Growing up and growing old

Briga, Michael

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Chapter 1

Environment, lifespan and aging: a synthesis

Michael Briga
Until now, the longest confirmed human lifespan ever recorded is that of a French woman, Jeanne Calment (1875-1997), who lived up to 122 years and 164 days (Whitney 1997). This exceptional lifespan lies at the very upper end of the human lifespan distribution (Fig. 1A). In humans and various other species, adult lifespan can vary up to tenfold between individuals (Jones et al. 2014, Fig. 1). This variation in human lifespan is only modestly heritable with approximately 25% being attributed to genetic differences\(^1\) (reviewed in Christensen et al. 2006). In the zebra finch *Taeniopygia guttata*, the model organism used in this study, adults also vary up to tenfold in lifespan (Fig. 1B) and I estimated its heritability to range between the 95%CI of 0 and 0.25\(^2\). This is considerably less that the heritability of, for example, body mass, for which, I estimated its heritability to range between the 95%CI of 0.20 and 0.69\(^3\). The low heritability of lifespan indicates that the environment is important. Indeed, for example, in the last 160 years, human life expectancy in various western societies has increased with several decades, even among societies’ oldest, and there is yet no sign of this trend slowing down (Oeppen and Vaupel 2002; Vaupel 2010). This change likely has an environmental origin, because it occurred too rapidly to be due to changes in DNA sequence. Thus individuals show variation in lifespan, which is to a large extent determined by the environment, a phenomenon that has important consequences for human society.

Genes can also play an important role in determining lifespan. According to the LongevityMap and GenAge, two extensive databases compiling the majority of genetic studies on lifespan and aging, researchers have currently identified over a hundred genes that are associated with lifespan in humans or that can extend lifespan in model organisms such as yeast, *Saccharomyces cerevisiae*, the nematode worm *Caenorhabditis elegans*, the fruitfly *Drosophila melanogaster* and the mouse *Mus musculus* (Budovsky et al. 2013; Tacutu et al. 2013). For example, there are a large number of long-lived mutants in the aforementioned model organisms (reviewed in Kenyon 2005; Kenyon 2010; Gems and Partridge 2013), some which can result in a doubling of the median lifespan, for example through the inhibition of the insulin or insulin-like growth factor signaling pathway (Kenyon et al. 1993; Garsin et al. 2003; Van Voorhies et al. 2005). These studies on model organisms indicate that certain genes can have a major effect on lifespan. However, the vast majority of these studies were carried out in laboratory environments, which can be very distinct from more natural environments.

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\(^1\) This refers to the narrow sense heritability, which technically, is the proportion of phenotypic variance among individuals in a trait that can be attributed to the additive effects of alleles that are independent of other alleles or loci (Kruuk et al. 2014).

\(^2\) Bayesian estimate using an ‘animal model’ approach (Kruuk 2004; Hadfield 2010) based on data from 440 cross-fostered individuals with a pedigree of 3 generations containing 839 half-sib bonds.

\(^3\) Both traits, lifespan and mass were measured with high accuracy: ± 1 day (Chapter 6) and 0.01 g respectively (Chapters 10 and 11).
Fig. 1 Variation in lifespan within a birth cohort. (A) Distribution of age at death, from age 10 onwards, for all humans born in France in 1875 (N=708,610). The black arrow indicates the lifespan of Jeanne Calment, currently the person with the longest recorded lifespan. Data are from Human Mortality Database (www.mortality.org/) (B) Distribution of age at death for zebra finches from this study. Shown here are the first three cohorts, i.e. those for which all individuals have died (N=338).

For example, laboratory environments are often characterized by a constant climate, minimal exposure to pathogens, no opportunity to reproduce (depending on the species) and *ad libitum* food that can be obtained with little or no physical effort. Therefore, the lifespan achieved by long-lived mutants in a laboratory environment is only one of the many possible lifespan phenotypes. The question then arises whether long-lived mutants would also display such a lifespan advantage in more natural environments. This issue is important, for example because humans are exposed to a variety of natural environments that are also distinct to the environment encountered in a laboratory setting. In chapter 2 we investigated the evidence for an environment specific lifespan advantage of long-lived mutants over their wild type controls under a range of laboratory conditions. We showed that in challenging environments (e.g. exposure to cold, pathogens or competition for food) the lifespan advantage of long-lived mutants disappeared or even that the wild type controls outlived their mutant counterparts. These results show that the role of environment in determining lifespan is also important when studying the genetic basis of lifespan. Hence, when studying the mechanistic basis of lifespan, attention should be payed to genotype x environment interactions. More controversially, these results suggest that genetic mechanisms generating lifespan variation in natural populations are different from those studied in the laboratory environments. Therefore, when studying variation in lifespan, careful consideration should be given in choosing
an environment that is relevant to the organism of interest. This obviously opens the question as to what defines a relevant environment. Below here we study in more detail one of several possible variables of interest, foraging costs, which are often encountered by free-living animals.

**Long term effects of developmental conditions**

The environment can exert effects on lifespan at many ages. However, the development phase is thought of as particularly important for adult lifespan and health (Lindström 1999; Metcalfe and Monaghan 2001; Lummaa and Clutton-Brock 2002; Bateson et al. 2004). For example in humans, Gambians born during the harvest season (i.e. with high food abundance) had a 20% higher chance to reach the age of 45 years relative to individuals born during a season with low food abundance (65 vs. 45% respectively; Fig. 2; Moore et al. 1997). Birth season effects, supposedly via food abundance, on lifespan were also shown in 20th century Austrians, Danes and Australians (Doblhammer and Vaupel 2001), although they were not found in 19th century Finns (Kannisto et al. 1997). More generally, there are several studies in a variety of human populations that have shown that cohorts with high childhood mortality are also characterized by a shorter adult lifespan (Kermack et al. 2001; Finch and Crimmins 2004; Crimmins and Finch 2006; Beltran-Sanchez et al. 2012; reviews in Galobardes et al. 2004; Lumey et al. 2011). Thus various cohort studies in humans have shown that adverse developmental conditions can negatively affect adult lifespan.

![Harvest season vs. Hungry season](image)

**Fig. 2** Harsh developmental conditions can negatively affect adult survival. Shown here as an example are the survival curves of three rural Gambian villages monitored from 1949–1994 (N=3102 births and 1077 deaths). The ‘hungry’ season refers to the wet season when food reserves are depleted (Moore et al. 1997). At the age of 45, individuals born during the ‘harvest season’ (solid line) had a survival 20% higher than those born during ‘hungry season’ (dashed line). Data from Moore et al. (1997).
In our model species, we manipulated the developmental conditions by experimentally manipulating brood size. In zebra finches developmental conditions affect survival in adulthood and, and other aspects of the phenotype that can be interpreted as being important for adult health (de Kogel 1997; Alonso-Alvarez et al. 2006; Griffith and Buchanan 2010; Holveck and Riebel 2010). During development, my collaborators and I performed brood size manipulations by cross-fostering chicks to either small or large broods (as in de Kogel 1997). Chicks that grow up in large broods show increased costly begging and diminished food reward relative to those from small broods (Kilner 2001; Neuenschwander et al. 2003; Kim et al. 2011; Redondo et al. in press; also in our study system: Box A). Hence, one consequence of growing up in large broods is that chicks are exposed to increased foraging costs. Chicks that grew up in large broods showed impaired growth (Box A), a result in concordance with previous studies (Griffith and Buchanan 2010). I therefore interpret large broods as a harsh developmental condition. Despite this, the brood size manipulation did not affect survival until adulthood, nor did we find an effect on adult survival (Chapter 3). The harsh developmental conditions generated by the brood size manipulation did thus impinge on development but not on survival in our zebra finches. While this may seem surprising at first, it is certainly not the only negative result of harsh developmental conditions on adult survival. In the blue footed boobies (Sula nebouxii), parents sometimes raise two young, and the second young suffers aggressive subordination and food deprivation relative to the first born young. However, both young have similar recruitment and survival rates (Drummond et al. 2011). Similarly, and in contrast to the aforementioned cohort studies in humans, there are various other studies that showed that human infants born in malnourished cohorts have similar juvenile and adult survival as those born just before or after the famine period (Kannisto et al. 1997; Lumey et al. 2011). These studies suggest that harsh developmental environments do not (always) produce the expected long lasting negative consequences on adult survival, including in our captive zebra finches.

Environmental conditions during adulthood

The environment during adulthood can also affect lifespan. For example, hard work during adulthood, which can be manipulated by increasing reproductive effort, can shorten lifespan (Santos and Nakagawa 2012; Boonekamp et al. 2014). Adult survival can thus be affected by the environment during development and in adulthood. At this point, the association between environment and lifespan can become more complex. Does a manipulation during adulthood, for example the increase in reproductive effort above, affect all individuals equally? Or are some individuals more sensitive than others the challenges during adulthood? There are two contrasting predictions on this matter. One scenario is that individuals from benign developmental conditions always perform
at least as well or better during adulthood relative to those from harsh developmental conditions, independent of the adult environment (the ‘silver spoon hypothesis’; Fig. 3A; Grafen 1988). However, developmental conditions may not only constrain, but can also lead to differential developmental, potentially adaptive, trajectories (Gilbert 2001; West-Eberhard 2003). In such cases, harsh developmental conditions could prepare individuals to specific challenges during adulthood, a ‘predictive adaptive response’ (PAR; Fig. 3B; Gluckman and Hanson 2004; Hanson and Gluckman 2014). However, when the developmental and adult environments do not match, these types of developmental adjustments may have negative consequences, for example on adult health and lifespan. Such mismatches have been suggested to be the at the root of health problems such as insulin resistance, type 2 diabetes mellitus and other components of the metabolic syndrome (Gluckman and Hanson 2004; Hanson and Gluckman 2014). Thus, the long-term effects of developmental conditions on adult lifespan and health may depend on the environmental conditions encountered during adulthood.

**Fig. 3** Schematic illustration of two scenarios showing how the long term effects of developmental conditions on adult fitness may depend on the environment during adulthood. Panel (A) illustrates a silver spoon outcome, i.e. individuals from benign developmental conditions always outperform individuals from harsh developmental conditions (Grafen 1988). Note that here we drew lines in parallel, but that according to the silver spoon hypothesis the advantage of benign over harsh developmental conditions need not be as big over the whole range of adult conditions. Panel (B) illustrates a match-mismatch scenario or predictive adaptive response (Bateson et al. 2004; Gluckman and Hanson 2004; Hanson and Gluckman 2014). In this scenario developmental conditions guide development such as to better prepare individuals to certain challenges during adulthood. These developmental adjustments however result maladaptive when there is a mismatch between the developmental and adult environment.

In the aforementioned cohort examples, harsh developmental conditions yielded low quality phenotypes that had shorter lifespans, suggesting that these were ‘silver spoon’ responses. However, many of these studies used only one type of environment during adulthood. For the studies in which the environment did vary, in the field for instance,
developmental and adult conditions are still likely to be correlated, for example because individuals that grew up in poor quality territories are more likely to settle in poor quality territories (van de Pol et al. 2006), or because of temporal correlations of environmental variables. Therefore, the ability of the majority of the previous studies (see below for exceptions) to distinguish ‘silver spoon’ effects from predictive adaptive responses scenarios is limited. The most robust approach to distinguish between these alternatives involves an independent experimental manipulation of the environment during development and in adulthood in a crossover design. To the best of our knowledge, there are only a few such experiments that have tested such effects on lifespan and most of these have failed to find interaction effects (Taborsky 2006; Barrett et al. 2009; Zajitschek et al. 2009; Auer 2010; Dmitriew & Rowe 2011; but see Saastamoinen et al. 2010). Unfortunately, all these studies used species with indeterminate growth and/or with developmental phases of flexible duration (i.e. insects and one study in fishes). Species with such developmental patterns can mitigate effects of harsh developmental conditions in ways that are not available to species with determinate growth, such as birds and humans. Thus to what extent lifespan is subject to match-mismatch versus silver spoon effects is unknown for species with determinate growth.

To test this I expanded upon the usual experimental manipulation of developmental conditions and added a foraging cost manipulation during adulthood in a full factorial (2x2) design. This expansion of the experimental design is interesting because manipulations of developmental conditions like ours are often used (Griffith and Buchanan 2010), but they always involve only standardized adult housing conditions. Furthermore, earlier studies have followed individuals until early adulthood only (e.g. one year de Kogel 1997) or allowed birds to reproduce (Alonso-Alvarez et al. 2006), possibly masking effects of developmental conditions on survival due to trade-offs between lifespan and reproduction (Santos and Nakagawa 2012; Boonekamp et al. 2014). Here, my collaborators and I monitored over 500 individual birds for up to 8 years (Chapter 3: Table 1) in conditions where they could never reproduce, and we exposed them to different experimental conditions until their natural death. We chose to manipulate foraging costs, defined as flight costs per food reward, because we believe that free-living animals often experience it (Koetsier and Verhulst 2011) and because it can have major effects on lifespan and/or reproduction. For example, in natural populations, foraging costs are manipulated by food supplementation experiments. It is generally thought that food supplementation increases survival and fecundity (Martin 1987; Boutin 1990) and several studies in birds and mammals have confirmed this (reviewed in: Robb et al. 2008; Prevedello et al. 2013; Ruffino et al. 2014). However, food supplementation interacts with other ecological factors such as population density,
For example, an increase in food availability is likely to reduce starvation risk but also reduces exposure to predators due to, among other things, a reduction in foraging time. Therefore, increased food availability could affect survival primarily through an effect on predation rate, with a negligible contribution of food intake *per se* (McNamara and Houston 1987). Perhaps because of these ecological confounds, a recent meta-analysis on 148 food supplementation experiments in small mammals found on average no effect of food supplementation on survival (Prevedello et al. 2013). Similarly, in birds, the association between food supplementation and adult survival remains debated (Robb et al. 2008). The complexity of the association between food availability and survival is further illustrated by the finding that dietary restriction in laboratory animals generally increases lifespan (Nakagawa et al. 2012). Thus, foraging costs are an ecologically relevant variable to manipulate, but the effects in isolation on survival in natural populations remain an open question.

In chapter 3 we thus investigated whether foraging costs shortened lifespan. We indeed found that high foraging costs shortened lifespan, but only for individuals that had grown up in large broods (Chapter 3: Fig. 1). The difference in lifespan was considerable: an approximately six months shorter life expectancy relative to an average life expectancy of approximately 3 years, which is a difference of 17%. These results thus show that the effect of high foraging costs on lifespan is conditional and only detectable in individuals that grew up in poor environments. Similarly, these results also show that the effect of developmental conditions on lifespan is conditional on the quality of the adult environment: birds from large broods suffer a shorter lifespan only when they are facing high foraging costs. These results can be put in the context of the contrasting predictions of the silver spoon and PAR scenarios. In our study, we found that birds from benign developmental conditions performed as well as or better than birds from harsh developmental conditions, and therefore our results are more consistent with the predictions of the silver spoon than the PAR scenario (Fig. 3). These results are in contrast with the many food supplementation in free living animals, which did not find an effect on survival (Prevedello et al. 2013) also indicate that, when everything else being equal, food supplementation can increase survival. Interestingly, our results are in contrast with dietary restriction results, which increases lifespan in model organisms in laboratory environments. We should note however that in laboratory rodents, dietary restriction is often applied by decreasing food intake. This is in sharp contrast with foraging costs manipulations, because both type of manipulations can have very different effects on the size and allocation of the energy budget (Carvalho et al. 2005; Wiersma et al. 2005; Schubert et al. 2008).
Population level dynamics in mortality and reproduction

Because individuals die only once, the dynamics of lifespan and mortality are population level phenomena. At this level, the parameters that are typically quantified are median lifespan or life expectancies, which we used when comparing groups that had experienced different environmental conditions (Chapter 3: Table 2). Unfortunately, these parameters capture only one time point and this may be insufficient when the differences between groups change with age. A useful mathematical approach to capture changing dynamics with age was developed by Benjamin Gompertz in 1825 (Gompertz 1825). In the Gompertz function \( M_t = Ae^{Bt} \) or, in the notation we use, \( \log(M) = \log(A) + Bt \), the force of mortality at time \( t \) \( (M_t) \) is a function of an age independent parameter \( A \) (baseline mortality rate) and increases exponentially with age according to the parameter \( B \) (actuarial senescence or aging rate). The Gompertz function shows that differences in lifespan between groups or populations can arise from two mutually non-exclusive reasons (Fig. 4). Populations may differ in the probability of dying at young age, which is reflected in a change in the baseline mortality rate. In addition, populations may differ due to a faster increase in mortality rate with age, which will be reflected in actuarial senescence. This difference is important, for example with regard to interventions that alter lifespan (Partridge et al. 2005). When an intervention changes lifespan through age independent mortality rate (Gompertz \( A \)), the effect is immediate. However, when an intervention changes lifespan through actuarial senescence (Gompertz \( B \)), the effect is cumulative. In such cases, applying the intervention will not change mortality abruptly. Instead, the intervention needs to be applied over longer time periods in order for differences in mortality to appear clearly (Partridge et al. 2005). Considerable research is devoted to finding via which parameter(s) a treatment affects lifespan. For example, dietary restriction in invertebrates extends lifespan via changes in Gompertz \( A \) (e.g. Mair et al. 2003; Nakagawa et al. 2012). In contrast, in laboratory rodents, dietary restriction changes lifespan via decreases in Gompertz \( B \) (Simons et al. 2013). These results suggest that in rodents, dietary restriction changes lifespan by slowing a cumulative process, while this seems not to be the case in invertebrates. Thus changes in lifespan may occur via distinct processes, either immediate and/or cumulative, and the Gompertz model is a useful tool in distinguishing these processes.

In chapter 3 we used this approach to distinguish whether the effect of our manipulations on lifespan arose via age-independent or age-dependent changes in mortality. We showed that our environmental manipulations increased mortality immediately and thus shorten lifespan via an increased age independent effect ‘\( A \)’ and despite diminished actuarial senescence ‘\( B \)’ (Chapter 3: Fig. 2). We also found that zebra finch females live shorter lives than males. This effect, however, arose cumulatively via actuarial
senescence ‘B’. Thus our environmental manipulations shortened lifespan via an age-independent effect on mortality, but the sex difference in lifespan arose because females aged faster (demographically, see below for explanation) than males.

Natural selection acts on individual contributions to future generations. Therefore, reproduction is an essential aspect of an individual’s life history. In the above studies, we have focused on lifespan, and the birds involved were not allowed to reproduce. This has the advantage that environmental effects on lifespan can be tested with less interference due to other life history traits such as reproduction. However, this may limit the ecological suitability of our foraging cost manipulation. In order to investigate whether birds facing our environmental manipulations were at all able to reproduce, we thus carried out two short-term breeding experiments. One study was carried out in autumn and winter, and we found that in the harsh treatment birds did not lay any eggs, while their benign counterparts readily did (Simons et al. 2014). We then carried out a follow-up study during spring (Chapter 4). We found that birds from both treatments readily reproduced, but that in the harsh treatment brood size was reduced and the young experienced a higher mortality. Thus birds in the high foraging cost manipulation could reproduce, but there were effects of seasonality on reproductive behavior and high foraging costs impaired chick development and survival.

The Gompertz model was developed a long time ago (published in 1825). Since then, many other equations have been developed to capture the dynamics of mortality (for an overview, see Colchero et al. 2012). Nevertheless, the Gompertz equation captures demographic patterns well and in many instances outperforms other demographic
models in terms of its fit with the data\(^4\), and this was also for our population (Chapter 3). However, the Gompertz and many other demographic models are limited in a biological sense: they are descriptive and therefore fail to include the underlying biology. This hampers connecting demography with the study of aging at the mechanistic or organismal level. A first step towards bridging this gap was developed by Gavrilov and Gravilova in 2001. They developed a demographic model using a bottom up approach: utilizing the concept of redundancy of elements and how these elements fail with age, demographic patterns emerge from their model (Gavrilov and Gavrilova 2001). Redundancy remains an abstract concept, but one way to think of it in biological terms is as an organ with redundancy being (the number or the functioning of) the cells in that organ. Redundancy decreases following a certain failure rate until it is depleted and the organ or organism dies. The redundancy model of aging has rarely been fitted to actual data (for exceptions see Boonekamp et al. 2013; Vural et al. 2014) and its fit has rarely been compared with that of other demographic models. In chapter 5 we make a first attempt to fit a mechanism-based demographic model on mortality data. We show that the redundancy model can fit demographic patterns well and in some cases even better than the traditional Gompertz model. Furthermore, we show that some common interventions that extend lifespan (dietary restriction and lowering ambient temperature) can be interpreted in terms of parameters of the redundancy model. For example, lowering ambient temperature increased lifespan in the fruitfly *Drosophila* through reductions in actuarial senescence (Mair et al. 2003). Following a simplified version of the redundancy model, increases in lifespan can be achieved by reducing failure rate, by increasing redundancy, or both. Fitting the redundancy model to the data indicated the role of failure rate while redundancy remained unchanged. Physiologically, this points towards a decrease in the production of physiological damage rather than a change in organismal strength or resilience. While using demographic changes to identify certain physiological processes remains a challenge, we hope that this study will motivate others to including mechanistic processes into demographic models.

Mortality not only changes with age, but also in response to extrinsic variables such as climate (Coulson et al. 2001). In general, when studying the biological consequences of climatic variables, the considered timescales are long, typically weeks, months or years (Stenseth et al. 2002; Parmesan 2006; Grosbois et al. 2008; Lawson et al. 2015). However, global warming is also associated with changes in climatic variability over much shorter time scales of typically days (Vose et al. 2005; Wang and Dillon 2014). For

\(^4\) Note however that the Gompertz model cannot explain late-life mortality plateaus, i.e. that among the oldest individuals, mortality rate remains constant with age (Carey et al. 1992). This demographic phenomenon can, however, be captured by the redundancy model of aging (Chapter 5: Fig. 2).
example, the diurnal temperature range (DTR), i.e. the difference between minimum and maximum temperature within one day, has increased with more than 2 °C since the 1960’s in Mexico, Bolivia, Patagonia, Madagascar, Indonesia, central Russia and the Western Himalaya (Yadav et al. 2004; Englehart and Douglas 2005; Jhajharia and Singh 2011; Wang and Dillon 2014). The demographic consequences of such changes for ectotherms are currently under investigation (Paaijmans et al. 2010; Raffel et al. 2012; Paaijmans et al. 2013; Vasseur et al. 2014; Zeh et al. 2014), but the consequences for endotherms are not yet known. In chapter 6 we address this by investigating whether DTR affects mortality in zebra finches. We find that an increase of 1°C in DTR can cause up to a twofold increase in mortality in zebra finches. This shows that temperature variability on short time scales can have a major impact on endotherm populations. This is to our best knowledge the first report of such an effect in endotherms, and we therefore believe that changes in short-term variability of climatic variables should be taken into account when estimating the possible consequences of climate change.

In our experimental set-up, however, the effect of DTR on mortality depended upon environmental quality. DTR increased mortality on days with low minimum temperature when foraging costs were low, but on days with high minimum temperature when foraging costs were high (Chapter 6: Fig. 4). This difference is important for two reasons. First, low foraging costs typically reflect a laboratory type of environment, while high foraging costs are typically encountered in more natural environments. Therefore, these results show that the effects of climatic variables can differ between a laboratory and a (semi-)natural environment, and thus highlight that testing the ecological consequences of climatic factors should be done in environments as natural as possible. Secondly, in the semi-natural environment, DTR decreased mortality on days with high minimum temperature. Global warming is associated with increases in minimum temperatures (Vose et al. 2005), and thus DTR effects will become increasingly important in a warming world.

**Individual aging**

In the population-level section, we focused on changes in lifespan and the dynamics of death. There we encountered a demographic quantification of aging or senescence, i.e. actuarial senescence or the increase in mortality rate with age. Aging, however, is more often referred to functionally as a decline in organismal functioning with age associated with decreases in fecundity and survival probability. Aging thus becomes a characteristic of individual functioning and we here further consider aging in this sense. Aging is a ubiquitous phenomenon, common in humans, model organisms and in the wild (Nussey et al. 2013; Belsky et al. 2015; Fontana and Partridge 2015). Aging is followed by death
and thus both processes (aging and death) are inevitably linked. However, aging is different from lifespan in that it explicitly refers to the decline in organismal functioning preceding death. Thus two individuals with the same lifespan can experience aging phases that differ in duration or intensity (Ricklefs 2010). Therefore lifespan and aging can be distinct phenomena (Williams 1999).

Because lifespan and aging are inherently linked, it is often assumed that both processes are consistently affected by the same factors (Williams 1999). For example, we may predict that longer-lived individuals may age later or at a slower pace. However, in humans, life expectancy has increased continuously since the 19th century, but it remains unclear to what extent this increase is accompanied by delays in aging (Christensen et al. 2009). Secondly, studies on model organisms in laboratory environments have shown that caloric and dietary restriction extend lifespan and can delay the onset of age-related pathologies such as type 2 diabetes, cancer and neurodegenerative diseases (Speakman and Mitchell 2011; Fontana and Partridge 2015). However, there are various examples in these same systems, showing that lifespan and aging can readily be uncoupled (Burger et al. 2007; Rueppell et al. 2007; Burger et al. 2010; Bansal et al. 2015). Thus, the assumption that aging and lifespan are in synchrony and affected by the same factors remains to be investigated (Williams 1999; Christensen et al. 2009; Kennedy et al. 2014; Bansal et al. 2015).

We therefore investigated the aging of individual zebra finches exposed the aforementioned brood size and foraging cost manipulations as part of section III of this thesis (Chapters 7-11). We then associated the above found experimental effects on lifespan with those on aging. Our starting hypothesis was the common assumption that the experimental group with the shortest lifespan aged fastest. The redness of the zebra finch bill (Chapters 7 & 8) is, in this context, a useful trait to study because it is a carotenoid-based sexual signal and therefore is expected to indicate individual ‘quality’ or ‘physiological state’ (Pérez-Rodríguez 2009; Simons et al. 2012). In brief, theory predicts that such costly signals can evolve when they are used as indicators of quality in mate choice (Zahavi 1975; Grafen 1990; Kotiaho 2001), as is the case for zebra finches (Simons and Verhulst 2011). In zebra finches it has, however, been suggested that bill color might be a poor indicator of quality in females (Price and Burley 1994). We thus first investigated to what extent the redness of the bill is an indicator of quality in male and female zebra finches, and found that males and females with redder bills live longer and reproduce more (Chapter 7). These results thus show that for the zebra finch bill, redder is ‘better’.
This cross-sectional association between bill redness and survival can arise because of two mutually non-exclusive explanations. On the one hand, it is possible that individuals with redder bills have an advantage over others at young age, creating a between individual change in population composition with age, i.e. selective disappearance. On the other hand, the cross-sectional association with survival might be due to aging, because bill color deteriorates with age within individuals. To separate between the contributions of these two processes, we collected longitudinal data of bill coloration. Between individuals we found that intermediate bill color lead to the longest lifespan, i.e. stabilizing survival selection. Within individuals, we found that bill color is maintained throughout life until a terminal decline in the last year before death (Fig. 5). Thus bill color showed aging. More generally, these studies illustrate the importance of using longitudinal over cross-sectional data when studying trait aging and their association with lifespan. That is because in the cross-sectional data (Chapter 7), we had found that individuals with the reddest bills live longest. However longitudinal data (Chapter 8) correctly show that this conclusion is confounded by within individual change and that individuals with intermediate redness lived longest. Thus, studying the individual aging and predicting lifespan is best done with longitudinal data, which is the approach used here below (Fig. 5).

Fig. 5 Mosaic aging in zebra finches. Shown here is a schematic representation of the age trajectories for five traits longitudinally quantified in this study. Individuals with high body mass lived longer than those with low body mass, but within individuals most birds showed a quadratic association with age. For BMR and SMR there is no selective disappearance. Within individuals, BMR linearly declined with age, while SMR increased until final year. For hematocrit, we did not find any evidence for age associated changes or selective disappearance. Bill color shows stabilizing survival selection before the terminal decline in the final year. Analyses are based on more than 20,000 measurements on 597 individuals monitored for up to eight years.

Evolutionary theory predicts that traits within one organism should age in synchrony (Williams 1957; Maynard-Smith 1962). The rationale behind this is that any trait that causes early death should be selected against, while there might be little benefit in investing in perfect trait functioning until ‘after death’. Unfortunately, this rationale
may well be overly simplified. Intuitively at least, it may seem likely that there are trait specific associations between age and survival and/or reproduction. This may arise for instance when an individual might benefit from altering its behaviour, physiology or life history decisions during late adulthood (McNamara et al. 2009). Various studies have indeed shown that an organism experiences heterogeneous declines in functioning with age between traits, tissues and cells, a phenomenon coined ‘mosaic aging’ (Herndon et al. 2002; Cevenini et al. 2008; Walker and Herndon 2010; Baris et al. 2015; Hayward et al. 2015). For example, in Drosophila, muscular functioning shows profound declines in functioning with age, whilst the functioning of nervous system appears age-independent (Herndon et al. 2002). Thus, within one organism, traits differ in how they change with age and the origins of this mosaic remains poorly understood.

Furthermore, traits can age following various shapes, with declines being gradual, accelerating or terminal, i.e. be triggered by time before death rather than age per se. We further call the shape of how a trait changes with age during adulthood the ‘age trajectory’. Note that age trajectories can be distinct from aging because traits can improve with age especially during early adulthood, for which there are many examples (e.g. Rebke et al. 2010; Robinson et al. 2012). For example, in wild mammals a variety of age trajectories have been described for mass: quadratic associations with a maximum in bighorn sheep Ovis Canadensis (Nussey et al. 2011), accelerating declines in Roe deer Capreolus capreolus (Nussey et al. 2011), terminal declines in Soay sheep Ovis aries (Hayward et al. 2015), accelerating and terminal declines in European badgers Meles meles (Beirne et al. 2015) and in male Alpine marmots Marmota marmota (Tafani et al. 2013). The origins of the between-species variation in these age trajectories remain unknown. Actually, even less is known about the level of biological organization at which variation in age trajectories should be described. Is a trait’s age trajectory fixed for a certain species or is it amenable to environment variation?

We thus investigated the age trajectories of several traits in zebra finches. Because the foraging cost manipulation changes an individual’s energy balance, we chose to quantify a series of traits that are known to be affected by energy intake or energy turn-over. We started with mass and found that mass showed a quadratic age trajectory in males that is independent of our environmental manipulations. Quadratic age trajectories for mass have been described previously in humans (reviewed in Kuk et al. 2009) and in laboratory rodents (Yu et al. 1985; Murtagh-Mark et al. 1995; Turturro et al. 1999; Miller et al. 2002). Laboratory rats however also show terminal declines a few weeks before death (McDonald et al. 1996; Black et al. 2003). In females, we found a quadratic age trajectory for mass in the benign foraging environment, but a linear mass age trajectory
in the harsh foraging environment, the slope of which depended upon the developmental conditions. For females from benign developmental conditions, mass increased linearly with age, while females from harsh developmental conditions showed the opposite pattern. These results thus show that the age trajectory of mass is not fixed, but subject to environmental variation, extending back as far as during development.

We then investigated energetic expenditure. Basal metabolic rate (BMR) is the minimum energy expenditure of a post-absorptive adult animal measured during the rest phase at thermoneutral temperatures (IUPS Thermal Commission 2001). Standard metabolic rate (SMR) is the same as BMR, except that the animal is at a temperature below the thermoneutral zone, and hence SMR includes energy for thermoregulation. At first it may seem redundant to measure energy consumption at two different ambient temperatures, because these two measures will likely be correlated. In chapter 10 we investigated this correlation. To do this we first needed to know to what extent these traits characterize an individual. This is done by quantifying the repeatability, i.e. proportion of total phenotypic variance that is caused by between individual variance (Falconer and Mackay 1996). Both BMR and SMR were repeatable over a period of years (r~0.3), showing that individuals can be characterized based on these traits. However, the correlation between the traits was poor (0.14<r<0.22), and thus BMR and SMR characterize different traits within an individual.

Once we knew that BMR and SMR are different traits, we could study aging of various components of the organism. In chapter 11, we quantified the age trajectories of BMR and SMR. We found that BMR declined with age (Fig. 5), and this is consistent with what was found other studies in birds and mammals (Elliott et al. 2015). In contrast to BMR, SMR increased with age until the terminal year (Fig. 5). This is new, and to our best knowledge, the first description of an SMR age trajectory. Thus BMR and SMR, two metabolic traits that quantify energy consumption and differ solely in the ambient temperature at which energy is consumed, age independently and in opposite directions. These results indicate that the aging of basal energy production is distinct from that of insulation and/or thermoregulation. For hematocrit, blood oxygen stores which can be important for metabolic activity (Petit and Vézina 2014), we found no evidence of any change with age, despite a high lifetime repeatability (r~0.6). Together, the above results show that different components the zebra finch organism age at different rates and follow a variety of age trajectories. Thus zebra finches show mosaic aging.

5 This refers to the phenotypic correlation. We note that here this weak phenotypic correlation was not due to the repeatability of ~0.3: correlation between SMRs at various ambient temperatures can be as high as 0.9 (see chapter 10 for further explanation).
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In our study, aging responses to our environmental manipulations were trait specific. Therefore, environmental factors affecting lifespan should be considered distinct from those that affect aging, which is similar to what has been suggested for genetic factors (Burger and Promislow 2006). Predicting for which traits an environmental variable that alters lifespan will also affect aging is currently difficult. One determinant factor is the shape of the age trajectory. For traits showing terminal declines, environmental factors that shorten lifespan will likely accelerate aging, as we found for SMR and for bill coloration. However, this assumes that the environment does not alter a trait’s age trajectory, which was the case here for most traits except for mass. Therefore, the association between lifespan and aging is trait specific and depends on a trait’s age trajectory, the environment and their interaction. I therefore believe that studying the age trajectories of a variety of traits is a fruitful approach to understanding the dynamics of aging, the factors affecting aging and the association between aging and lifespan.
References


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