

The pattern of DMM with age of TL resembles the patterns reported for other biomarkers of aging (Fig.4.2), confirming its generality. This resemblance raises the question whether, like TL, the DMM of BP, CHOL and BMI also results from diminishing redundancy with age. This is not obvious, given that the analogy that exists between redundancy elements and telomeres is not clear for these other biomarkers. On the other hand, we do not consider it likely that there are directly measurable redundancy "elements" existing within a single physiological structure or system. Instead, we consider redundancy to be an abstraction comprising a multitude of aspects of physiological state that together determines the body's capacity to absorb damage. When our interpretation is correct that diminishing somatic redundancy with age is causal to the aging process we would predict each biomarker of aging to reflect diminishing redundancy. However, whether this interpretation applies to BP, CHOL, and BMI remains to be verified.

Our findings are in agreement with the assumption that diminishing redundancy is causal to aging, but DMM with age of TL could also arise if the relation between TL and mortality is non-linear. When only a certain range of TL is associ-

ated with mortality, then TL may no longer predict mortality in the surviving subjects with TL outside this range. Because we found no evidence for non-linearity within the studies included in our meta-analysis, we consider it realistic to assume that mortality risk is linearly related with TL.

We recognize however that our evidence for diminishing redundancy as causal agent of aging is circumstantial, and it is important to note therefore that the redundancy model yields an additional prediction regarding biomarkers of aging. Due to the reduction in redundancy variance between individuals with age, redundancy element failure rate becomes increasingly important in predicting mortality. Verifying whether this prediction is supported by data would thus be a key test of the redundancy model of aging, and such a test may in particular be feasible using telomeres, because for this biomarker the rate of telomere attrition can be used as proxy for element failure rate. The data for a comprehensive test of this prediction using TL are unfortunately not yet available, but we note that promising preliminary support comes from one recent study showing that at old age telomere shortening more accurately predicted mortality than TL itself (Epel *et al.* 2009), in accordance with the redundancy model of aging.

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SUPPORTING INFORMATION

SI.I META-ANALYSIS PROCEDURES

A. Search and selection of studies:
We searched papers with (i) ISI Web of Knowledge and Google Scholar using combinations of multiple keywords: human, telomeres, telomere length, age, ag(e)ing, survival, mortality, and (ii) by checking references of relevant papers. In addition, (iii) we checked all the papers that cited (Cawthon *et al.* 2003), the first paper showing an association of human telomere length with mortality. The last search was carried out on 2-Feb-2012.

From the retrieved papers we selected studies that contained human leucocyte telomere length (TL) measurements combined with a follow-up period in which mortality was recorded. Further inclusion criteria were: (i) the study used "healthy" subjects, i.e. studies in which subjects were not selected for carrying a particular disease or other health problem. Causes of death were unfortunately available in only a few cases, thus we could not take into account whether these were aging-related or not. We note however that since this increases measurement error, this makes our test more conservative, i.e. decreases type-I error probability. (ii) Whether the necessary data could either be extracted from the paper, or received after contacting the authors, which was the case for each otherwise eligible study. See Table S4.1 below for an overview of the studies and study-specific details on data extraction.

B. Data extraction and effect size calculations:
From each study we extracted: the natural logarithm (ln) of the hazard ratio and its 95% confidence interval associated with TL, the mean age of the study population at TL sampling, the length of the follow up period, and the TL assay method (qPCR, Southern Blot, or flow-FISH). Studies differed in the number of covariates included in the survival analysis, possibly rendering the TL estimates across studies to be incomparable. Therefore, we used only the simplest survival models reported, in which besides TL only age was taken into account.

Studies varied in whether they used TL as continuous variable or instead compared TL quantiles, which in principle renders the hazard ratio estimates to be incomparable, because the units of analysis differ (Kavvoura & Liberopoulos 2007). We therefore determined for each study the unit of analysis and converted the HR's accordingly (see Table S4.1 for details). For example, 1.23 in table S4.1 denotes that the HR was based on 1.23 kbp TL difference (if this was not reported in the paper we estimated it based on the reported mean TL and standard devia-

Table S4.1 Studies used in the meta-analysis. Sample sizes are the total numbers of individuals sampled. Ln HR (C.I.) denotes the natural logarithm of hazard ratio of TL with the 95% confidence interval in brackets. The letters a-c denote method of HR extraction: a=HR directly from paper, b=HR from author, c=HR calculated by us (see below for details). TL assay denotes whether TL was determined using quantitative pcr (q-pcr), southern blot (s-blot), or flow-cytometry (flow-FISH). Mean age denotes the mean age in years at blood draw of the sampled subjects. Follow-up is the number of years after blood draw during which survival was recorded. Unit of analysis denotes the difference in TL in kbp that was used as unit of analysis in the study's survival analysis (as determined by us). Corrected ln HR (C.I.) denotes the study ln HR corrected for the unit of analysis, i.e. the study HR divided by the unit of analysis factor.

Study	Sample size	LN HR (C.I.)	TL assay	Mean age	Follow-up	Unit of analysis (KBP)	Corrected LN HR (C.I.)
Bakaysa *et al.* 2007	350	0.531 (0.182:0.956) a	s-blot	78.8	6.9	1.23	0.430 (0.148:0.774)
Bischoff *et al.* 2006[1]	42	-0.101 (-0.375:0.189) c	s-blot	101.0	6.0	1.00	-0.101 (-0.375:0.189)
Cawthon *et al.* 2003	143	0.621 (0.199:1.040) a	q-pcr	71.9	15.0	1.55	0.402 (0.129:0.676)
Epel *et al.* 2009	235	0.329 (-0.067:0.725) b	q-pcr	73.7	12.0	1.55	0.213 (-0.044:0.471)
Fitzpatrick *et al.* 2011	1,136	0.278 (0.095:0.451) a	s-blot	73.9	8.1	1	0.278 (0.095:0.451)
Harris *et al.* 2006	190	0.092 (-0.147:0.330) b	q-pcr	79.0	5.0	1	0.092 (-0.147:0.330)
Honig *et al.* 2006[2]	132	-0.223 (-1.204:0.588) a	q-pcr	81.4	NA	2.08	-0.107 (-0.578:0.282)
Houben *et al.* 2011	203	-0.215 (-0.673:0.248) a	q-pcr	78.5	7.0	0.9	-0.239 (-0.747:0.276)
Kimura *et al.* 2008[1]	548	0.211 (-0.030:0.446) a	s-blot	78.8	7.3	1	0.211 (-0.030:0.446)
Martin-Ruiz *et al.* 2005	598	0.000 (-0.166:0.236) a	q-pcr	89.8	13.0	2	0.000 (-0.083:0.118)
Martin-Ruiz *et al.* 2011	751	0.365 (-0.198:0.936) b	q-pcr	85.0	1.5	1.45	0.252 (-0.137:0.646)
Njajou *et al.* 2009	2,721	0.000 (-0.105:0.095) a	q-pcr	73.6	10.0	1	0.000 (-0.105:0.095)
Strandberg *et al.* 2011	622	0.000 (-0.186:0.174) b	s-blot	75.6	7.0	1	0.000 (-0.186:0.174)
Willeit *et al.* 2010	787	0.467 (0.010:0.673) a	q-pcr	62.6	10.0	1.07	0.435 (0.009:0.626)
Woo *et al.* 2008	2,006	0.166 (-0.493:0.878) a	q-pcr	72.4	4.0	4.9	0.034 (-0.099:0.176)
Zekry *et al.* 2011	444	0.058 (-0.261:0.365) a	flow-FISH	85.3	5.0	2.08	0.028 (-0.125:0.175)

[1] The cohort of Danish twins analyzed by Bischoff *et al.* was studied again later by Kimura *et al.* However, the paper by Bischoff et al included a separate analysis for centenarians, which was not replicated and we used only this study effect size in our meta-analysis. Survival and TL of centenarians were taken from figure 1 in the paper of Bischoff *et al.* HR was determined using a Cox-proportional hazard model without censoring of the data (all individuals died).

[2] The total sample of individuals analyzed by Honig *et al.* was selected for the prevalence of Alzheimer's disease, which led us to only include the HR of the control group in our meta-analysis.

tion, assuming a normal distribution). All analyses and figures were based on these converted HR values, but we note that this conversion had only minor effects on the results.

C. Meta-analysis:
We performed meta-analyses using the Metafor package (Viechtbauer 2010) in R (Anon 2009) using a random-effects model fitted with restricted maximum likelihood. Sampling variances were calculated from the confidence intervals, and we used 1/s.e.^2 as weighting factor in the meta-analysis (Hedges & Olkin 1985). Heterogeneity was evaluated using Q tests. With respect to testing whether the association of TL and mortality diminished with age we used the natural logarithm of age rather than age, because when the ln HR declines with age it can be expected that it will asymptotically approach zero, and this is better captured by ln age when compared to age.

D. Meta-analysis results:
We tested for publication bias using a funnel plot in combination with a rank test (Viechtbauer 2010), and no publication bias was detected (Fig. S4.1 below; Kendall's tau = 0.150; P = 0.450). There was significant heterogeneity among effect sizes (Q = 33.1; P = 0.005). Residual heterogeneity was substantially reduced when adding subject sampling age to the model, but remained significant (–18%, Q = 27.2; P = 0.018), suggesting that in addition to subject sampling

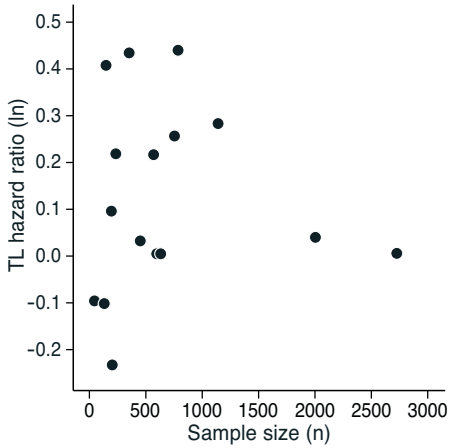

FIGURE S4.1 Funnel plot of the studies in the meta-analysis on the association of TL and mortality

age, differences in study methods and, or, population differences may affect the association of TL and mortality. We tested for such study differences, i.e. TL assay method and study follow-up period, but these were not significant as main effect (TL assay method $P = 0.502$, follow-up period $P = 0.767$), or interacting with age ($P = 0.678$ and $P = 0.148$ respectively).

SI.II SIMULATION STUDY PROCEDURES

A. General simulation procedures:
With the simulation models of biological age and somatic redundancy (described in SI–III and –IV respectively), we simulated survival times per individual per study, using the number of individuals, mean subject sampling age, and follow-up period as in the studies used in the meta-analysis. In the simulation, we generated individual survival data from one age to the next by using the age and TL specific mortality probability (determined by either one of the model equations 1, 2, or 3 described in SI–III and –IV) and a random value drawn from the uniform distribution $U(0,1)$. Each study was simulated 50 times and we calculated the HR of TL using Cox's proportional hazards with right censoring (Kleinbaum & Klein 2005) per simulation cycle, and subsequently we averaged these HR's over the 50 simulations. Thus, we obtained a simulated data set for each parameter combination for each of the models. We then optimized the parameters to maximize the resemblance between the simulated data and the meta-regression line of the real data, and subsequently compared which of the models generated data that best matched the observed pattern.

B. Model optimization:
To enable a quantitative comparison with the meta-regression results we optimized the model parameters for the simulated HR values to yield the closest possible fit to the meta-regression line of the observed studies. This was achieved by minimizing the sum of the weighted squared differences between the simulated study HR's and the meta-regression line fitted through the observed HR's. The weight factor that we applied to these squared differences was the same weight factor as used in the meta-analysis of the corresponding empirical studies, i.e. $1/\text{s.e.}^2$. To find the optimal parameter values we started with a wide range of parameter combinations and applied bisectioning to find the optimal parameter values.

Theoretically, a good fit of the simulated data to the meta-regression line of the observed studies could be based on lifespan distributions in the simulated data that strongly deviate from the empirically observed lifespan, which would render the model uninformative. We avoided this problem by additionally fitting the simulated lifespan distributions to the observed lifespan distribution obtained from the Dutch bureau of statistics (Anon n.d.) and omitted all model parameter combinations that yielded a fit of $r < 0.90$. We were limited to this selection of models, because a quantitative approach, i.e. directly optimizing the simulation model to the observed lifespan, requires the lifespan data of the studies that we used in our meta-analysis, and these are unavailable. Since all studies were done in recent years, and in Western countries, we consider it is safe to assume that these distributions are sufficiently similar when compared to our selection criterion. We calculated *r* as follows

$$r = 1 - \mathrm{SSe}/\mathrm{SStot}$$

where *SSe* is the sum of the squared differences between the observed and simulated probability density lifespan distributions, and *SStot* is the sum of squared differences between the observed probability density lifespan distribution and its mean. For the calculations of *r* we used matched age ranges of the simulated- and the observed lifespan distributions, and thus observed age at deaths of age <63 and simulated age at deaths of age >98 were ignored.

C. Model comparison:
To formally compare the fit of the simulation models to the observed pattern we performed additional meta-regression analyses of the observed hazard ratios, pooled with the hazard ratios generated by one of the simulation models with the optimized parameters. Pooling data and then fitting one meta-regression is informative, because when the simulated data fit the observed data less well this results in a poorer total fit. As measure of goodness of fit we used Akaike's "An Information Criterion" (AIC) (Anon 1974), calculated on the basis of the maximum log-likelihood (Metafor package in R). Following general convention, we considered models to fit equally well if their AIC's differed by less than two (Anon 2002).

SI.III Simulation model 1: biological age

A. Weibull:

We here describe how in our model TL determines biological age and how this was implemented in the Weibull distribution. At the start of each simulation, for each study, TL was generated from a normal distribution with the mean and SD approximating the mean TL and SD of the actual studies (TL mean=6.6 kbp, SD=1.0 kbp). TL shortening was included of 40 base pairs per year, which approximates the measured TL shortening rate in some longitudinal studies, e.g. (Aviv *et al.* 2009; Chen *et al.* 2011; Ehrlenbach *et al.* 2009; Houben *et al.* 2011). We stress however that the exact value has no effect on the outcome of the simulations, because the entire distribution shifted to shorter TL with increasing age, but the exact same range and relative differences between means were maintained. As measure of TL we used the age-specific deviation from the mean TL as follows:

$$\delta T_t \equiv T_t - \overline{T}_t$$

where T_t is TL (kbp) at age t, and T_t is the population mean TL at age t. Subsequently we defined biological age as

$$t' \equiv t - b_1 \delta T_t$$

where t is age in years and $b_1 > 0$ is the parameter indicating how many years the age is adjusted per δT_t. This would generate negative biological ages early in life but not in our simulations in which the lowest age is 63 years. At young age (after birth) we assume the effect of TL on biological age to increase non-linearly with age, levelling-off at medium to older ages, but note that we cannot test this because data on ages < 63 are unavailable.

We based equation (1) below on the Weibull distribution, which has been shown to describe the distribution of human life span well (Weibull 1951), but for comparison repeated the analysis using the Gompertz distribution (see below). We assumed the hazard rate $h(t)$ to increase with biological age t' as follows:

$$(1) \qquad h(t) = \lambda p (\lambda t')^{p-1}$$

where λ and p are the Weibull scale and shape parameters respectively. In this model the effect of TL on mortality diminishes with age, because for $p > 1$ mortality increases as a power function of age while the modulating effect of TL on mortality does not. This results in TL becoming relatively less important for

survival, because the mortality risk of other factors increases with age, suggesting qualitative agreement between the biological age model and the observed pattern. The parameter range that we tested for this model was [$\lambda(10{\cdot}10^{-3}$, $17{\cdot}10^{-3}$); $p(1, 10)$; $b_1(1, 10)$]. The optimal parameter values were: $\lambda = 14.29{\cdot}10^{-3}$; $p = 4.0$; $b_1 = 3.0$, resulting in a fit to the meta-regression line with AIC = –46.8 (calculated as described in SI–II.C); see Fig. S4.2 below.

B. Gompertz:
Alternatively we based our model of biological age on the Gompertz function, because some discrepancy between these functions exists when fitting to old ages (Juckett 1993). We used the same definition of biological age as previously described, and in the Gompertz model the hazard rate $h(t)$ increases with biological age t' as follows:

(2) $\quad h(t) = Re^{at'}$

where R is the initial mortality rate and a is the age dependent mortality. The parameter range that we tested for this model was [$R(1{\cdot}10^{-4}$, $1{\cdot}10^{-3}$); $a(0.01$, $0.2)$; $b(1\text{-}15)$]. The optimal parameter values were: $R = 5{\cdot}10^{-4}$; $a = 0.045$; $b_1 = 10.1$, resulting in a fit to the meta-regression line with AIC = –45.3 (calculated as described in SI–II.C); see Fig. S4.2 below).

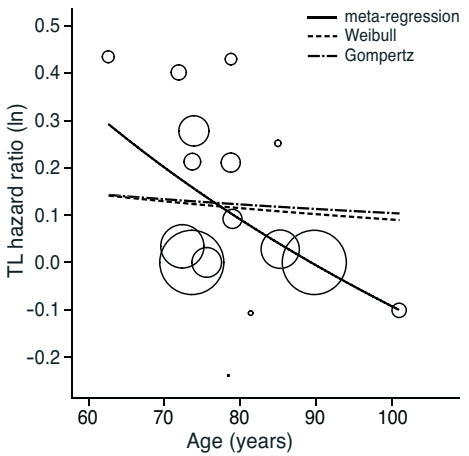

FIGURE S4.2 HR of TL according to the biological age model based on the Weibull and Gompertz distributions. The data points are the study HR values and the solid line is the meta-regression line as in Figure 4.1 of the main paper.

SI.IV SIMULATION MODEL 2: SOMATIC REDUNDANCY

The initial number of redundancy elements and the rate at which these fail characterizes a redundancy system. We considered TL as index of the number of redundancy elements, and we assumed the redundancy element failure rate to be constant, i.e. independent of age. This results in that the cumulative survivorship of a single redundancy element with failure rate k decreases with age exponentially ($S(t)=e^{-kt}$) and the hazard function of an organism with multiple redundancy elements is therefore given by:

$$h(t) = \frac{nke^{-kct}(1 - e^{-kct})^{n-1}}{1 - (1 - e^{-kct})^{n}} \quad (3)$$

where

$$n \equiv a + b_2\delta T$$

where t is age in years, k is the constant (age-independent) failure rate of n redundancy elements, and c is a scaling factor (Gavrilov & Gavrilova 2001). As measure of TL we used the deviation from the population mean TL (kbp) at sampling age ($\delta T \equiv T - \overline{T}$). We set a to 500, meaning that the average redundancy (at mean TL) at the start of our simulation was 500, and $b_2 > 0$ determines the redundancy per unit . In this model the effect of TL on mortality diminishes with age because the variation in the number of redundancy elements between individuals diminishes with age, because individuals with a high level of redundancy also lose more elements per unit of time, compared to individuals with a low redundancy level. We optimized the parameters of equation (3) using the same procedure as used for the previous model (see SI II.C). The parameter range that we tested was [k (0.18, 0.25); c (0.25, 0.35); b_2 (15, 110)]. The optimal parameter values were: k = 0.235; c = 0.328; b_2 = 90, resulting in a fit to the meta-regression line with AIC = –50.8 (see SI–II.C for details on calculations of the AIC).

The fit of the redundancy model in Fig.4.1 is a meta-regression fit using the simulated data, which explains why the line goes below zero at ages > 92, instead of approaching zero asymptotically. For the exact outcome of the model see Fig. S4.3 below.

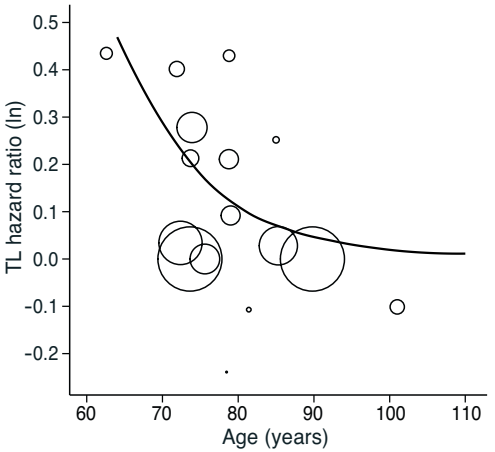

FIGURE S4.3 Exact ln HR of TL as calculated by the redundancy model (solid line). The data points are the study HR values as in Figure 4.1. The line asymptotically approaches zero because the rate at which total redundancy diminishes asymptotically approaches the redundancy element failure rate. This results in that at very old age all individuals face the same mortality risk, because mortality risk is then equal to redundancy element failure rate.

CHAPTER 5

SOCIAL CAREERS IN JACKDAWS: SOCIAL DOMINANCE INCREASES WITH AGE FOLLOWED BY A TERMINAL DECLINE

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SUBMITTED

Abstract

Social dominance increases access to resources, and can thereby increase fitness. In many species older individuals have higher social ranks. This pattern may be due to shorter lifespan of sub-dominants and / or an increase in social dominance with age, in which case social dominance could mitigate effects of physiological senescence. We studied the social careers of individual free-living jackdaws over a twelve-year period, and found that (i) independent of age, larger males attained higher ranks. (ii) Social rank increased with age within individuals, and (iii) high-ranked individuals had shorter lifespan suggesting that maintaining or achieving high rank comes at a cost. Lastly, (iv) social rank declined substantially in the last year an individual was observed in the colony, indicating that high social rank cannot be maintained in the final life-stage. This finding corresponds to our earlier finding that jackdaw telomere shortening is accelerated in the last year of life. We suggest that behaviour affecting the ability to secure resources is integral to the senescence process via resource effects on somatic state, where behaviour includes social dominance but probably also other behaviours such as learning, memory, perception and (sexual) signalling.

INTRODUCTION

Optimality theories of senescence (Partridge & Barton 1993) predict that senescence emerges because natural selection prioritizes early-life benefits over longevity (Medawar 1952; Hamilton 1966; Williams 1957). Key to this theory is the assumption that resources are limited, because this imposes a trade-off between reproductive investment and somatic maintenance or repair, causing senescence (Kirkwood & Rose 1991). One important aspect here is that individuals show large variation in their ability to secure environmental resources (ref), and this inherently shapes the scope for allocation between reproductive investment and somatic maintenance or repair. Therefore, understanding what determines an individual's success in securing resources is essential in order to understand the process of senescence in natural populations.

It is difficult to disentangle factors that determine the ability to secure resources, because they interact with each other (Fig.5.1). For example, body size, a component of physiological state, is positively correlated with the ability to compete over food, e.g. (Berdoy *et al.* 1995; Favre *et al.* 2008). Increased access to food in turn facilitates increased investment in somatic growth, maintenance or repair, positively affecting state and hence the ability to secure resources itself. The interaction between state and the ability to secure resources is likely modified by age, because (i) physiological state first improves with age due to development, and later in life declines with age due to senescence (Nussey *et al.* 2011), and (ii) the ability to secure resources in part depends on learning or experience that is gained over time, e.g. (McComb *et al.* 2001; McComb *et al.* 2011). Lastly, social dominance, or any other behaviour affecting the ability to secure resources, is integral part of these relationships (Fig.5.1). It is of interest therefore to verify what determines dominance when aiming to understand the senescence process. If dominance is a life history trait in the sense that one could actively invest in

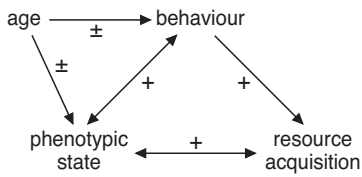

FIGURE 5.1 The relationship between phenotypic state, behaviour, and the ability to secure resources (social dominance). Behaviour in the context of the present paper is social dominance, i.e. the ability to win conflicts over resources, but can also be taken to be other components of behaviour related to e.g. perception and cognitive abilities.

dominance, gaining resource holding potential (Parker 1974), this could modify the balance between somatic maintenance and repair and other competing demands, shaping senescence.

Social dominance has often been shown depend on age, with older individuals being more dominant (Arcese & Smith 1985; Henderson & Hart 1995; Weiss *et al.* 2011; Berdoy *et al.* 1995; Aujard & Perret 1998; Favre *et al.* 2008; McComb *et al.* 2001; McComb *et al.* 2011; Thouless & Guinness 1986; Bridge & Field 2007), also in jackdaws, the subject of the present study (Henderson & Hart 1995). However, these studies are cross-sectional, i.e. between individuals, and on the basis of cross-sectional data it cannot be decided whether the observed pattern is due to changes within individuals as opposed to other not mutually exclusive processes. For example, high ranked individuals may have longer lifespan resulting in selective disappearance of lower ranked individuals. This would result in a positive correlation between age and dominance in cross-sectional studies, even if dominance were fixed within individuals (van de Pol & Verhulst 2006). Alternatively, dominance may depend on queuing for higher rank (Ens *et al.* 1995; Bridge & Field 2007; Wiley & Rabenold 1984; East & Hofer 2001). Queuing may arise when all individuals start at the bottom of the social ladder, and can only advance to a higher position when a dominant individual disappears from the population. If such a queuing effect occurs then the resulting pattern is that dominance increases with age within individuals, despite that this process may be entirely independent of competition, experience, or development. Longitudinal studies are required to disentangle these non-mutually exclusive effects, but only a few such studies have been performed. These studies showed that indeed dominance increases with age within individuals (Weiss *et al.* 2011) and that this increase is highly variable between individuals (DuVal 2012; Schubert *et al.* 2007).

We collected longitudinal data of social dominance in a colony of jackdaws over a period of twelve years. Each year we collected data on pre-breeding contest behaviour over food. A risk of this approach is that the outcome of conflicts may be determined by hunger level rather than resource holding potential, but earlier work in our colony has shown that social dominance over food is strongly correlated with the ability to secure nest boxes (Röell 1978). This indicates that social dominance in conflicts over food reflects something other than hunger level, and we assume this to be resource holding potential. We determined dominance only in jackdaw males, because they are dominant over females and the outcome of conflicts between pairs is determined by the rank of the male (Röell 1978; Wechsler 1988), and as a consequence female rank cannot determined independently. In the present analysis we disentangled the effects of within- (delta age) from between- (average age) subject age (van de Pol & Wright 2009) to determine if

dominance increased with age within subjects. This approach also allowed us to test whether social dominance is associated with life span, because this is reflected in the mean age at which they were observed. Furthermore, we previously found in jackdaws that telomere shortening rate – a biomarker of senescence – was highly elevated in the year preceding death (Salomons *et al.* 2009), and we therefore also tested for a terminal decline in social dominance.

METHODS

STUDY POPULATION

We studied free-living jackdaws in the colony at the Zoological Laboratory in Haren, The Netherlands, a semi-urban environment. Jackdaws are highly social medium long-lived birds with a strong and stable dominance hierarchy (Salomons *et al.* 2008; Verhulst & Salomons 2004; Henderson & Hart 1995; Wechsler 1988; Tamm 1977; Röell 1978) and therefore a good species to study for lifetime factors of social dominance. The colony was established in 1965 and enlarged to 36 nest boxes in 1996 when the study was resumed. Individual birds were marked with colour rings and a metal numbered ring before the breeding season. Estimates of adult age are exact for individual birds first ringed as fledglings or yearlings, the latter distinguishable from older adults through brown plumage colouration. Birds of unknown age were assigned a minimum age of 2 years. For this project it was sufficient to know the exact age difference between years, because our primary interest was (change in) social status within individuals. Biometry (tarsus, wing length, and mass) was measured, and a small blood sample (± 60 μl) was collected by puncture of the brachial vein for molecular sex determination and other analyses. We used the tarsus length as a measure of body size because this reflects skeletal size and does not change with age.

DOMINANCE

Social dominance was determined the month before the breeding season (March and first days of April) in the years 1998–2009, with the exception of 1999 and 2002, see (Salomons *et al.* 2008) for details. In brief, we staged social interactions with the use of 2 feeding pits (filled only during observation), 30 meters apart, where only one jackdaw could eat at a time. Social dominance was determined by the outcome of displacement, threat, or fight interactions between males, which were scored for each male that could be identified (mean ± s.e. = 59.5 ± 7.4 interactions / male). As in our previous studies, relative rank was calculated using David's score (Gammell *et al.* 2003).

Statistical analyses

We performed mixed-effects logistic regression analyses using ML-win v2.02. Relative rank measures were first transformed to numerical values ranging between 0-1, where 0 was assigned to the most dominant individual. Rank was nested within individual birds across years and therefore bird ID was included as a random effect. We first tested the effects of tarsus, age, age-squared, and [returned?], i.e. whether individuals returned the subsequent year (1 when they returned and 0 when they did not). To investigate the shape of the relationship between age and social dominance, and whether this depended on body size, we also tested the two-way interactions. Next, we disentangled queuing from within-individual effects, by testing the effect of colony composition. If queuing determines the increase of dominance with age, then the effect of age will depend on the fraction of new or disappeared individuals. To test this hypothesis we included the effects of [%dead in hierarchy], [%new in hierarchy], and tested the two-way interactions with delta age. Results are reported as model slopes ± standard errors (s.e.).

Separating within- from between-subjects effects

In a standard linear regression the estimated effect of age is the combined effect of the within- and between-subject age effects (model 1). Relative rank y_{ij} of measurement i from subject j is given by:

$$(1) \qquad y_{ij} = \beta_0 + \beta_1 x_{ij} + u_{0j} + e_{0ij}$$

where β_0 is the intercept and β_1 the dependency of dominance y_{ij} on age x_{ij}. The terms u_{0j} and e_{0ij} denote the random intercept and residual variance. To disentangle the within- from the between-subjects age components we transformed the model (1) into a model with average age and delta age (model 2) as previously described (van de Pol & Verhulst 2006; van de Pol & Wright 2009; Snijders & Bosker 2011). Average age $\bar{x}$ of subject j was obtained by taking the mean of ages i over the years an individual was ranked, resulting in one value for average age per individual $\bar{x}_j$. Delta age is defined as the difference between the age at which individuals are ranked and their average age $(x_{ij} - \bar{x}_j)$, resulting in multiple values for delta age per individual. Thus, the average age describes how relative rank is related with average age between individuals, while delta age describes the change in dominance with age within individuals. The dependency of relative rank on age is than described by:

$$(2) \qquad y_{ij} = \beta_0 + \beta_w(x_{ij} - \bar{x}_j) + \beta_B \bar{x}_j + u_{0j} + e_{0ij}$$

where β_w is the within individual effect of delta age ($x_{ij} - \bar{x}_j$), and β_B the between individual effect of average age $\bar{x}_j$. To test for occurrence of selective disappearance, we transformed the model (2) into a model with average age $\bar{x}_j$ and age x_{ij} as follows:

$$(3) \quad y_{ij} = \beta_0 + \beta_w x_{ij} + (\beta_B - \beta_w)\bar{x}_j + u_{0j} + e_{0ij}$$

If the estimate of average age $\bar{x}_j$ is significant, than individuals do selectively disappear from the colony, because this implies that the slopes of within- versus between-subject age differ significantly (van de Pol & Wright 2009). A random intercept model with u_{0j} as described above allows individuals to vary in the intercept of the relationship between relative rank and age. However, this model renders the slope of age to be fixed across individuals. Potentially such a fixed slope confounds inference with respect to the question whether dominance increases with age within individuals on the population level (Schielzeth & Forstmeier 2009). To account for this, we included the interaction of bird ID X delta age as a random term in the model as following

$$(4) \quad y_{ij} = (\beta_0 + u_{0j}) + (\beta_w x_{ij} + u_{Wj}) \cdot (x_{ij} - \bar{x}_j) + \beta_B \bar{x}_j + e_{0ij}$$

where u_{Wj} describes the variance around the random slope, allowing the relationship between relative rank and delta age to vary between individuals. The random slope explained a negligible amount of the variance (0.013 ± 0.027) and did not change the estimate of delta age (–0.228 versus -0.221), and therefore we omitted u_{Wj}, simplifying the model to eq. 3.

RESULTS

SIZE, AGE AND DOMINANCE

Larger birds (with a longer tarsus) were more dominant (Model A, Table 5.1, Fig.5.2). Tarsus length is stable during life, and therefore a between subject effect. Average age was not related to dominance (Model A, Table 5.1). The estimate of average age did not change when excluding delta age from the model (β_B = –0.06 ± 0.05), indicating that there was no cross-sectional relationship between age and social dominance in our population. Dominance increased with age within individuals (delta age β_w = –0.221 ± 0.069, P = 0.001, Model A, Table 5.1), showing that there was a strong longitudinal relationship between age and relative rank. Please note that 0 was assigned to the most dominant individual

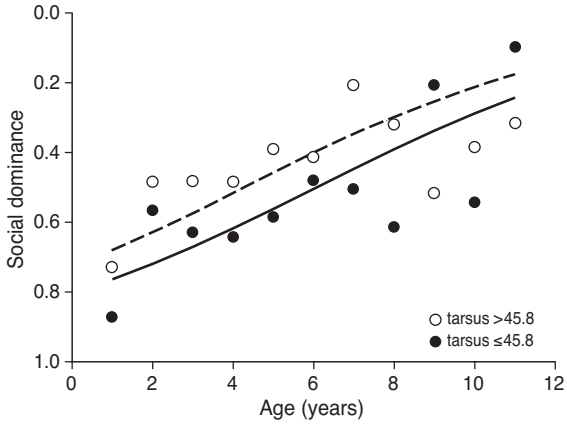

FIGURE 5.2 Social dominance in relation to age and size (tarsus length). For graphical purposes only, individuals were grouped by tarsus size (tarsus ≤ 45.8; or >45.8). Data-points were then calculated by averaging relative rank values of individuals for different age classes. Lines represent the predicted values of the logistic regression (eq. 2, Table 5.1), by using the average tarsus size (44.8; or 46.8) of each group. Low rank (0) indicates high dominance.

TABLE 5.1 Model A. Social dominance in relation to age and body size. The effect of delta age $\beta_W(x_{ij} - \bar{x}_j)$, average age $\beta_B(\bar{x}_j)$ (equation 2), tarsus size, and the variable [returned?], i.e. whether individuals return to the colony in the subsequent year. n = 149 bird years, of 69 individuals. Deviance denotes the -2 loglikelihood value of the model fit.

Model	deviance	fixed effect	slope	s.e.	P-value
Null	83.94	intercept (β_0)			
Final	57.12	intercept (β_0)	11.135	4.225	<0.001
		tarsus	–0.229	0.092	0.013
		delta age ($\beta_W (x_{ij} - \bar{x}_j)$)	–0.221	0.069	0.001
		average age ($\beta_B \bar{x}_j$)	0.052	0.048	0.276
		returned?	–0.581	0.281	0.038
Rejected terms in order					
		average age * average age	0.009	0.016	0.577
		delta age * delta age	0.001	0.028	0.998
		average age * returned?	0.098	0.110	0.377
		tarsus * returned?	–0.037	0.213	0.860
		average age * tarsus	–0.023	0.037	0.524

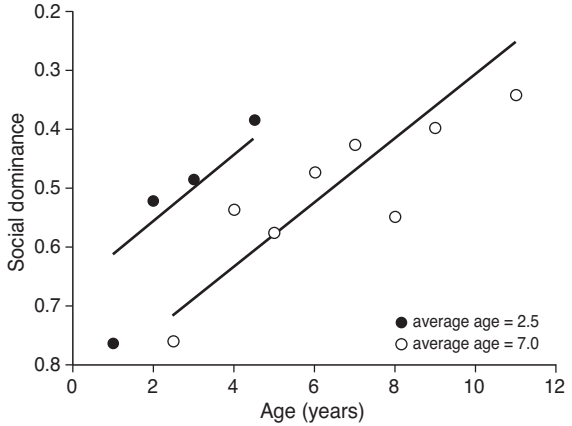

FIGURE 5.3 Social dominance in relation to age and life span. Individuals were first grouped by mean average age as relative index of their life span (low mean average age of 2.6, or high mean average age of 6.8). Data-points were then calculated by averaging relative rank values of individuals in each age category. The lines represent the predicted values of the logistic regression (eq. 3), by using the mean values of average age (2.6; or 6.8). Low rank (0) indicates high dominance.

TABLE 5.2 Model B. Testing whether between - and within-individual effects on dominance are significantly different. The effect of age (x_{ij}), average age ($\bar{x}_j$) (eq. 3 in the methods section), tarsus size, and the variable [returned?], on social dominance. n = 149 bird years, of 69 individuals. Deviance denotes the -2 loglikelihood value of the model fit.

Model	deviance	fixed effect	slope	s.e.	P-value
Null	83.94	intercept (β_0)			
Final	56.12	intercept (β_0)	11.004	4.217	<0.001
		tarsus	–0.226	0.092	0.014
		age (β_W (x_{ij}))	–0.231	0.069	<0.001
		average age (($\beta_B - \beta_W$) $\bar{x}_j$)	0.178	0.085	0.037
		returned?	–0.589	0.281	0.035

and hence that a negative slope signifies increasing dominance with age. The relation between age and social dominance is not necessarily linear, and to investigate the shape of the relationship between dominance and the different age components we tested for quadratic effects of age (Table 5.1, Model A). However, none of the quadratic terms or other two-way interactions were significant.

To test whether the within- and between-subject effects of age significantly differed from each other we transformed the model A with average age and delta

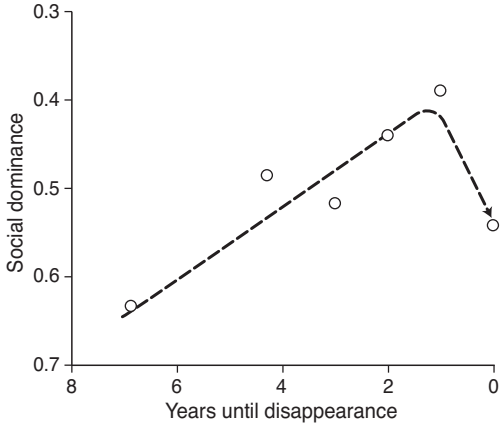

FIGURE 5.4 Social dominance in relation to years prior to disappearance (death). Individuals were first categorized by 'years before disappearance'. Data-points were then calculated by averaging relative rank values of individuals per 'years before disappearance' category. The line is fitted manually, representing the effect of [returned?] in the logistic regression (model B). Low rank (0) indicates high dominance.

age (eq. 2) into a model with age and average age (eq. 3; Model B, Table 5.2) as described in the methods section. The within- and between-subjects effect of age differed significantly from each other and the slope of average age was positive (Model B, Table 5.2, Fig.5.3). This shows that birds with low average age had higher dominance than birds with high average age. Hence, dominant birds disappeared at a younger age from the colony when compared to subordinates (Fig.5.3).

We previously showed that telomere shortening rate is elevated in the year before disappearance / death, indicating a terminal decline (Salomons *et al.* 2009). We tested whether social dominance shows a terminal decline, by including the variable [returned?] in model A that also included age (birds got a 1 when they did return the subsequent year and a 0 when they did not). This test showed that birds decreased in rank when they did not return to the colony the subsequent year (returned = –0.581±0.281, P = 0.038, Table 5.1, Fig.5.4).

ARE BIRDS THAT DISAPPEARED DEAD?

The pattern that dominant males disappear at younger ages (Fig. 5.3) may reflect their shorter lifespan, but alternatively dominant males may disperse more often, which could yield the same pattern. We do not have direct estimates of dispersal, but here use an alternative approach to verify to what extent disappearance could be due to dispersal rather than death. Divorce is very rare in jackdaw pairs

(Lorenz 1931), and our unpublished observations, and hence we can assume that the return of only one pair member to the colony reflects the death of the partner. On average of pairs where males did not return, females returned to the colony in 48,6 % of cases, which is lower than the known survival rate in our study area (66%) (Boonekamp *et al.* submitted). However, a jackdaw colony consists of resident birds that each year return to the colony to breed, and intruders that may return or disperse to breed elsewhere (Röell 1978). If we compare the percentage of returned females for resident birds, i.e. birds that bred in the colony more than one year, females that lost their males came back in 65% of cases, close to the observed annual survival rate of 66%. This is in agreement with the interpretation that disappeared males are dead in residents.

We repeated the analysis in Table 5.2, model B with residential males that bred in the colony more than one year, to determine if migration of intruder birds could confound our age estimates of dominance. We found that this analysis using the subset of resident males produced similar results compared to the total dataset (average age ($\beta_B - \beta_w$) = 0.193 ± 0.089 versus 0.178 ± 0.085; residents versus total).

DOES DOMINANCE INCREASE VIA QUEUING?

Queuing may arise when the increase in dominance of lower ranked subjects depends on the disappearance of dominant males. Therefore, when queuing is apparent we expect that the increase in dominance with age depends on changes in the colony composition, with individuals increasing in rank more in years that there are many new individuals in the colony. We examined the effect of the composition of the colony by testing the variables [%newrank] (The percentage of new birds per year in the colony), [%deadrank] (The percentage of disappeared birds per year from the colony), and the two-way interactions with delta age. None of these variables significantly decreased the deviance (Table 5.3), indicating that dominance did not increase with age due to changes in colony composition (queuing).

TABLE 5.3 The effect of colony composition variables on social dominance, when added to the final model in table 5.1. Variables are explained in the methods section.

fixed effect	slope	s.e.	P-value
%newrank	–0.002	0.006	0.689
%deadrank	–0.007	0.010	0.452
%deadrank * delta age	0.003	0.007	0.627
%newrank * delta age	–0.003	0.004	0.489

Discussion

Success in competition over resources links resource abundance to phenotypic state (Fig.5.1). We studied social dominance in relation to size, age and population composition in a colony of free-living jackdaws. Larger individuals were more dominant (Fig.5.2), which is consistent with previous findings in other species, (Verhulst & Salomons 2004; Berdoy *et al.* 1995; Favre *et al.* 2008). We found no effect of average age showing that dominance is not related to age when comparing between individuals. However, dominance rank clearly increased with age within individuals (Fig.5.3), except that individuals lost dominance in the last year before disappearing from the colony.

The increase in dominance with age could be the result from learning / experience (Arcese & Smith 1985; Berdoy *et al.* 1995; DuVal 2012), and / or queuing (East & Hofer 2001; Wiley & Rabenold 1984; Bridge & Field 2007). If queuing determines the increase of dominance with age then the fraction of new, or disappeared, individuals in the colony would affect the effect of delta age on social dominance. We did not find such an effect and we therefore conclude that the increase of dominance with age is the result from changes with age within individuals rather than the result of changes in population composition.

Socially dominant males disappeared from the colony at younger ages (Fig.5.3) suggesting that dominant individuals may pay a cost for being dominant in terms of reduced lifespan. Natural selection is expected to favour investment in dominance only if the benefits of dominance outweigh the costs of reduced lifespan. Given that extra-pair fertilizations are practically absent in jackdaws(Henderson *et al.* 2000; Liebers & Peter 1998), only reproductive success with the males' partner could outweigh the negative effect of a shortened lifespan. However, in contrast to the general pattern, we previously showed that dominant jackdaws in our colony achieved lower reproductive success than subdominants, and hence it was concluded that overall the more dominant birds had lower fitness (Verhulst & Salomons 2004). Our finding in the present study that more dominant birds have shorter lifespans indicates that the negative fitness effect of dominance in our colony is even stronger than we assumed on the basis of data on reproductive success. One explanation that remains untested is that the nest boxes in our colony were closely placed with only 1.5-3m distance between boxes and we previously argued that this may lead to increased aggressive interactions and testosterone titres, mitigating reproductive success (Verhulst & Salomons 2004). In birds, high testosterone has been show to reduce survival (Dufty 1989) and this may be the cause of reduced lifespan of dominant jackdaws. Furthermore, testosterone titres have previously been show to be lower in

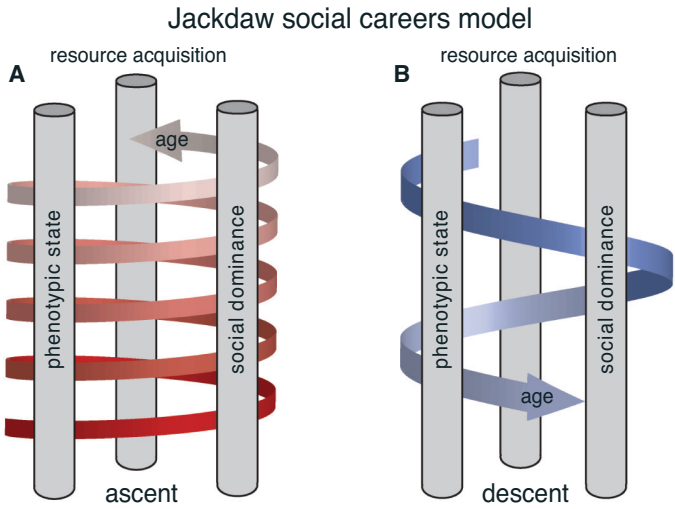

FIGURE 5.5 Reinforcement effects up and down the social ladder. Positive (A) and negative (B) reinforcement leading to increasing and decreasing social dominance with age at different life stages. Each pillar (phenotypic state, dominance, and the ability to secure resources) will positively affect the next pillar, causing an upward spiral with age (A). Physiological state deteriorates with age due to senescence, ultimately turning the direction of the effects (B).

old compared to young males, attenuating dominance (Aujard & Perret 1998). Reduced testosterone in old jackdaws could explain why dominance decreased in the year prior to disappearance. Therefore, the close distance of adjacent nest boxes in our colony may have far reaching consequences.

We previously showed that the rate of telomere attrition is strongly elevated in the year before disappearance from the colony (Salomons *et al.* 2009), and speculated that this could reflect a more general physiological collapse heralding death. If such a terminal decline indeed characterizes jackdaw senescence, we expected that this would also be apparent on the behavioural level. In agreement with our results on telomeres, we found that birds that were in their last year in the colony substantially lost dominance status, which contrasted with the steady increase observed in the years up this point (Fig.5.4). This finding is in line with a study in lemurs showing that the oldest individuals could not maintain high social status (Aujard & Perret 1998). Terminal declines have been reported in several traits (Coulson & Fairweather 2001; Rattiste 2004; Reed *et al.* 2008; Nussey *et al.* 2011) and we here show that it is also apparent in social dominance.

Phenotypic state, dominance, and resource acquisition can be viewed as three interacting factors, which may all be independently affected by age (Fig.5.1). This

interaction can be positive, with animals climbing up the social ladder, which through knock-on effects on resource acquisition enhances social dominance via positive resource effects on somatic state (Fig.5.5A). We found that dominance increased with age within individuals, and we interpret this to be at least in part the result of a positive reinforcement loop across these three factors (Fig.5.5A). An increase in phenotypic state will support higher dominance, which in turn increases the availability of resources for somatic maintenance or repair. An upward spiral over time can thus be envisioned (Fig.5.5A), counteracting negative age effects on physiological state (i.e. physiological senescence). However, at some point in time, physiological state may deteriorate sufficiently due to senescence to cause a decline in social dominance, at least in jackdaws where the more dominant individuals also participate in more agonistic interactions (Verhulst & Salomons 2004), suggesting that dominance becomes too costly to maintain. When such a threshold is crossed, the reinforcing loop may reverse, leading to a collapse through negative reinforcement, with reduced dominance leading to lower resource acquisition, leading to lower phenotypic state and so on (Fig.5.5B).

Behaviour is not generally considered as part of the senescence process, probably because most senescence research is carried out in model organisms in the laboratory. Behaviour is of limited importance in captivity, because social competition for resources is usually minimized and predators are absent. In contrast, we propose that under natural conditions different aspects of behaviour may delay or accelerate the senescence process. Senescence may be delayed due to increasing knowledge and experience, while senescence may be accelerated when an initial decline in performance is amplified due to downstream effects on, for example, the ability to secure resources. Cognitive abilities may be as important in this context as competitive abilities; indeed in figure 5.5 behaviour can be replaced with cognitive abilities, and perhaps also with other aspects of behaviour such as sexual signalling. Thus we suggest that behaviour is an integral part of the ageing syndrome and studying behaviour may therefore be important to understand the senescence process.

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Samenvatting

DE EVOLUTIE THEORIE VAN VEROUDERING

Hoewel het leven zeer divers is hebben organismen tenminste één ding gemeen – aan elk leven komt een einde. Eindig leven lijkt een evolutionaire paradox, want wanneer een individu sterft dan ontneemt dit alle toekomstige voortplantingsmogelijkheden. Natuurlijke selectie zou dus moeten leiden tot de evolutie van een steeds langere levensduur. Levensduur wordt echter niet alleen door interne (genetische) factoren bepaald, maar ook door externe factoren zoals predatie en ongelukken welke grotendeels afhangen van de omgeving waarin een individu leeft. In het geval dat het sterfterisico door deze externe factoren heel hoog is, zal een individu gemiddeld genomen een korte levensduur hebben. Dit heeft twee implicaties voor de evolutie van levensduur. (i) De eerste implicatie betreft de slagkracht van natuurlijke selectie die afneemt met leeftijd omdat door externe mortaliteit er maar weinig individuen zijn die oud worden. Dit fenomeen wordt ook wel de selectieschaduw genoemd. Het leidt er toe dat mutaties met negatieve effecten die specifiek op hoge leeftijd tot uiting komen kunnen accumuleren in een populatie, omdat er geen of nauwelijks selectie tegen dit proces is. Als een mutatie met negatieve effecten op hoge leeftijd eveneens positieve effecten vroeg in het leven heeft, dat wordt deze mutatie zelfs door selectie bevoordeeld, doordat positieve effecten vroeg in het leven gemakkelijk opwegen tegen negatieve effecten laat in het leven. De mate van externe mortaliteit heeft dus rechtstreeks invloed op de evolutie van negatieve effecten die gepaard gaan met hogere leeftijd, hetgeen kenmerkend is voor veroudering. Eveneens zal door externe mortaliteit evolutie naar snelle reproductie (vroeg in het leven) plaatsvinden, omdat uitstel van reproductie kan leiden tot sterfte voor het moment van reproductie. (ii) De tweede implicatie voor evolutie heeft betrekking op het beperkt beschikbaar zijn van levensmiddelen zoals voedsel in de natuur. Hierdoor is elke investering in reproductie kostbaar, omdat deze geïnvesteerde middelen niet voor andere doeleinden gebruikt kunnen worden. Wanneer externe mortaliteit hoog is, leidt dit zoals hierboven is uitgelegd tot de evolutie van hoge reproductieve inspanning vroeg in het leven. Dit resulteert in hogere kosten van reproductie ten opzichte van individuen die pas later in hun leven reproduceren en bevordert daarmee mogelijk het verouderingsproces.

De kosten van reproductie zoals hierboven genoemd kunnen op vele manieren tot uiting komen. De disposable soma (wegwerp lichaam) theorie veronderstelt een mechanistische link tussen reproductieve investeringen en veroudering. Elke investering in reproductie (nageslacht) gaat ten koste van investeringen en onderhoud aan het soma (lichaam), omdat primaire levensmiddelen als voedsel beperkt beschikbaar zijn. Hoge externe mortaliteitsrisico's leiden er toe dat individuen al

vroeg in hun leven reproduceren. Dit heeft tot gevolg dat ze intern sneller verouderen doordat er geen toereikende middelen beschikbaar zijn om het lichamelijk onderhoud te waarborgen om deze versnelde veroudering tegen te gaan. De disposable soma theorie is een aantrekkelijke hypothese en wordt veelal als raamwerk gebruikt in wetenschappelijke studies. Op dit moment is er echter onvoldoende bewijs dat deze theorie juist is. In dit proefschrift beschrijf ik onder meer de resultaten van een studie waarbij ik op experimentele wijze de hypothese test dat verhoogde reproductieve inspanning leidt tot versnelde veroudering (hoofdstuk 2).

DEZE KAUWENSTUDIE

Onderzoek aan vrijlevende dieren voegt een nieuwe dimensie toe aan het onderzoek naar veroudering, doordat deze dieren onderhevig zijn aan een veelvoud aan kosten en baten die eenvoudigweg afwezig zijn in gevangenschap. Vogels, en met name ook kauwen, zijn in deze bijzonder geschikt doordat 'bekende' individuen gedurende meerdere jaren relatief eenvoudig kunnen worden geobserveerd en gevangen. Hierdoor ontstaat er een link tussen fysiologische informatie en belangrijke life-history gegevens. In mijn studie heb ik gebruik gemaakt van een vrijlevende kauwenpopulatie in een gebied ten zuiden van Groningen. Kauwen zijn strikt monogaam, wisselen zelden van partner en zijn semi-koloniale holenbroeders. Natuurlijke holen komen nauwelijks voor in het studiegebied en zodoende waren er bij aanvang van deze studie geen natuurlijke kauwenkolonies. Deze eigenschappen zorgden voor hoge kwaliteit onderzoeksgegevensgegevens over de overleving en het reproductief succes van de kauwen, omdat dispersie en buitenechtelijke reproductie te verwaarlozen zijn. De eerste studiekolonie werd in 1996 opgericht door het plaatsen van nestkasten. Vanaf 2004 zijn ook op andere locaties nestkast kolonies opgericht en de gegevens in dit proefschrift zijn gebaseerd op 7 verschillende locaties / kolonies. Elk voorjaar werden alle kauwen gevangen op het moment dat de jongen circa 2 weken oud waren. Wanneer een kauw voor het eerst gevangen werd kreeg deze een combinatie van kleurringen en een metalen Vogeltrekstationring zodat de kauw individueel herkend kon worden zonder de noodzaak om de kauw opnieuw te vangen. Vlak voor het moment van uitvliegen werden op dezelfde wijze alle jongen uitgerust met unieke ringcombinaties. In totaal zijn er meer dan 1600 individuen geringd en hebben meer dan 2700 vangsten plaatsgevonden. Veelal werden uitgevlogen jongen niet meer waargenomen binnen het studiegebied, onder meer door de hoge sterfte van jonge onervaren dieren. Sommige adulte vogels werden daarentegen wel 13 jaar achtereenvolgens gevangen.

Om de hypothese te testen dat verhoogde reproductieve inspanning veroudering versnelt hebben Martijn Salomons (mijn voorganger) en ik sinds 2005 systematisch broedselgroottemanipulaties uitgevoerd. Deze manipulatie verhoogt reproductieve en fysieke inspanning doordat de ouders meer voedsel moeten verzamelen om hun jongen groot te brengen. Hierbij is het een belangrijk gegeven dat ouders geen onderscheid lijken te maken tussen hun eigen en vreemde jongen. Deze manipulatie kan je interpreteren alsof individuen een genetische mutatie dragen die hun ouderlijke zorg verhoogt of verlaagt. Een dergelijke mutatie zou de reproductieve inspanning beïnvloeden gedurende het hele leven. In overeenstemming hiermee hebben we de broedselgroottemanipulaties levenslang uitgevoerd: kauwen met verkleinde broedsels hebben elk opvolgend jaar dat ze terugkeerden een verkleind broedsel gekregen en hetzelfde geldt voor kauwen uit de vergrote manipulatiecategorie. Vervolgens heb ik de effecten van deze manipulaties op de snelheid van veroudering bestudeerd (hoofdstuk 2).

Kauwen die herhaaldelijk vergrote broedsels grootbrachten hadden gemiddeld 34% kortere levensduur in vergelijking tot kauwen die verkleinde broedsels grootbrachten (hoofdstuk 2). Dit verschil werd veroorzaakt doordat sterfte drie maal zo snel toenam met leeftijd in kauwen die vergrote broedsels grootbrachten. De basale sterftekans was daarentegen identiek. Deze resultaten zijn uniek, want voorgaand onderzoek bij invertebraten wees uit dat reproductieve inspanning juist geen effect heeft, of alleen effect heeft op de basale sterftekans in plaats van de snelheid van veroudering. Mijn resultaten zijn in overeenstemming met de 'disposable soma' theorie van veroudering, omdat we op basis van deze theorie verwachten dat verhoogde reproductieve inspanning leidt tot versnelde veroudering. Veroudering gaat meestal gepaard met een verminderd reproductief succes. Ook dit aspect heb ik bij de kauwen bekeken: vroegtijdige analyses duiden er op dat de manipulatie van reproductieve inspanning hier geen effect op had (resultaten hiervan zijn niet opgenomen in dit proefschrift). Reproductieve inspanning versnelt veroudering door een acceleratie in de kans van sterven, maar waarschijnlijk niet in verminderd reproductief succes.

GEDRAG IS ONDERDEEL VAN HET PROCES VAN VEROUDERING

Een belangrijke aanname bij de 'disposable soma' theorie van veroudering is dat primaire levensmiddelen als voedsel beperkt beschikbaar zijn. Deze beperking zorgt er namelijk voor dat levensmiddelen verdeeld moeten worden over verschillende "belangen" zoals het lichamelijk onderhoud en reproductie. Individuen verschillen echter in hun vaardigheid om levensmiddelen te verkrijgen, bijvoor-

beeld doordat de één meer succesvol is in competitie om levensmiddelen dan de ander. Eveneens kunnen cognitieve capaciteiten invloed hebben op het succes van het verkrijgen van primaire levensmiddelen. Gedrag is zodoende onderdeel van het verouderingsproces, maar krijgt als zodanig weinig aandacht in verouderingsstudies. In hoofdstuk 5 laat ik zien dat bij kauwen de dominantie in voedselcompetitie toeneemt met leeftijd. Dit heeft daarmee mogelijk het effect dat veroudering vertraagd wordt doordat de beschikbaarheid van voedsel toeneemt met leeftijd. Dit is een spannende hypothese die meer aandacht verdient. Hoge beschikbaarheid van voedsel d.m.v. dominantie in voedselcompetitie heeft een positieve invloed op de fysiologische staat doordat meer kan worden geïnvesteerd in het lichamelijk onderhoud. Dit kan op zijn beurt de dominantie positief beïnvloeden wat vervolgens leidt tot een hogere beschikbaarheid van voedsel e.d., waardoor zodoende een stijgende spiraal ontstaat. Echter in het laatste levensjaar van de kauw is de dominantie sterk gedaald (hoofdstuk 5). Een verklaring hiervoor is dat de fysiologische staat onvermijdelijk verslechtert door veroudering. Als de fysiologische staat dusdanig is verslechterd dat het niet langer de hoge dominantie kan ondersteunen, dan is een reductie in dominantie onvermijdelijk. Lagere dominantie leidt op zijn beurt weer tot verminderde competitie om voedsel en dit zorgt voor een verder verslechterde fysiologische staat. Een neerwaartse spiraal tegen het einde van het leven lijkt daarmee onontkoombaar. Dominantie is een gedragseigenschap met brede “life history” impact en in hoofdstuk 5 benader ik dit aspect in het kader van veroudering. Echter, zeer waarschijnlijk hebben andere vormen van gedrag en cognitieve eigenschappen eveneens invloed op veroudering. Verouderingsonderzoek zou hiervan kunnen profiteren door gedrag onderdeel te maken van het wetenschappelijke repertoire.

TELOMEREN ALS BIOMARKER VAN VEROUDERING

Veroudering kan gemeten worden door individuen gedurende hun leven te volgen en hun sterfte als eindpunt te nemen (hoofdstuk 2). Deze methodiek noodzaakt een lange duur van de studie en bemoeilijkt de studie aan veroudering bij langlevende soorten zoals de mens. Biomarkers van veroudering kunnen hier een oplossing bieden. Een biomarker van veroudering is een meetbare fysiologische eigenschap die verandert over de tijd, en deze verandering hangt samen met fysiologische veroudering. Biomarkers van veroudering voorspellen sterfte en kunnen zodoende fungeren als eindpunt van een studie in plaats van sterfte. Zo zou ook bij langlevende soorten (zoals mensen) onderzoek plaats kunnen vinden naar de oorzaken van veroudering.

Theorieën met betrekking tot de fysiologische oorzaken van veroudering zijn talloos, en 'oxidatieve schade' speelt mogelijk een belangrijke rol. Oxidatieve schade treedt op doordat instabiele moleculen geproduceerd worden als onvermijdelijk bijproduct van energiemetabolisme. Deze instabiele moleculen hebben hoge affiniteit met celcomponenten zoals onder meer de chromosomen, vetzuren en eiwit complexen in celmembranen, en induceren door binding breuken in het DNA of vervormingen van vetzuren en eiwitten. Telomeren (de 'uiteinden' van chromosomen) worden gedurende het leven korter in delende cellen onder andere door deze oxidatieve schade. Bij mensen is vastgesteld dat telomeerverkorting samenhangt met levensstijl, stress en verscheidene ouderdomsziekten. Dit laat zien dat telomeerlengte een veelbelovende kandidaat is als biomarker van veroudering. Deze studies laten echter nog niet zien dat telomeerlengte ook informatief is over de resterende levensduur.

Bij mensen hebben tot (de datum van mijn studie) een 16-tal studies plaatsgevonden gericht op de functie van telomeerlengte als biomarker van sterfte, door te kijken naar de relatie tussen telomeerlengte en de daaropvolgende sterfte. In slechts 4 van deze studies werd een significante correlatie aangetoond en dit doet de vraag rijzen of telomeerlengte daadwerkelijk informatief is over resterende levensduur. Met behulp van een meta-analyse laat ik in hoofdstuk 4 zien dat gemiddeld genomen over deze 16 studies telomeerlengte wel degelijk geassocieerd is met sterfte. Verder bleek dat de mate waarin telomeerlengte geassocieerd is met sterfte afneemt met leeftijd: op relatief jonge leeftijd is telomeerlengte een beter voorspeller van sterfte dan op hogere leeftijd (hoofdstuk 4). Bij een leeftijd van 88 jaar was deze associatie lager dan 5% hetgeen ik interpreteer als de leeftijd waarop telomeerlengte niet meer informatief is als biomarker van veroudering.

Ook bij vrijlevende vogels is herhaaldelijk aangetoond dat telomeerlengte gecorreleerd is met sterfte. Het is echter nog onduidelijk welke factoren invloed hebben op telomeerverkorting. Onderzoek hieraan wordt bemoeilijkt doordat de verkorting van telomeren bij adulten dusdanig laag is, dat deze nauwelijks te meten is. Bij nestjongen daarentegen is telomeerverkorting relatief hoog en daardoor ook beter te meten. In hoofdstuk 3 heb ik onderzocht wat het effect is van opgroeiomstandigheden op telomeerverkorting bij kauwenjongen gedurende de nestfase. Hierbij heb ik gebruik gemaakt van de broedselgroottemanipulatie-experimenten die ik heb toegepast om het effect van reproductieve inspanning te onderzoeken op de snelheid van veroudering bij adulte kauwen. Kauwenjongen uit vergrote broedsels hebben een lager uitvlieggewicht en verliezen meer baseparen (telomeerverkorting) ten opzichte van jongen uit verkleinde broedsels (hoofdstuk 3). Bij zebravinken in gevangenschap is recentelijk gebleken dat

telomeerlengte in de nestfase al een voorspeller is voor de resterende levensduur. Bij kauwenjongen bleek juist telomeerverkorting een goede voorspeller voor overleving na het uitvliegen tot de leeftijd van rekrutering (~2.4 jaar oud). Dit effect was onafhankelijk van het uitvlieggewicht en de manipulatiecategorie. Verder onderzoek moet opheldering verschaffen in de fysiologische oorzaken van telomeerverkorting en de relatie hiervan met overleving.

Telomeerlengte bij kauwen jongen was niet geassocieerd met overleving na het uitvliegen (hoofdstuk 3) terwijl in adulte kauwen (en ook in andere soorten) telomeerlengte wel de overleving voorspelt. Dit doet de vraag rijzen waarom de relatie tussen telomeerlengte en overleving zou verschillen tussen jongen en adulte kauwen. Een mogelijke verklaring hiervoor is dat telomeerlengte niet volledig bepaald wordt door de met veroudering samenhangende DNA-schade processen, maar ook door de initieel aangeboren telomeerlengte. Deze aangeboren telomeerlengte bevat geen informatie over de snelheid van schade accumulatie / verouderingsprocessen, omdat veroudering immers nog niet heeft plaatsgevonden. Variatie in deze aangeboren telomeerlengte kan zodoende de nauwkeurigheid van telomeerlengte als biomarker van veroudering reduceren, met name op jonge leeftijd. Op hoge leeftijd zal het aandeel in variatie in telomeerlengte meer bepaald zijn door de hoeveelheid telomeerverkorting die heeft plaatsgevonden gedurende het leven waardoor telomeerlengte steeds informatiever wordt m.b.t. veroudering. Bij mensen nam de relatie tussen telomeerlengte en sterfte juist af met leeftijd (hoofdstuk 4) en in het volgende deel zal ik uitleggen hoe dit kan en waarom dit niet tegenstrijdig hoeft te zijn met het idee dat telomeerlengte juist informatiever wordt met leeftijd.

SOMATISCHE REDUNDANTIE ALS MECHANISME VAN VEROUDERING

Het redundantiemodel van veroudering is een mechanistisch model waarin individuen in abstracte zin zijn opgebouwd uit elementen welke eenzelfde functie hebben. Deze elementen zijn dus uitwisselbaar, ofwel redundant. Wanneer het lichaam schade ondervindt (door veroudering) en redundante elementen kapot gaan, dan heeft dit geen invloed op het biologisch functioneren, tot op het moment dat het laatste overgebleven element kapot gaat. Het model heeft in feite twee parameters die samen bepalen hoe lang een individu leeft (i) de hoeveelheid redundante elementen en (ii) de snelheid waarmee deze elementen kapot gaan. Wanneer de snelheid waarmee de elementen kapot gaan constant is gedurende het leven, dan neemt het sterfterisico op organismeniveau toch toe met leeftijd (ongeveer exponentieel). Dit komt doordat het aantal intacte elementen steeds

kleiner wordt. Deze toename in sterftekans blijft echter niet exponentieel toenemen doordat de maximale sterftekans van het organisme beperkt is tot de kans per tijdseenheid waarmee de redundante elementen kapot gaan: immers de hoogste sterftekans van het individu is wanneer er maar 1 element over is en dus bepaalt de kans dat dit element kapot gaat de sterftekans van het gehele individu. Deze eigenschappen van het redundantiemodel levert sterftepatronen op die goed overeenkomen met sterftedemografie van onder andere mensen.

In hoofdstuk 4 beschouw ik telomeerlengte als maat voor het aantal redundante elementen. Telomeerverkorting is in overeenstemming met het redundantiemodel, omdat de snelheid waarmee het verliezen van baseparen door schade afhangt van telomeerlengte ofwel het aantal baseparen. In het redundantiemodel gaan er eveneens meer elementen per tijdseenheid kapot wanneer er meer elementen zijn. Door middel van simulaties heb ik onderzocht hoe telomeerlengte gemodelleerd als maat voor redundantie zich gedraagt als predictor van sterfte op verschillende leeftijden. Uit deze simulatiestudie bleek dat telomeerlengte correleert met sterfte, maar dat deze correlatie gradueel verdwijnt met leeftijd, hetgeen zowel kwalitatief als kwantitatief overeenkwam met het patroon van de meta-regressie analyse (hoofdstuk 4). De afname in de associatie tussen telomeerlengte en sterfte wordt veroorzaakt doordat de variatie in het aantal redundantie elementen tussen individuen afneemt met leeftijd, omdat iedereen uiteindelijk convergeert naar dezelfde staat waarin maar 1 element resteert.

Dat telomeerlengte zich gedraagt als maat voor somatische redundantie kan twee dingen betekenen: (i) telomeren fungeren als redundantiesystemen op zichzelf, of (ii) mensen zijn opgebouwd als redundantiesysteem en de afname van het aantal intacte elementen veroorzaakt veroudering. Deze twee verklaringen zijn moeilijk te testen en beide kunnen waar zijn. Echter, wanneer mensen in abstracte zin zijn opgebouwd als redundantiesysteem dan wordt verwacht dat (alle) andere biomarkers zich hetzelfde gedragen als telomeerlengte m.b.t. de afnemende mate waarin biomarkers sterfte voorspellen over de leeftijdsrange. In de literatuur is dit fenomeen inderdaad beschreven bij enkele goed bestudeerde biomarkers zoals cholesterol, body-mass-index en bloeddruk. Hoewel er tot op heden nog geen verklaring werd toegekend aan dit fenomeen, duidt mijn studie aan dat de afname in redundantie ervoor zorgt dat deze biomarkers op hoge leeftijd minder informatief zijn over sterfte dan op lage leeftijd. Dit is een belangrijke indicatie dat de oorzaak van veroudering te vinden is in een verminderde redundantie naarmate je ouder wordt.

Een belangrijke voorspelling van het redundantiemodel is dat hoewel het aantal elementen steeds minder informatief is over de resterende levensduur, de correlatie tussen de snelheid waarmee deze elementen kapot gaan (parameter ii

in het model) en sterfte juist toeneemt met leeftijd. Dit komt doordat de maximale sterftekans van het organisme wordt bepaald door de kans dat een redundantie element kapot gaat in de situatie dat er nog slechts één enkel element intact is. In mijn ogen is de verificatie van deze predictie een belangrijke test of mensen inderdaad fungeren als redundantiesystemen. Een veelbelovend resultaat van een voorgaande studie is dat sterfte op hoge leeftijd beter werd voorspeld door snelheid van telomeerverkorting dan telomeerlengte, in overeenstemming met de predictie van het redundantiemodel.

Zoals ik hierboven uitleg zou telomeerlengte op jonge leeftijd (bij geboorte) juist het sterkst gecorreleerd zijn met sterfte. Bij mensen zijn de gegevens om deze predictie te testen helaas niet beschikbaar, omdat er nog geen volledige dataset is die telomeerlengte vanaf de geboorte relateert aan levensduur. In hoofdstuk 3 laat ik bij kauwen zien dat telomeerlengte in de nestfase geen voorspeller van overleving is, maar daarentegen dat telomeerverkorting juist sterk gecorreleerd is met overleving. Deze discrepantie valt te verklaren met behulp van het redundantiemodel. De hypothese dat het aantal redundantie elementen, ofwel telomeerlengte, het beste sterfte voorspelt op jonge leeftijd is onder de aanname dat er geen variatie is tussen individuen in de snelheid waarmee redundantie elementen kapot gaan. Dit is echter zeer onwaarschijnlijk, omdat we weten dat telomeerverkorting zeer variabel is tussen individuen. In het geval dat er (veel) variatie bestaat tussen individuen in de snelheid waarmee redundantie elementen kapot gaan, dan verwacht ik dat de associatie tussen telomeerlengte en sterfte eerst toeneemt met leeftijd waarna deze relatie vervolgens weer afneemt op hoge leeftijd (hoofdstuk 1; Fig. 1.3). Een dergelijk patroon met een optimum is te verklaren doordat variatie in redundantie tussen individuen eerst toeneemt met leeftijd in het geval dat de snelheid van schadeprocessen verschilt tussen individuen. Deze variatie neemt vervolgens weer af met leeftijd door de convergentie naar de fysiologische staat met slechts een enkel resterend element. Bij mensen is het dus ook zeer aannemelijk dat er een optimum leeftijd is waarbij de resterende levensduur het beste te voorspellen is.

TOT SLOT

De belangrijkste beslissingen in het leven zijn gecentreerd rond de vraag “Wanneer neem ik hoeveel kinderen?”. Natuurlijke selectie leidt tot de optimalisatie van deze beslissingen in de context van de omgeving waarin we leven. Hoewel we door dit proefschrift nu weten dat het herhaaldelijk grootbrengen van vergrote broedsels tot gevolg heeft dat individuen sneller verouderen op demogra-

fisch niveau, weten we nog nauwelijks hoe dit effect tot stand komt. Versnelt reproductieve inspanning het fysiologische verouderingsproces, of is er een indirect effect op de sterftekans? Waarom is er geen effect van reproductieve inspanning na 1 jaar van manipulatie? Immers dit zou betekenen dat individuen 1 jaar harder kunnen werken zonder de negatieve effecten hiervan te ondervinden. Gelukkig wordt het kauwenonderzoek tenminste 3 jaar voortgezet om wellicht antwoord te vinden op deze vragen. Met name de vraag of reproductieve inspanning de fysiologische veroudering versnelt is hierbij van belang. De relatie tussen telomeerdynamica en andere fysiologische variabelen en sterfte in het kader van het broedselmanipulatie-experiment zal hier een leidende rol in spelen. Tevens worden de schattingen van fitness steeds nauwkeuriger doordat de studiepopulatie steeds meer rekruten bevat, hetgeen het mogelijk maakt om te onderzoeken wat de gevolgen van telomeerverkorting op fitness zijn. Met dit proefschrift hoop ik een bijdrage te hebben geleverd aan het begrijpen van het hoe en waarom we verouderen. Het model dat ik toets in dit proefschrift leert dat een verouderd lichaam geen resterende capaciteit heeft om schade te incasseren. Dit heeft de implicatie dat medisch ingrijpen bij een falend aspect hiervan (bijvoorbeeld hartfalen) de levensduur nauwelijks verlengt, omdat de behandeling van één fysiologisch aspect de algehele capaciteit om schade te incasseren niet doet genezen. In feite zou het gehele lichaam opgelapt moeten worden hetgeen vanuit technisch oogpunt onmogelijk lijkt. Een gezonde levensstijl minimaliseert schade-accumulatie en minimaliseert daarmee veroudering. Lang en gezond leven is iets wat vele van ons nastreven. Om het maximale hierin te bereiken luidt mijn advies: leef gezond!

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