

# Species Selection in Comparative Studies of Aging and Antiaging Research

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*A great range of life histories are observed among mammals. Understanding why different species age at different rates may provide clues about the mechanistic and genetic basis of aging. This work focuses on animal species and their use in comparative studies of aging. Firstly, I debate how to compare aging across different species, including physiological parameters and statistical analyses. Afterwards, a selection of species for use in the comparative biology of aging is suggested. Taking into account that the ultimate aim of research on aging is to benefit people, my selection is largely based on primates. Primates feature such a vast range of aging phenotypes that comparative studies of aging need not employ other more distant species, except perhaps a few rodents. A number of animal species that may serve as models of antiaging strategies are also presented. Examples include species that appear not to age, such as some turtles, and animals featuring regenerative mechanisms absent from humans. Studying the genetic and molecular basis of these traits may have potential implications for antiaging research. Sequencing the genome of these species should then be a priority.*

## Introduction

Different species of animals age at radically different paces. A salmon will degenerate and die within days after spawning while some turtles, studied for decades, do not show signs of aging. Among mammals too a great range of life histories are observed. Even under the best laboratory conditions a mouse (*Mus musculus*) will not live past its 5th birthday, the oldest person on record—Jean Calment—died at age 122, but a bowhead whale (*Balaena mysticetus*) may live over 200 years (George *et al.*, 1999). Equally impressive, similar species tend to show great differences in longevity and aging rates. Among primates, for instance, while humans may live over 100 years, dwarf and mouse lemurs do not commonly live more than 20 years and, in their second decade of life, show age-related pathologies typical of elderly people.

Despite great differences in lifespan, the aging phenotype is remarkably similar across mammals (Finch, 1990; Miller, 1999). For example, aged (8–11 year-old) mouse lemurs (*Microcebus murinus*) show senile plaques comparable to those witnessed during human cerebral aging (Bons *et al.*, 1992). Consequently, the principle behind the comparative biology of aging is that studying why different species age at different rates may provide clues about the mechanistic basis of aging. Moreover, identifying which genetic factors determine the pace of aging in mammals could open the possibility of delaying aging and/or age-related diseases in people.

The focus of this work is on animal species and their selection for use in comparative studies of aging. Recently, we developed AnAge, an aging-oriented database featuring over 3,000 animals (de Magalhaes *et al.*, 2005a). All species mentioned in this chapter are featured in our database, and hence additional information and references are available in AnAge. My aims in this chapter are to (1) briefly discuss how to compare the aging process between different species; (2) provide a selection of species for comparative studies of aging; and (3) suggest species that may be models of antiaging strategies. Importantly, the purpose of biomedical research is to improve the human condition. The goal of gerontology is to preserve human health and well-being, and extend healthy life. Consequently, the choice of models employed by researchers must always have humans as the ultimate goal, and this is reflected in this work.

Although the focus of this chapter is not on how to perform comparative studies of aging, but rather on which species to employ, the selection process must take potential experiments into consideration. Numerous parameters may be studied between species with different rates of aging: DNA repair rates, cellular stress resistance, antioxidant concentrations, and many others (Finch, 1990). Some of these experiments require captive populations of the species under study, which may not be readily available and might even be impossible to obtain. Although *in vivo* studies may be highly informative, these may be difficult to conduct in many of the species mentioned herein—such as in bowhead whales. Modern high-throughput technologies like genomics, however,

do not require captive populations or in vivo studies. Furthermore, cellular studies have been proposed as a means to study long-lived species (Austad, 2001; de Magalhaes, 2004), such as stem cells differentiating in culture. Therefore, intact organism studies may not be necessary in comparative biology, particularly since I predict that comparative genomics will become a major tool for comparative studies of aging (de Magalhaes and Toussaint, 2004). My choice of species hence does not take into account potential husbandry costs and difficulties.

## Measures for Comparing Aging

In this work, lifespan is defined as the period of time in which the life events of a species typically occur. So far, I have been mostly referring to maximum lifespan (*t<sub>max</sub>*) as a means to compare aging among different species. There are multiple problems, however, in using *t<sub>max</sub>* as an estimate of aging. For example, feral animal populations may have their *t<sub>max</sub>* limited by predation, accidents, or starvation. Even differences in *t<sub>max</sub>* in captivity may reflect husbandry difficulties, and several species are impossible to maintain in captivity. Therefore, and since *t<sub>max</sub>* is not the only way of comparing aging, it is worthwhile to consider how aging rates can be compared across species before selecting them. After all, since aging is one of the variables under study in comparative studies of aging, we must at least roughly quantify the rate of aging if we are to design appropriate experiments. As an example, I will examine the closest human relative, chimpanzees (*Pan troglodytes*). Chimpanzees live a maximum of 73 years while humans live 122 years, so *t<sub>max</sub>* suggests that chimpanzees age about twice as fast as humans.

## PHYSIOLOGICAL AGING

Aging can be defined as an age-related decline in physiological function (Austad, 2005). Studying aging in model organisms may include numerous anatomical, physiological, and biochemical age-related changes (Finch, 1990). Consequently, one way to determine rate of aging is to study the pace of age-related changes and/or the onset of age-related pathologies (Miller, 2001; de Magalhaes *et al.*, 2005b). This is arguably the most accurate and informative way of studying aging in a given species.

Briefly, the basic aim of physiological studies in aging animals is to investigate typical human age-related changes. These include the major human killers in old age: cancer, heart and neurodegenerative diseases. In fact, while it is not a measure of aging, it is informative to know what animals die of, particularly in captivity where the effects of accidents and predation are minimized. Determining common causes of death for model organisms is insightful regarding the onset of aging and regarding which age-related pathologies in a given

animal model are similar to those seen in people. Equally relevant are reproductive changes with age, which include testis and ovary changes as well as the onset of reproductive senescence such as age of menopause. Many age-related changes and pathologies can also be studied and compared to human aging. A few parameters of interest include, but are not limited to, fat deposits, hormonal levels such as those of growth hormone, insulin and insulin-like hormones, and dehydroepiandrosterone (DHEA), atherosclerotic lesions, osteoporosis, arthritic changes, changes in reaction times with age, changes in senses, and the presence of cerebrovascular  $\beta$ -amyloid protein (Finch, 1990). There are also examples of comparative studies aimed at specific age-related pathologies, and readers should consult other chapters in this book.

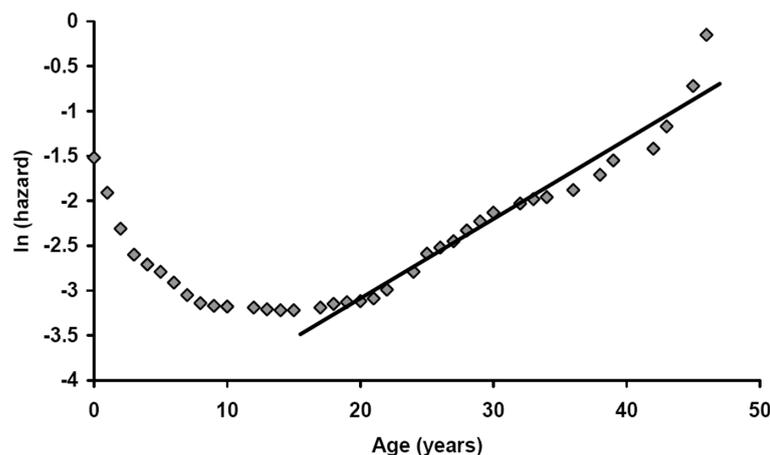
In the case of chimpanzees, physiological deterioration appears to occur at earlier ages than in humans (Finch, 1990; Hill *et al.*, 2001; Erwin *et al.*, 2002). This is obvious in, for instance, bone aging: chimpanzees generally develop bone aging—such as fractures and loss of bone density—at earlier ages than people do (Morbeck *et al.*, 2002). Chimpanzees also show tooth erosion at earlier ages than humans (Hill *et al.*, 2001). While chimpanzees, in general, appear to show signs of aging at earlier ages than humans, little is known about the pace of aging in chimpanzees, some age-related changes typical of humans may not occur faster in chimpanzees. For example, cancer rates do not appear to be higher in chimpanzees, though little is known about age-related cancer rates in chimpanzees (Erwin *et al.*, 2002).

## DEMOGRAPHIC AGING

Changes in physiological parameters are interesting for comparative studies of aging but they are potentially expensive and difficult to study. One alternative is to employ demographic measurements of aging. Aging can also be defined as an age-related increase in vulnerability and decrease in viability (Comfort, 1964). One of the features of aging in species with gradual senescence, like most mammals, is an exponential increase in mortality after maturity. For example, in humans, our chance of dying roughly doubles every 8 years after about age 30. This is remarkably similar among different human populations, independently of average lifespan (Finch 1990). Therefore, one way to compare rates of aging across different species is to calculate the rate at which mortality increases with age, which gives a measure of senescence (Pletcher *et al.*, 2000). As an example, Figure 2.1 shows the hazard function—which represents the probability of death—of chimpanzees according to age based on published mortality rates (Hill *et al.*, 2001).

In chimpanzees, hazard rates begin to increase near the end of the second decade of life, while human hazard rates generally begin to climb at the end of the third decade of life. This time it takes for mortality rates to climb has also been suggested as a measure of aging (Finch, 1990),

## 2. Species Selection in Comparative Studies of Aging and Antiaging Research



**Figure 2.1.** Natural logarithm of chimpanzee mortality rates as a function of age. The straight black line represents the estimated adult mortality trajectory based on Gompertz parameters calculated using a weighted linear regression. The data comes from five field studies of chimpanzees (Hill *et al.*, 2001) and was fitted using the T4253H smoothing algorithm from the SPSS package (SPSS Inc., Chicago, IL).

and in this case suggests aging commences earlier ages in chimpanzees than in humans. To calculate the age-related increase in mortality, the Gompertz function is typically used. Although other functions have been proposed (Wilson, 1994), the Gompertz function is generally the most adequate for these calculations, particularly when using small populations as is common in studies of higher vertebrates. It is also the most widely used function, making it a good term for comparisons. The Gompertz equation is  $R_m = R_0 e^{\alpha t}$  where  $R_m$  is the chance of dying at age  $t$ —i.e., the hazard rate— $R_0$  is the nonexponential factor in mortality, and  $\alpha$  is the exponential parameter. Based on the Gompertz equation it is possible to calculate the mortality rate doubling time (MRDT), which is an estimate of rate of aging given by  $MRDT = 0.693/\alpha$  (Finch, 1990; Mueller *et al.*, 1995).

Depending on the quantity and quality of the data, there are different ways of calculating the Gompertz parameters (Mueller *et al.*, 1995), and a certain amount of subjectivity is unavoidable. In this case, and as previously described (de Magalhaes *et al.*, 2005b), the weighted linear regression was obtained from the ln-transformed Gompertz equation:  $\ln(R_m) = \ln(R_0) + \alpha t$ . The chimpanzee curve was estimated as:  $\ln(R_m) = -4.56 + 0.0798t$  with  $r^2 = 0.81$ . Hence,  $\alpha = 0.0798$  with 95% confidence intervals of 0.0627 and 0.0969. This means that the MRDT for chimpanzees is around 8–9 years, similar to that of humans.

The great advantage of the Gompertz function and estimating  $\alpha$  and the MRDT is that it allows us to quantify the rate of aging. As shown above, however, MRDT estimates indicate chimpanzees and humans age at similar paces, which may not be true from a physiological level. Our results from rodents also suggest that MRDT is a good but not perfect estimate of rate of aging (de Magalhaes *et al.*, 2005b). Therefore, even though the MRDT is a useful measurement of aging rates,

it should be used in conjunction with physiological observations.

### CONCLUDING REMARKS

While not being measurements of aging, lifehistory traits such as developmental schedules are relevant for comparative studies of aging. For instance, long development in mammals is typically associated with a long adult lifespan, independently of body size (Harvey and Zammuto, 1985). Age at sexual maturity, gestation or incubation time, and litter or clutch size are all important features of animals, particularly in the context of ecology and to understand the evolutionary forces that shape lifespan.

Other estimates of aging have been used such as adult mortality rates, average longevity, and adult lifespan. Adult mortality rates and average longevity did not correlate well with the MRDT or with physiological aging parameters in rodent cohorts (de Magalhaes *et al.*, 2005b). In the context of comparative studies of aging, there is no strong reason to use these estimates rather than maximum lifespan, though maximum adult lifespan may sometimes be more appropriate than  $t_{max}$ . In contrast, the large amounts of  $t_{max}$  data available make it a good term for comparisons.

In conclusion, the most adequate measure of aging is still  $t_{max}$ . Faster aging organisms will not be able to live as long as slower or nonaging species, which will be reflected in  $t_{max}$ . In fact,  $\alpha$  has been shown to correlate with  $t_{max}$  (Finch and Pike 1996). We also recently showed that  $t_{max}$  correlates with MRDT in rodent cohorts (de Magalhaes *et al.*, 2005b). Certainly, there are inherited problems in quantifying aging using  $t_{max}$  and, at least for species with high mortality rates in the wild,  $t_{max}$  should be estimated from captive populations. Nonetheless, while the use of the methods described above is encouraged, particularly descriptions of physiological,

biochemical, and anatomical changes with age,  $t_{max}$  will continue to be the most widely used estimate of rate of aging. In the remaining of this chapter,  $t_{max}$  is commonly used.

### Species for the Comparative Biology of Aging

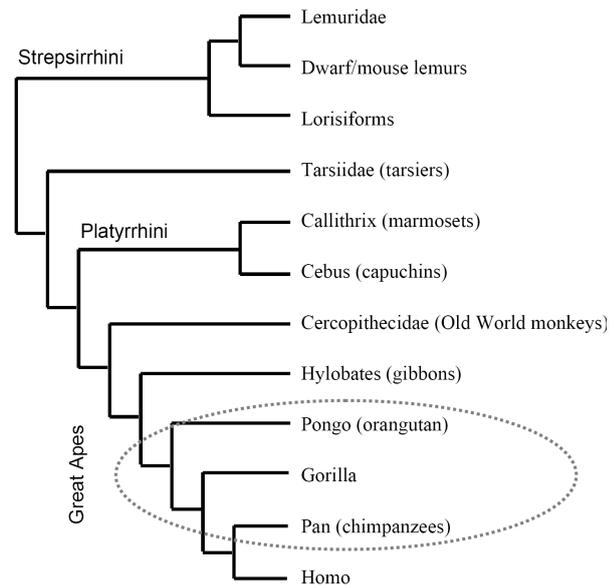
As mentioned above, the ultimate objective of aging research is to benefit people. Consequently, choosing species for the comparative biology of aging must be done having *Homo sapiens* in perspective. Whether model organisms are representative of the human aging process has been debated by many others (Gershon and Gershon, 2000). It is possible that mechanisms of aging are conserved across distant species, and it is possible that they are not (de Magalhaes, 2004). Since there is still no definitive answer to this debate, my position in this work is that species biologically and evolutionarily more distant from humans are less likely to share mechanisms of aging with people, and thus an effort was made to select species closer to humans.

Since among mammals there is a great diversity in the pace of aging, there is no scientific reason to employ nonmammalian species in the comparative biology of aging. On the contrary, incorporating nonmammalian species may lead to the use of species with different biology than humans and thus of more dubious use to understand human aging. In fact, mammals feature unique traits associated with aging such as diphyodont replacement—i.e., two sets of teeth—which is surprisingly common in mammals and is associated with tooth erosion, and a lack of oocyte regeneration, which makes reproductive senescence inevitable in all studied female mammals. These traits suggest that the evolution of aging in mammals may have had unique features (de Magalhaes and Toussaint, 2002). While there may be practical and economical reasons to employ nonmammalian species in aging research, these must be considered as secondary choices and more error-prone than mammalian models.

#### PRIMATES AND RODENTS

If the focus of gerontology is on the human species, then it makes sense for us to select our closest relatives as models of aging, provided these species indeed age differently than humans (Figure 2.2).

As mentioned above, our closest living relative is the chimpanzee, in which aging appears to occur earlier than in humans. Similarly, all the great apes show signs of aging at younger ages than humans, though it is unknown whether they age differently from each other (Erwin *et al.*, 2002). As we move further away from humans and great apes, species tend to be smaller, less intelligent, and shorter-lived. This is apparent among Old World monkeys (family: Cercopithecidae), which are generally shorter-lived than apes. Two species of Old World



**Figure 2.2.** Primate phylogeny highlighting potential models for comparative studies of aging. The Platyrrhini infraorder represents New World monkeys. Phylogeny was drawn based on Goodman *et al.* (1998). Branch lengths are not to scale.

monkeys have already been studied in the context of aging: rhesus monkeys and baboons. Both appear to age considerably faster than humans and great apes, making them potentially useful models for comparative studies of aging. Interestingly, baboons have an MRDT of roughly 4 years (Bronikowsky *et al.*, 2002) while rhesus monkeys appear to have an MRDT not smaller than that of humans (Finch, 1990). Nonetheless, physiological studies suggest that rhesus monkeys age about twice as fast as humans (Finch, 1990; also see Chapter 38 by Roth and colleagues in this book and references in AnAge). Baboons and rhesus monkeys demonstrate how comparing aging rates among different species can be difficult and how the MRDT is not always an accurate estimate of rates of aging.

Among New World monkeys, also termed Platyrrhini, we find species much shorter lived than apes and humans. Marmosets are a good example, such as the common marmoset *Callithrix jacchus*. The record longevity for these animals is little over 16 years, and numerous age-related changes have been reported in their second decade of life (see AnAge for references). They also reach sexual maturity at about one year of age—which is much sooner than apes—suggesting shorter generation cycles, shorter lifespans, and hence in accordance with a faster aging process. Therefore, if our choice of species is aimed at discovering what determines rate of aging among primates, with humans as our ultimate goal, then these shorter lived primates are certainly a good choice. In contrast, some New World monkeys are longer lived, attaining sexual maturity at older ages. Examples include members of the genera *Alouatta*, *Ateles*, *Cacajao*,

## 2. Species Selection in Comparative Studies of Aging and Antiaging Research

and *Cebus*. The white-faced capuchin (*Cebus capucinus*) is a good example with a record longevity of almost 55 years and attaining sexual maturity with at least 5 years of age. Therefore, New World monkeys offer a variety of aging phenotypes suitable for comparative studies of aging. The large variation in rates of aging among such closely related species argues, once again (Miller, 1999), that genetic factors determine rate of aging in primates and makes New World monkeys a valuable source of models of aging.

Moving further away from humans, tarsiers (family: Tarsiidae; genus: *Tarsius*) also appear to be short-lived with short generation cycles (Austad, 1997a). In contrast, a greater diversity is found among Strepsirrhini, one of the two primate suborders (Figure 2.2). Lemurs of the *Lemuridae* genus are relatively long-lived when compared to their closest relatives. The brown lemur can live 37 years, which is impressive considering it reaches sexual maturity at about age 2. It was also reported that a hybrid between a brown and a black lemur lived for 39 years (Jones, 1982). In contrast, dwarf and mouse lemurs (family: Cheirogaleidae) do not live more than 20 years, and age-related changes have been described in their second decade of life (see AnAge). For instance, the fat-tailed dwarf lemur (*Cheirogaleus medius*) has been argued as an example of a fast-aging primate (Austad, 1997a). Lorisiforms such as the slender loris (*Loris tardigradus*) also appear to be short-lived with a fast development (Austad, 1997a), although the slow loris (*Nycticebus coucang*) has been reported to live over 26 years. As in New World monkeys, the Strepsirrhini suborder appears to feature a variety of aging rates.

While there are reasons to focus only on primates in comparative studies of aging (Austad, 1997a), rodents may also serve as a potential models. First of all, mice and rats (*Rattus norvegicus*) are well-established models in biomedical and aging research. Secondly, rodents and primates diverged roughly 58 million years ago (mya), not long before the two primate suborders—Strepsirrhini and Haplorrhini—diverged 49 mya (Springer *et al.*, 2003). Lastly, the short life cycles and fast aging processes of mice and rats have not been observed in any primate. By incorporating rodents and primates, we thus obtain a range of aging rates close to that of the entire Mammalia class. In Table 2.1 I recap all of the species mentioned above.

If we aim to investigate the factors regulating aging in primates having the human species as our priority, then our choice of species need not go further than primates and rodents. As argued before (Austad, 2005), more is learned by the study of closely-related species that differ considerably in the trait of interest. Of course, diversity is always welcomed and other species can be incorporated into the comparative biology of aging. Nonetheless, solely using primates and rodents in the comparative biology of aging may be adequate to determine which genetic factors regulate the human aging rate. Certainly, there are major

difficulties in studying primates, but that depends on which factor is being studied. In the modern age of genomics it may be necessary, not to keep animals in captivity, but rather to have their genome sequenced. It is with this prospect that I suggest these animals as choices for aging research. Hopefully, some of these animals, like short-lived primates, may also be incorporated as experimental models, as suggested before (Austad, 1997a).

### THE INFLUENCE OF BODY SIZE

A number of factors correlate with *t<sub>max</sub>*, and while this work is about species selection, not the methodology of comparative biology, there is one factor that must be mentioned: body size (Promislow, 1993). Clearly, bigger species, including mammals, live longer, on average, than shorter-lived species (Austad, 2005). Exceptions exist and, for example, gorillas (*Gorilla gorilla*) are typically bigger than humans and still do not live longer than us. Likewise, bats live longer than predicted from their body size. Nonetheless, when comparing parameters across species it is crucial to take body size into consideration. Otherwise we could make the mistake of correlating some physiological factor with body size, not with longevity or aging. For example, early studies indicated that DNA repair capacity was higher in longer-lived mammals, arguing that DNA repair was a factor in aging (Hart and Setlow, 1974). Yet it has been argued that the correlation between DNA repair and longevity is due to the fact that bigger animals live longer and, for reasons unrelated to aging, have better DNA repair mechanisms (Promislow, 1994). In other words, the evolution of aging rates and DNA repair may have been related to body size and thus independent from one another. Therefore, body size is a factor that comparative studies of aging must take into consideration. It is necessary that we devise appropriate methods to exclude or at least minimize the impact of body size in such studies, and a careful selection of species may also minimize these problems.

Among primates, longer-lived species tend to be bigger with bigger brains, and hence the problems cited above must also be taken into consideration. One way to minimize these problems is the inclusion of negative controls. For example, pairs of species that age similarly but that differ in body size may be employed: gorillas may age at the same pace as chimpanzees even though the former are considerably bigger. Choosing different species with similar aging processes may then be necessary. Likewise, choosing species that live longer than expected for their body size is important: the white-faced capuchin is a good example of a relatively small primate with a *t<sub>max</sub>* comparable to that of apes (Table 2.1).

Assuming mechanisms of aging are conserved between rodents and humans, which is debatable in itself, it may be worthwhile to consider other rodents besides rats and mice. For instance, long-lived rodents like porcupines (Erethizontidae and Hystricidae families), which may live over 20 years, and the naked-mole rat (*Heterocephalus*

TABLE 2.1  
Species with potential interest for comparative studies of aging, including comparative genomics

Taxon <sup>a</sup>	Name	Species	$t_{max}^b$	$t_{sex}^c$	$M^d$	Observations
Primates	Humans	<i>Homo sapiens</i>	122.5	13	60	
Apes	Chimpanzee	<i>Pan troglodytes</i>	73	9	45	
	Gorilla	<i>Gorilla gorilla</i>	54	9–15	140	
	Orangutan	<i>Pongo pygmaeus</i>	59	8	65	
	Gibbons	<i>Hyllobates</i> genus	40–47	6–8	6–8	
	Hamadryas baboon	<i>Papio hamadryas</i>	45	3–5	15–30	Examples of Old World monkeys
New World monkeys	Rhesus macaque	<i>Macaca mulatta</i>	40	4–6	8	
	Common marmoset	<i>Callithrix jacchus</i>	16.8	1–1.5	0.2–0.4	
	Golden lion marmoset	<i>Leontopithecus rosalia</i>	30	2–3	0.65	Longest-lived marmoset
	White-faced capuchin	<i>Cebus capucinus</i>	54.8	4–8	2	Example of a long-lived Platyrrhini
Tarsiidae	Tarsiers	<i>Tarsius</i> genus	15	1–2	0.1–0.2	Generally short-lived
Strepsirrhini	Brown lemur	<i>Eulemur fulvus</i>	37	2	2	Longest-lived lemur
	Fat-tailed dwarf lemur	<i>Cheirogaleus medius</i>	19.3	1	0.3	
	Lesser mouse lemur	<i>Microcebus murinus</i>	15.5	1	0.06	Fast aging for a primate
Lorisidae	Galago	<i>Galago senegalensis</i>	18.8	<1	0.2	
	Slender loris	<i>Loris tardigradus</i>	16.4	1	0.2–0.3	
	Slow loris	<i>Nycticebus coucang</i>	26.5	2	1	
Rodentia	House mouse	<i>Mus musculus</i>	5	0.1–0.2	0.02	One of the fastest aging mammals
	Norway rat	<i>Rattus norvegicus</i>	6	0.25	0.2	
	Slender-tailed cloud rat	<i>Phloeomys cumingi</i>	13.6	–	2	Long-lived murids
	Muskrat	<i>Ondatra zibethicus</i>	10	0.5–1	1	
Other rodents	Naked mole-rat	<i>Heterocephalus glaber</i>	28	<1	0.03	Longest-lived rodent
	Old World porcupine	<i>Hystrix brachyura</i>	27.3	1	8	
	European beaver	<i>Castor fiber</i>	25	2	25	

<sup>a</sup>Species are typically listed according to their evolutionary distance to humans.

<sup>b</sup>Maximum lifespan in years.

<sup>c</sup>Age at sexual maturity in years. Typical or range of values is displayed.

<sup>d</sup>Adult body mass ( $M$ ), a standard measure of body size, in kilograms. Typical or range of values is displayed.

*glaber*), which may live up to 28 years, could be useful models. Likewise, while mice and rats are short-lived, some species of the Muridae family can live up to 10 years or more, namely, the slender-tailed cloud rat (*Phloeomys cumingi*) and the muskrat (*Ondatra zibethicus*). The phenotypic variation we witness across mammalian orders may bias comparative studies of aging, so again primates and rodents should be preferred (Table 2.1). Of course, we can consider other mammalian orders, and for practical reasons these may even be necessary. Still, I maintain my opinion that studies, such as comparative genomics studies, based on primates and

rodents should suffice to study aging and are the most appropriate to identify genetic factors influencing human aging.

### Putative Models of Antiaging Strategies

Due to obvious practical and economical reasons, the most widely employed models of aging are short-lived. It has been argued, however, that studying short-lived species may be irrelevant to humans because whatever mechanisms limit the lifespans of these species have been evolutionarily “solved” by long-lived species like us.

## 2. Species Selection in Comparative Studies of Aging and Antiaging Research

Studying long-lived species may then be potentially more beneficial to people (Strehler, 1986).

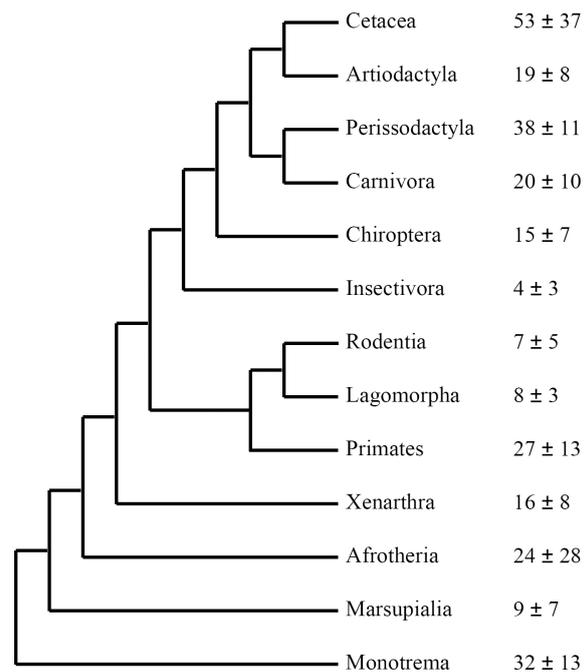
### MAMMALIAN EXAMPLES OF LIFE-EXTENSION

It is well-established that longevity increased in the lineage leading to humans, yet the evolution of longevity occurred in other mammalian lineages as well (Figure 2.3).

Thus it is possible, and even likely, that life-extending strategies vary according to phylogeny. In other words, different mechanisms for long life may have evolved independently in different mammalian lineages. Identifying these mechanisms could potentially allow us to employ them in human medicine.

The best example is certainly the bowhead whale, which has been reported to live over 200 years (George *et al.*, 1999). There is little knowledge of diseases affecting these animals. Still, given that bowhead whales weigh over 75 tons, they must feature some sort of anticancer mechanism(s) to prevent cancer from developing among their huge mass of cells (Austad, 1997b). Other whales too appear to have long lifespans. Animals of the Balaenopteridae family are generally long-lived: examples include the blue whale (*Balaenoptera musculus*) and the fin whale (*Balaenoptera physalus*) which may live over a century. Understanding why these species live so long may yield clues about antiaging mechanisms that are absent from humans, such as anticancer mechanisms. Similar examples include elephants (*Elephas maximus* and *Loxodonta africana*), which can live up to 80 years, and the dugong (*Dugong dugon*), which can live up to 70 years. All these mammals feature a long lifespan, a rate of aging, from what we know, comparable to that of humans, and are considerably bigger than us. The hippopotamus (*Hippopotamus amphibius*), which has a maximum lifespan of 61 years and an MRDT of 7 years, rhinoceros (*Ceratotherium simum* or *Rhinoceros unicornis*), which can live up to 50 years, and maybe even horses (*Equus caballus*), which can live nearly 60 years, may also fit this category. Identifying anticancer mechanisms in these species is thus a promising prospect.

Other specific life-preserving mechanisms may exist in several mammals. For example, the nabarlek (*Petrogale concinna*), a wallaby from northern Australia, apparently features continuous tooth development, termed polyphyodonty (Department of the Environment and Heritage, 2000). Elephants also feature an unusual scheduling of tooth eruption and species of the Sirenia order—i.e., manatees (genus: *Trichechus*) and the dugong—may also feature some form of polyphyodonty (Finch, 1990). While progress in stem cells may allow teeth replacement in humans sooner than later (Ohazama *et al.*, 2004), animals like the nabarlek and manatees demonstrate how numerous species may feature unique mechanisms to cope with nearly universal age-related diseases among mammals that also afflict humans. Even among strains of a given species there may be potentially useful phenotypes,



**Figure 2.3.** Maximum lifespan phylogenetic tree for different mammalian orders. Obtained from AnAge, values represent the average  $t_{max}$  for species of each mammalian order and are expressed in years  $\pm$  standard deviation. Afrotheria is not an order but rather a clade of mammals proposed, based on DNA analysis, to have a common origin. It includes the following families: Macroscelidea ( $n = 4$ ), Tubulidentata ( $n = 1$ ), Sirenia ( $n = 3$ ), Hyracoidea ( $n = 3$ ), Proboscidea ( $n = 2$ ), and Tenrecidae ( $n = 7$ ). Thus, it is normal for Afrotheria to feature a bigger standard deviation than other taxa. Phylogeny was drawn based on Springer *et al.* (2003). Branch lengths are not to scale.

as exemplified in the regeneration capacity observed in the MRL mouse (Heber-Katz *et al.*, 2004).

### Potential Nonmammalian Models of Antiaging Strategies

Long-lived nonmammalian species may also feature antiaging mechanisms of potential use in human medicine that obviate, at least, some human age-related pathologies. The best examples are species that appear not to age, such as many types of turtles (order: Testudines). Species like Blanding's turtle (*Emydoidea blandingii*) and the painted turtle (*Chrysemys picta*) have been reported not to show signs of aging in studies lasting decades (Congdon *et al.*, 2001, 2003). An increased reproductive output with age was also reported, in accordance with reports of *de novo* oogenesis in adult reptiles (Finch, 1990; Patnaik, 1994). Understanding the physiological basis of this phenomenon, also termed negligible senescence (Finch, 1990), has tremendous implications for gerontology but has so far been neglected. Further examples include the Aldabra tortoise (*Geochelone gigantea*) and the Galapagos tortoise (*Geochelone elephantopus*), which likely live over

a century. Anecdotal evidence suggests the Galapagos tortoise reaches sexual maturity only after at least two decades, making it one of the vertebrates with the longest developmental period. Unfortunately, work on turtles is limited. There is some evidence that telomere biology is different in turtles (Girondot and Garcia, 1999), and some results suggest that the brains of turtles have enhanced mechanisms to protect against reactive oxygen species formation and damage (Lutz *et al.*, 2003). Likewise, neurogenesis may be predominant in reptiles (Font *et al.*, 2001). Since other turtles may feature negligible senescence and oocyte regeneration (Finch, 1990), turtles are promising models for antiaging medicine (de Magalhaes, 2004).

Apart from turtles, other species with negligible senescence include bullfrogs (*Rana catesbeiana*), certain fishes such as rockfishes (genus: *Sebastes*) and sturgeons (family: Acipenseridae), as well as many lower life forms (Finch, 1990). It is possible, of course, that many other species feature negligible senescence, or at least slower rates of aging than humans, of which we know nothing about. Since all studied mammals age, incorporating nonaging species in studies of the biology of aging is auspicious. Species with negligible senescence are also promising models for identifying mechanisms that can be used to fight specific human age-related pathologies. For example, it was shown that the rainbow trout (*Oncorhynchus mykiss*) features high levels of telomerase and a continuous molting which may be partly responsible for its continuous growth and negligible rate of aging (Klapper *et al.*, 1998). Moreover, species with negligible senescence are important in understanding how the genetic program, the genome, can be optimized for long-term survival. Certainly, there are great difficulties in studying, for instance, an animal that outlives humans and that is probably why most of these species have not been studied in detail. Modern high-throughput technologies, however, give researchers a host of new experimental opportunities (de Magalhaes and Toussaint, 2004). Sequencing the genome of these species should then be a priority.

Turtles are clearly the reptiles with the greatest potential as models of antiaging strategies. Nonetheless, while some short-lived reptiles show signs of aging, other long-lived reptilian species may be of interest. For example, *de novo* oogenesis has been reported in different reptiles, including alligators and lizards, plus the aforementioned turtles (Patnaik, 1994). The ability to regenerate oocytes in adulthood is crucial to avoid reproductive senescence and, according to evolutionary models, essential for the emergence of negligible senescence. Increased reproductive output with age has also been reported in other reptiles apart from turtles, such as in the northern fence lizard (*Sceloporus undulatus*) and in king snakes (*Lampropeltis getulus*), as well as other species (Finch, 1990; Patnaik, 1994). With the exception of turtles,

the longest-lived reptile is the tuatara (*Sphenodon punctatus*), which lives at least 77 years, but possibly much longer (Patnaik, 1994). Tuataras are the only living descendants from the Rhynchocephalia order and thus have no closely related species. They are found only in New Zealand. Even though tuataras rarely exceed one kilogram in weight, they are long-lived, attaining sexual maturity after at least 10 years. Due to their unique evolutionary history and features, the tuatara is a potential model of antiaging strategies.

There are no confirmed birds with negligible senescence, though fulmars and the Andean condor age very slowly, if they age at all. The northern fulmar (*Fulmarus glacialis*), for example, likely ages more slowly than humans (Gosden, 1996). The longest-lived bird, however, is reported to be the Andean condor (*Vultur gryphus*), which can live up to 75 years. Senescence has not been described in these animals, though detailed studies are lacking (Finch, 1990). In the arctic tern (*Sterna paradisaea*) too no senescence has been demonstrated so far (Gosden, 1996). The record longevity for this species is only 34 years, but this particular 34-year-old individual appeared in excellent health and was actually released in the wild (Terres, 1980). Such cases again suggest that there may be many species aging more slowly than humans and about which we know little. The African grey parrot (*Psittacus erithacus*), the mute swan (*Cygnus olor*), the southern ground hornbill (*Bucorvus cafer*), and the Manchurian crane (*Grus japonensis*) have all been reported as living around 70 years. Interestingly, it has also been suggested that long-lived birds feature enhanced mechanisms of neurogenesis, protection against oxidative damage, and mechanisms against the formation of advanced glycosylation end products (Holmes *et al.*, 2001).

Another bird of potential interest to gerontologists is the raven (*Corvus corax*), a passerine. Typically, Passeriformes, corvids, and other species of the genus *Corvus* are short-lived, but ravens are clearly an exception. In the wild, ravens generally only live a few years, but in captivity their lifespan is likely above 70 years, with anecdotal reports of one raven living up to 80 years in captivity (Boarman and Heinrich, 1999). It would be interesting to know what physiological and genetic mechanisms make ravens live so much longer than their closest relatives. Likewise, more rigorous studies may reveal other long-lived species in the genus *Corvus*.

Although amphibians are not reported to be as long-lived as reptiles or mammals, they may prove useful for gerontology. The longest-lived amphibian is the Japanese giant salamander (*Andrias japonicus*), which reportedly can live up to 55 years. While this pales in comparison to whales and tortoises, amphibians do have some unique traits of potential use to medicine. One of them is how regenerative mechanisms in amphibians are more advanced than those of mammals. For example,

## 2. Species Selection in Comparative Studies of Aging and Antiaging Research

amphibians can regenerate entire limbs while mammalian tissues, such as muscle, can regenerate only as isolated entities (Carlson, 2003). Limb regeneration has been particularly well-studied in newts, and it may have future applications in antiaging research. In one study, protein extracts derived from newts were able to dedifferentiate mouse muscle cells into stem cells. This process of dedifferentiation of adult cells appears then to be modulated by appropriate factors that can be potentially isolated in newts (McGann *et al.*, 2001). Future studies to implement the regenerative capacity of some amphibians to humans are of great medical interest. Furthermore, while short-lived amphibians show signs of aging, long-lived amphibians may feature negligible senescence, polyphodonty, and oocyte regeneration (Kara, 1994).

Among the large diversity of animals in the world there are certainly multiple processes that can be useful to prevent age-related pathologies in humans. For example, loss of auditory hair cells (AHCs) is a major cause of deafness in people and hence regeneration of these cells has considerable medical interest (Hawkins and Lovett, 2004). It is interesting to note that most mammals, contrary to most birds and amphibians and maybe even some bats (Kirkegaard and Jorgensen, 2002), lose the capacity to regenerate AHCs early in life. Therefore, it has been suggested that genomic tools may be used to understand the basis of this regenerative capacity and eventually apply it to mammals (Hawkins and Lovett, 2004). Another example is heart regeneration. Mammals and amphibians typically have a limited regenerative capacity of the heart muscle. The zebrafish (*Danio rerio*) heart, however, appears to have a robust capacity for regeneration based on the proliferation of cardiomyocytes which can avoid scar formation and allow cardiac regeneration (Poss *et al.* 2002). Thus, zebrafish may also be a powerful system to study antiaging or life-prolonging strategies of specific human age-related diseases (see chapters 27 and 28 in this book about the zebrafish). With the emerging age of genomics, it may soon be possible to employ genomic tools to identify life-extending genes and pathways absent from humans. For example, mammals appear to have lost the CPD-photolyase DNA-repair enzyme (Thoma, 1999). Certainly, other such genes exist and some may turn out to have life-extending functions.

These examples are only the tip of the iceberg. Among the extraordinary diversity of life forms on earth, including the thousands of vertebrates, we are likely to find many novel antiaging strategies. In Table 2.2 I present the examples cited above plus the longest-lived species for a number of vertebrate classes and selected mammalian orders. These represent species in which longevity likely evolved and hence may feature antiaging mechanisms. In theory, the longest-lived animals in each mammalian family are capable of delaying aging in relation to similar species, and thus studying these

animals may allow us to identify not only genetic factors regulating aging rates but even life-extending mechanisms.

### Conclusion

Maximum lifespan will likely continue to be used as the measure of aging in animal species. Even though other measures exist and should be implemented, *t<sub>max</sub>* does give an estimate of rate of aging; it is the easiest method presently at our disposal, and *t<sub>max</sub>* data are widely available—including in AnAge—making comparisons straightforward. It is not a perfect measure for comparing aging across species, but it is arguably the best.

More animal diversity is necessary in comparative studies of aging. Implementing novel models of aging should be welcomed independently of the species used. In the context of comparative studies of aging, though, there is no need to drift away from primates and rodents and certainly not from mammals. With sequencing technology becoming cheaper, in a near future it will become possible to sequence the genome of multiple species, and I predict comparative genomics to become the predominant tool in comparative studies of aging (de Magalhaes and Toussaint, 2004). Choosing which species to investigate will then become crucial. My rationale is that the best way to perform comparative genomics studies of aging is by focusing on primates plus a few rodents (Table 2.2).

Mammalian species, with a major bias toward primates and rodents, may allow us to understand the genetic factors that determine the pace of aging. Yet numerous other species, including long-lived mammals, reptiles, amphibians, fishes, and birds, may hold secrets to delay human age-related pathologies and maybe even the aging process itself. From species that appear to have escaped senescence, to animals featuring extreme forms of regeneration, passing by animals possessing specific traits that may be used to delay human age-related pathologies, multiple species may feature applications to antiaging research.

### Recommended Resources

Readers are encouraged to visit the Human Ageing Genomic Resources (<http://genomics.senescence.info>), which features the AnAge database (<http://genomics.senescence.info/species/>).

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TABLE 2.2  
Species with potential interest as sources of antiaging strategies

Taxon <sup>a</sup>	Name (species)	<i>t</i> <sub>max</sub> <sup>b</sup>	Observations
Mammalia: Cetacea	Bowhead whale ( <i>Balaena mysticetus</i> )	211	Longest-lived vertebrate
	Fin whale ( <i>Balaenoptera physalus</i> )	116	
	Blue whale ( <i>Balaenoptera musculus</i> )	110	Biggest animal
Artiodactyla	Hippopotamus ( <i>Hippopotamus amphibius</i> )	61	Long-lived artiodactyls
	Camel (genus: <i>Camelus</i> )	50	
Perissodactyla	Horse ( <i>Equus caballus</i> )	60	Long-lived perissodactyls
	Rhinoceros ( <i>Ceratotherium simum</i> or <i>Rhinoceros unicornis</i> )	50	
Afrotheria	Elephant ( <i>Elephas maximus</i> or <i>Loxodonta africana</i> )	80	Biggest land animal
Sirenia	Manatees (genus: <i>Trichechus</i> ) and the dugong ( <i>Dugong dugon</i> )	>50	May feature polyphyodonty
Marsupialia	Nabarlek ( <i>Petrogale concinna</i> )	>10	Appear to feature polyphyodonty
Reptilia: Testudines	Aldabra tortoise ( <i>Geochelone gigantea</i> )	152	
	Mediterranean spur-thighed tortoise ( <i>Testudo graeca</i> )	127	
	Galapagos tortoise ( <i>Geochelone elephantopus</i> )	>100	Long developmental phase
	Blanding's turtle ( <i>Emydoidea blandingii</i> )	75	Featuring negligible senescence
	Painted turtle ( <i>Chrysemys picta</i> )	61	
Diapsida	Tuatara ( <i>Sphenodon punctatus</i> )	90	Longest-lived reptile that is not a turtle
Aves	Andean condor ( <i>Vultur gryphus</i> )	75	Longest-lived bird
	African grey parrot ( <i>Psittacus erithacus</i> )	73	
	Northern fulmar ( <i>Fulmarus glacialis</i> )	48	Likely age very slowly
	Arctic tern ( <i>Sterna paradisaea</i> )	34	
Passeriformes	Raven ( <i>Corvus corax</i> )	69	Longest-lived passeriform
Amphibia	Japanese giant salamander ( <i>Andrias japonicus</i> )	55	
	Common European toad ( <i>Bufo bufo</i> )	40	
	Bullfrog ( <i>Rana catesbeiana</i> )	16	Can increase reproductive output with age
	Red-spotted newt ( <i>Notophthalmus viridescens</i> )	15	One of several species capable of limb regeneration
Osteichthyes	Lake sturgeon ( <i>Acipenser fulvescens</i> )	152	
	Rockfish ( <i>Sebastes aleutianus</i> )	>150	Featuring negligible senescence
	Orange roughy ( <i>Hoplostethus atlanticus</i> )	149	Slow-growing, long-lived species
	Zebrafish ( <i>Danio rerio</i> )	5.5	Capable of heart regeneration

<sup>a</sup>Species are typically listed according to their evolutionary distance to humans.

<sup>b</sup>Maximum lifespan in years.

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João Pedro de Magalhães

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