Proposal to Sequence Genomes of Unique Interest for Research on Aging: *Heterocephalus glaber, Cebus capucinus,* and *Balaena mysticetus*

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Summary

Aging is not only a major puzzle of biology but it has a profound impact on medicine with age-related diseases like heart diseases, type II diabetes, cancer, and neurodegenerative diseases among the leading causes of death in modern societies. Recent research has revealed several gene systems that can regulate longevity in model organisms and appear evolutionary conserved. Nonetheless, the longevity effects of these genes are modest when compared to the lengthening of lifespans during evolution. Among mammals there is at least a 40-fold variation in maximum longevity. We still do not know why different species of similar body plan, biochemistry, and physiology can age at such different rates, but these differences must be seated in the genome.

In this white paper, we champion the idea that longevity should also be taken into consideration when choosing target organisms for sequencing, and we propose the sequencing of three organisms (in order of priority): the naked mole-rat (*Heterocephalus glaber*) whose record longevity of 28.3 years makes it the longest-lived rodent, the white-faced capuchin monkey (*Cebus capucinus*) which can live over 50 years, and the bowhead whale (*Balaena mysticetus*), possibly the longest-lived mammal with an estimated longevity of over 200 years. In particular *Heterocephalus* and *Cebus* have a much longer lifespan than expected for their body size.

Model organisms have been typically chosen based on their ability to replicate human disease. *Heterocephalus* is extraordinary because when compared to similar-sized species, such as mice and rats that live up to 4-5 years, it can live >28 years. Therefore, sequencing its genome will provide the molecular biology tools necessary for *Heterocephalus* to be used not only as another model of human disease but primarily as the first model of resistance to chronic diseases of aging. Interestingly, *Heterocephalus* is extremely resistant to neoplasia.

Cebus is, after humans and apes, the best example of a long-lived primate lineage. The convergence of *Cebus* and humans in extreme longevity, as well as in other traits such as a large brain, makes *Cebus* an ideal organism to identify mechanisms and genes that contributed to these convergent traits. Finding clues regarding which genes and mechanisms contributed to the evolution of longevity in primates will increase our knowledge of the biology and genetics of human aging and age-related diseases.

Balaena is the only mammal reported to outlive human beings. Due to our inability to conduct in vivo studies in *Balaena*, obtaining its genome sequence is a first step in uncovering clues about the genetic and molecular mechanisms that allow some organisms to live longer than humans.

Overall, these resources will help establish *Heterocephalus* as the first long-lived model for biomedical research. Because these organisms were chosen to dovetail with existing mammalian sequencing projects, sequencing their genome will also provide researchers with pairs of closely-related species with divergent lifespans that will be powerful tools for studying the evolution of longevity and consequently help guide experiments in more traditional models. If we could understand the genetic basis of differences in longevity between humans and closely-related species, it would open a whole new paradigm for biomedical research on aging and age-related diseases with countless potential applications for improving human health.

Introduction

Complete genome sequences are now available for several organisms, including the human species, and multiple mammalian target organisms have been chosen for genome sequencing at different levels of coverage

(http://www.genome.gov/10002154). In this white paper, we argue that longevity should also be taken into consideration when selecting target organisms for sequencing and we propose the sequencing of three organisms of unique interest for aging research (in order of priority): the naked mole-rat (*Heterocephalus glaber*) whose record longevity of 28.3 years makes it the longest-lived rodent (Buffenstein, 2005), the white-faced capuchin monkey (*Cebus capucinus*) which can live over 50 years (Weigl, 2005), and the bowhead whale (*Balaena mysticetus*), possibly the longest-lived mammal with estimates suggesting it may live over 200 years (George et al., 1999). In particular *Heterocephalus* and *Cebus* have a much longer lifespan than expected for their body size (Figure 1).

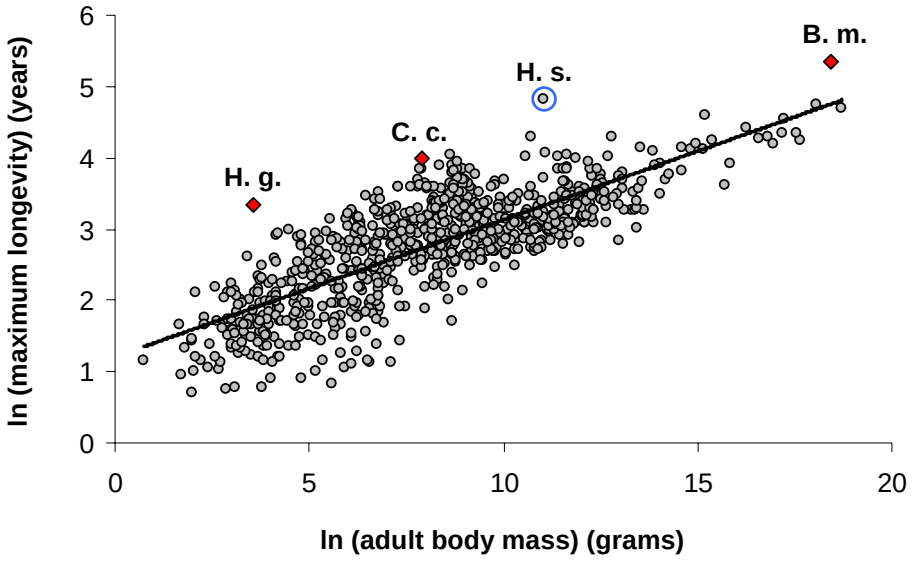

Figure 1: Plot of the relationship between ln-transformed maximum longevity and typical adult body mass for non-flying mammals. Red squares represent the proposed organisms (H. g. = *Heterocephalus glaber*; C. c. = *Cebus capucinus*; B. m. = *Balaena mysticetus*). Each gray circle is an individual species (*n* = 891). H. s. = *Homo sapiens*; highlighted in blue. Data from de Magalhaes et al., 2007.

Aging is a major biological process with a profound impact on human medicine and society. Age-related diseases such as diseases of the heart and malignant neoplasms are the leading causes of death in modern societies. In fact, the incidence and morbidity of most diseases increases with age (Hoyert et al., 2006). Moreover, neurodegenerative diseases, like Alzheimer's and Parkinson's disease, are among the major health concerns of aging adults and are recognized as important targets of biomedical research.

Recent strides have been made concerning our understanding of aging. Research has now elucidated several gene systems that can regulate longevity, and often aging and multiple age-related diseases, in short-lived model organisms including rodents (Guarente and Kenyon, 2000; Finch and Ruvkun, 2001). Interestingly, some of these genes have been shown to determine longevity in very different species, such as in roundworms and in mice (Holzenberger, 2003; Liu et al., 2005). Therefore, at least some mechanisms of aging appear to be evolutionary conserved and may thus have potential therapeutic applications (Baur et al., 2006). The human genome sequence has also made it possible to identify the gene responsible for progeria (Eriksson et al., 2003), and several genes have been associated with human longevity and/or survival in old age (Vijg and Suh, 2005; for a list of aging- and/or longevity-associated genes please refer to the GenAge database at http://genomics.senescence.info/genes/). Nonetheless, the longevity effects of these genes are modest relative to the lengthening and shortening of lifespans during evolution. Animals of different species age at remarkably different rates (Finch, 1990; Miller, 1999). For instance, among mammals there is at least a 40-fold variation in maximum longevity even for animals in captivity under adequate husbandry conditions (Austad, 1997). Given our present inability to quantify aging in a systematic and efficient way, such differences in maximum longevity are indicative of differences between species in terms of disease susceptibility, in particular susceptibility to age-related diseases (de Magalhaes, 2006). Understanding the genetic, cellular, and molecular mechanisms by which animals of different species age at different paces would provide important clues about human aging and age-related diseases (Austad, 2005).

Short-lived model organisms have been typically chosen to study aging and age-related diseases. Indeed, gerontological research using traditional models has benefited from the high-throughput approaches made possible by genome sequences (Murphy et al., 2003; Hamilton et al., 2005). Many have argued, however, the

necessity to study aging in long-lived organisms and investigate why they live so long (Strehler, 1986), yet the difficulties have so far outweighed the scientific merits. Cellular studies have been proposed as an alternative to in vivo studies (Austad, 2001). Though some results hint of correlations between longevity and some cellular biomarkers, such as stress resistance (Kapahi et al., 1999; Harper et al., 2007), the mechanistic and genetic causes for species differences in aging and age-related diseases remain largely, if not totally, unknown. It remains a mystery why different species of similar body plan, biochemistry, and physiology can age at such different rates, yet these differences must be seated in the genome (Miller, 1999). One approach brought about by the current explosion of data in the life sciences involves comparative genomics to gather insights about the evolutionary mechanisms determining lifespan and derive testable hypotheses (de Magalhaes and Toussaint, 2004). As detailed below, we think this approach, coupled with experiments in traditional model systems, has the potential to change our understanding of aging with multiple implications for biomedical research.

There have been some preliminary studies on the evolutionary mechanisms of longevity. For example, mitochondria have for long been hypothesized to be key players in aging and researchers have employed the mitochondrial DNA of multiple species in an attempt to correlate some genomic feature under study with longevity (de Magalhaes, 2005; Samuels, 2005; Khaidakov et al., 2006; Lehmann et al., 2006; Rottenberg, 2006 & 2007). Because the mitochondrial genome covers only a tiny fraction of an organism's genetic information, however, these studies—albeit promising—provide only a limited number of insights and candidate alleles regarding the evolutionary mechanisms of longevity. Having the complete genome sequence would allow the analysis of mitochondrial nuclear-encoded genes. In another approach, because aging appears to be delayed in humans when compared to chimpanzees, studies of human-chimpanzee gene pairs have been conducted to identify candidate genes. With only two species, however, the limit of detection is still confined to relatively strong signals (de Magalhaes and Church, in press).

In this proposal, we champion the idea that longevity, largely ignored so far when choosing target organisms for sequencing, should also be taken into consideration. By studying the evolution of longevity we will gather clues about the genetic basis of age-related diseases afflicting humans. As one of the longest-lived animal species, it is in our interest to study the genetic features that contribute to the evolution of longevity and determine our susceptibility to age-related diseases like heart diseases, type II diabetes, cancer, and neurodegenerative diseases. Therefore, as a first step we propose 6x genome sequencing of *Heterocephalus glaber* and *Cebus capucinus* and 2x sequencing of *Balaena mysticetus.*

Rationale

Independently of environmental conditions, mammals of different species age at remarkably different paces (Finch, 1990). For example, a mouse will age about 25-30 times faster than a human being, independently of how well the mouse is taken care of (Miller, 1999). Even primates, such as dwarf and mouse lemurs that do not commonly live more than 20 years, can exhibit in their second decade of life age-related changes and pathologies found in elderly human patients (de Magalhaes, 2006). Consequently, it is clear that genetics plays a major role in longevity differences between species, in differences in rate of aging, and in differences in the susceptibility to age-related diseases (Cutler, 1979; Miller, 1999; Partridge and Gems, 2006). In fact, mammals show major species differences in a host of age-related diseases, like cancer, type II diabetes, and neurodegenerative diseases, which may be traced to specific gene differences (Finch, 1990). If we could understand why different species, particularly species biologically and evolutionary similar to us, have different susceptibilities to many age-related diseases this would open a whole new paradigm for biomedical research on aging and age-related diseases. In other words, if we could identify which genes contribute to determine differences in the incidence of diseases across species, including humans, it would provide important insights into the mechanisms of human diseases that could lead to better a diagnosis and treatment.

The projected genome sequencing of a large number of mammalian species, including several primates, will offer extraordinary opportunities for studying genome evolution and to better understand the structure and dynamics of the human genome. For example, it is possible to analyze sequence data from species with different brain sizes to obtain candidate genes (Gilbert et al., 2005). Several other studies have identified genes that could be involved in the evolution of unique human traits (Finch and Stanford, 2004; Vallender and Lahn, 2004). With the most important biomedical model organisms already sequenced or chosen for sequencing (http://www.genome.gov/10002154), and particularly now with progress in sequencing technology (Church,

2006), we think it is opportune to expand comparative genomics studies to longevity. Ideally, we would like to study closely-related species with different lifespans. As a result, our rationale while selecting organisms was not just to choose long-lived species, but identify long-lived organisms that can be compared to closely-related, shorter-lived species already approved for sequencing.

Clearly, longevity evolved in the lineage leading to humans (Cutler, 1979), and it would be of great biological and biomedical interest to identify the genes involved. Nevertheless, studies using the currently available primate genome sequences to identify, for instance, signatures of evolutionary changes are not powerful enough to determine if the signatures are related to longevity—rather than other human-specific traits—because species closer to humans will tend to have a longer lifespan. One way to obviate this lack of power and precision is to use other long-lived primate lineages in the analysis of the genetic changes shaping the long-lived human lineage. To put it another way, to identify which genomic features are specifically involved in the evolution of longevity requires additional genome sequences from at least another long-lived primate lineage. Because, as shown in Figure 1, body size strongly correlates with longevity and is hence a potential source of bias in comparative analyses (Speakman, 2005), such long-lived lineage should preferably be composed of relatively small animals.

Some—so far unknown—information in the genome determines longevity to a large degree and we think it is computationally possible to employ the genome sequence of organisms with different lifespans to gather novel insights about the evolution of longevity and identify candidate genes that can be experimentally tested. Potentially, this may allow researchers to identify the genes that contribute to the unique features of human aging, longevity, and disease susceptibility. Having the genome sequence of long-lived organisms would also facilitate the ability of researchers to do experiments and investigate the unique biochemistry, physiology, and cell biology of these organisms. Moreover, considering longevity as a factor when selecting organisms for genome sequencing will allow researchers to start studying the genetic factors that allow other species to be more resistant than humans to certain diseases, like cancer. The idea that other organisms possess unique features whose understanding may help alleviate human diseases is not new, as exemplified by the ample and exciting research conducted in species with a greater regeneration ability than humans (McGann et al., 2001; Poss et al. 2002; Hawkins and Lovett, 2004; Voskoboynik et al. 2007). Therefore, we also wanted the proposed resources to allow researchers to identify unique genes and mechanisms that contribute to longevity and disease resistance in other species, which may provide insights into our own aging process and potentially be used to develop therapies for improving human health.

Proposal

This proposal, written on behalf of a large national and international community of researchers studying aging and age-related diseases (Appendix I), proposes the complete sequencing of *Heterocephalus glaber* and *Cebus capucinus* and the low coverage sequencing of *Balaena mysticetus*. These organisms were chosen by taking into consideration species already being sequenced and then trying to fill the gaps by considering longevity as an additional criterion. The proposal was widely circulated through the scientific community, particularly among biogerontologists but also evolutionary biologists and experts working on each of the proposed species, and made available in a website (http://genomics.senescence.info/sequencing/) for further discussion.

Based on the aforementioned rationale, it is clear to us that at least one more long-lived primate species should be sequenced. After careful consideration, we think that the best of such species is *Cebus capucinus*. Other species were considered such as the Brown lemur (*Eulemur fulvus*) that can live up to 35.5 years and the golden lion tamarin (*Leontopithecus rosalia*), a New World monkey that is also long-lived (maximum longevity is 31.6 years) when compared to other marmosets and tamarins (family: Callitrichidae). Although sequencing these and other long-lived primate species would be extremely useful for understanding the evolution of longevity (see below), we think *Cebus capucinus* is the best target organism primarily because it can live over 50 years (Weigl, 2005). Among primates, only humans, apes, and *Cebus capucinus* have been reported to live over 50 years (Weigl, 2005; de Magalhaes, 2006), but unlike humans and apes, *Cebus capucinus* has not been chosen as a target organism for sequencing (Figure 2).

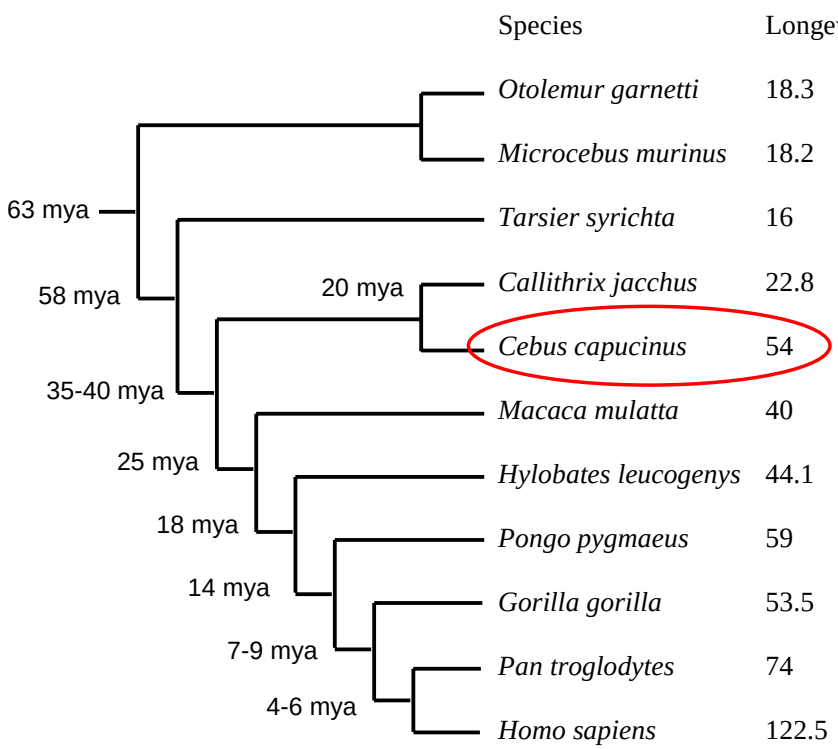

Figure 2: Phylogeny and longevity of selected primate species. Longevity refers to maximum longevity and was obtained from build 9 of the AnAge database (de Magalhaes et al., 2007). Human longevity, of course, could be overestimated due to the much larger sample size. Nonetheless, it is clear that longevity increased in the human lineage (Cutler, 1979). Except *Cebus capucinus*, all these species have been chosen as target organisms for sequencing. Phylogeny from Goodman, 1999. Branch sizes are not to scale. mya = million of years ago.

Cebus capucinus is a small-sized (adults weight about 4-8 kilograms) New World monkey (infraorder: Platyrrhini) that diverged from the lineage leading to humans (infraorder: Catarrhini) about 35-40 million years ago (Figure 2). *Cebus* monkeys have a large brain for their body size and show convergent evolution with humans of several behaviors such as hunting, food sharing, high reliance on social learning, and tool use. In fact, *Cebus capucinus* are known for their social complexity (Fragaszy et al., 2004). One wild-born male specimen lived 53.5 years in captivity, making it at least 54 years of age when it died (Weigl, 2005).

Heterocephalus glaber is an underground dwelling, eusocial rodent native to north Eastern Africa (Sherman et al., 1991). *Heterocephalus* belongs to the Bathyergidae family, which in turn is part of the Hystricognathi suborder of rodents, that includes the new world caviomorpha and the old world phiomorpha. Fossil evidence suggests that this suborder diverged from the Sciurognathi suborder to which mice and rats belong about 55 million years ago, though estimates based on molecular clocks have produced controversial results (Huchon et al., 2002; Faulkes et al., 2004). Despite their small size (adult body mass is on average about 35 grams) *Heterocephalus* are the longest-lived rodents with a record longevity of over 28.3 years in captivity. Furthermore, these mouse-sized rodents continue to breed well into their third decade of life and show attenuated age-related changes in physiological function (O'Connor et al., 2002, Buffenstein, 2005, Csiszar et al., in press). Strikingly, cancer has not been observed to date in *Heterocephalus* (Buffenstein, 2005). The only other known small mammals with such a long lifespan for their body size are bats, namely those of the *Myotis* genus which are already being sequenced (http://www.genome.gov/10002154).

Balaena mysticetus is a cetacean, a marine mammal currently restricted to a few stocks in the northern Atlantic and Pacific oceans (Burns et al., 1993; Nowak, 2003). Adult animals can weight more than 100 tons, making *Balaena* the second largest animal on earth, surpassed only by the blue whale *Balaenoptera musculus* (Nowak, 2003). Studies in large whales using indirect methods have suggested that these animals can live over 100 years (Nowak, 2003). One carefully-designed study estimated one *Balaena mysticetus* male specimen to be over 200 years-old (George et al., 1999). Therefore, with our current—albeit admittedly incomplete—knowledge, *Balaena* is the longest-lived mammal, and the only mammal for which there is some evidence—even if indirect—of outliving human beings. In addition, very few obvious signs of pathology have been found in old *Balaena* specimens (George et al., 1999). Considering the long lifespan and large body size of *Balaena*, it is likely that it has a very low cancer incidence and mortality when compared to humans. In the specimens

analyzed so far, tumors have been rarely observed in whales in general and in *Balaena* in particular. Mortality due to cancer is suspected to be very rare (Burns et al., 1993).

As detailed below, these resources will provide researchers with powerful tools for establishing *Heterocephalus* as the first long-lived model for biomedical research and for uncovering the genomic basis of the evolution of longevity. Our proposal to sequence these three species fits the two major goals of the NHGRI in that it will improve the annotation of the human genome, particularly using the sequence of *Cebus capucinus*, and may allow, for instance, the identification of functional elements in the human genome associated with longevity. Furthermore, the proposed resources will help understand genome evolution. In particular, they will aid in providing information about a major physiological innovation that is the evolution of longevity and resistance to diseases—and age-related diseases in particular.

Specific biological/biomedical rationales for the utility of new sequence data

Improving human health and understanding human disease

Certainly, these three organisms are unlikely to give us a full picture of how evolution extends lifespan. Species differ not only in longevity and disease susceptibility but in many other physiological, developmental, and anatomical features. Nonetheless, having the genome sequence of the proposed organisms will allow us to start prodding the mechanisms behind the evolution of longevity in mammals. As abovementioned, mechanisms of aging have been reported to be conserved across large evolutionary distances. Identifying even a fraction of the genomic features determining longevity and disease susceptibility in mammals, and in primates in particular, would be a major breakthrough that would lead to a better diagnosis and treatment of multiple age-related diseases. Several age-related diseases have a genetic basis and understanding why the incidence of these diseases varies across species will help us understand the basic biological processes responsible for human diseases. Potentially, such findings can help improve human health not only by providing insights about the genetic basis of human pathology but by providing genetic tools to fight age-related diseases like cancer, type II diabetes, or neurodegenerative diseases. For example, nearly all vertebrates share the amyloid-beta peptide sequence associated with Alzheimer's disease in humans, yet the accumulation of Alzheimer-like changes varies widely among species (Finch and Stanford, 2004). What other gene differences, one may ask, modulate the effect of the amyloid gene during aging?

Informing human biology and expanding our understanding of the basic biology of aging

Cebus is, after humans and apes, the best example of a long-lived primate lineage (Figure 2). In fact, among anthropoid primates, *Cebus* and *Homo* have the greatest longevity after body and brain mass are controlled for. A convergence of *Cebus* and *Homo* in extreme longevity as well as in other traits, such as a relatively large brain, has been observed (Judge and Carey, 2000). Having the genome sequence of *Cebus capucinus* will thus allow researchers to investigate genetic factors associated with these phenotypes in primates, including the evolution of a long lifespan. Although several human evolutionary signatures have been reported, the genome sequence of *Cebus* will allow researchers to identify evolutionary signatures specifically related to longevity thus providing novel insights regarding human evolution and biology. Considering how some mechanisms of aging have been shown to be conserved from roundworms to mice, it is reasonable to assume that at least some of the mechanisms and genes that contributed to the extension of longevity in the human lineage also contributed to the evolution of longevity in the *Cebus* genus. Finding clues as to what these genes and mechanisms might be will increase our knowledge of the biology of human aging. For example, which genes show similar evolution patterns in humans and *Cebus* when compared to other lineages? Candidate genes can then be further studied at the functional and molecular level or in genetic association analyses of longevity in humans. The *Cebus* genome will also allow studies of other convergent phenotypes, such as studying if brain-expressed genes show similar patterns of selection in humans and *Cebus*. Because marmosets (*Callithrix jacchus*) appear to age considerably faster than *Cebus* and their genome is currently being sequenced (Figure 2), comparisons between *Cebus* and *Callithrix* may be informative about the evolution of longevity. Lastly, primate genome sequences are crucial to annotate the human genome and thus the *Cebus* genome sequence will also be valuable to further identify and help decipher conserved functional elements in the human genome.

Similarly, while we do not know for a fact that evolution used the same mechanisms to extend the longevity of *Heterocephalus* as it did to extend human longevity, it is plausible that having the *Heterocephalus*

genome sequence will, too, provide clues about the evolution of longevity and about the mechanisms and genes that contributed to the extended human longevity. Having the genome sequence of *Heterocephalus* will permit researchers to identify putative genes responsible for the longevity and unique disease-resistance of *Heterocephalus* that can be experimentally tested in, for instance, mice. This concept is exemplified in the skin cancer protection offered by creating transgenic mice expressing one or two CPD-photolyases, repair enzymes evolutionary lost in placental mammals (Jans et al., 2005). For example, we would like to study if *Heterocephalus* evolved innovative DNA repair genes and has differences in the content of putative aging-related genes. Because and mice and rats age so much faster than *Heterocephalus*, having the full genome sequence of *Heterocephalus* will provide researchers with a rodent pair of similar species with highly divergent lifespans. Interestingly, another bathyergid, *Cryptomys*, has been chosen as a target organism to be sequenced (http://www.genome.gov/10002154). *Cryptomys* have been known to live nearly 20 years in captivity, making them long-lived rodents, though not quite as long-lived as *Heterocephalus*. *Heterocephalus* probably split from other bathyergids about 40 million years ago (Huchon et al., 2002; Faulkes et al., 2004), which means that having the genome of these two species may help identify genes associated with the unique traits of bathyergids. It might be useful, however, to obtain a reference point that is shorter-lived than *Cryptomys* and more closely-related to *Heterocephalus* than mice and rats. The Cape mole-rat (*Georychus capensis*) is a solitary furry bathyergid (Faulkes et al., 2004). Its lifespan has been estimated to be about 3 years in the wild (Nowak, 1999), and they have been reported to live up to 11.2 years in captivity (Weigl, 2005). We are currently investigating unpublished claims that these animals develop signs of aging in their first decade of life.

Lastly, *Balaena* is a fascinating species in that it is the only mammal for which there is evidence of outliving humans. As argued for the other species in this proposal, we do not know the genetic, physiological, or molecular reasons underlying the evolution of longevity in *Balaena*, yet again we think that having the *Balaena* genome sequence will allow us to gain insights into the evolution of longevity and thus potentially about the genes and pathways that contributed to the evolution of human longevity. Another cetacean, the bottlenose dolphin (*Tursiops truncates*), is currently being sequenced (http://www.genome.gov/10002154). While there are few detailed studies, *Tursiops* are not known to live more than 50 years in the wild (Nowak, 1999), and their record longevity in captivity is 51.6 years (Wiegl, 2005). Having the full genome sequence of *Balaena* will provide researchers with a new pair of related species with highly divergent lifespans. Whales and dolphins diverged about 30 million years ago (Springer et al., 2003). Another hypothesis we are currently exploring would be to use one of the right whales (genus: *Eubalaena*) for comparisons with *Balaena* since *Eubalaena* are the closest living relatives of *Balaena* and are thought to live about half as much as *Balaena* (Best and Schell, 1996; Hamilton et al., 1998).

While our proposal focuses on longevity, aging, and age-related diseases, the proposed organisms may also be useful to inform about the genetic factors determining the rate of development, another unexplained difference between mammals. In fact, there is a very strong relationship between maximum adult lifespan and rate of development, measured as age to sexual maturity or growth rates (de Magalhaes et al., 2007). The three proposed organisms have a slow pace of development, such as long time to sexual maturity for their body size, and thus obtaining their genome sequence will be helpful to study what determines the pace of development.

<u>Providing long-lived model organisms for experimentation and facilitating the ability to do experiments</u>

Mammalian model systems have been typically chosen to study specific human diseases, with an emphasis on short-lived genotypes that show early onset conditions. This is especially true for rodent models such as mice and rats that live up to 4-5 years even in protected environments (Finch, 1990). It seems timely for us to consider very long-lived models. *Heterocephalus* is an excellent choice for a long-lived model organism due to its small size, long lifespan (when compared to other rodents), and a lower incidence of many diseases that typically affect humans and mice, such as a low cancer incidence and maintenance of vascular youthfulness well into old age (Csiszar et al., in press). In other words, we do not suggest *Heterocephalus* will be used as a model of human disease, but rather as a model of resistance to certain diseases.

Heterocephalus can be kept and bred in captivity (Buffenstein, 2005) and many studies are already underway (see Appendix II). For example, *Heterocephalus* has been used to study abnormal lens differentiation and its role in eye morphogenesis (Nikitina et al., 2004), neural plasticity and cortical remodeling in the brain (Catania and Remple, 2002), as well as vomeronasal organs, which may make *Heterocephalus* a unique model to study this sensory system (Smith et al., 2007). Moreover, because *Heterocephalus* is virtually poikilothermic

(Buffenstein and Yahav, 1991), it is an exceptional model for studying thermoregulatory features of circadian cycles (Herold et al., 1998) and mammalian metabolic regulation (Goldman et al., 1999). Sequencing the genome of *Heterocephalus* will also help study its unusual traits such as its social system and reproductive patterns. What makes *Heterocephalus* such a promising model for biomedical research, however, is its longevity. The studies conducted so far in *Heterocephalus* demonstrate how little we know about the evolution of longevity and what determines rate of aging. Briefly, young *Heterocephalus* produce similar amounts of reactive oxygen species to shorter-lived rodents (Labinskyy et al., 2006; Lambert et al., in press) and surprisingly have high levels of oxidative damage (Andziak et al., 2005). It has been reported that antioxidant activity is unlikely to explain the extreme longevity of these animals when compared to mice (Andziak et al.,2006). However, these rodents do show attenuated age-related changes in accrued oxidative damage (Andziak and Buffenstein 2006) and also show marked resistance to oxidative insults (such as treatment with hydrogen peroxide) as well as vascular resistance to proapoptotic stimuli (Labinskyy et al., 2006). Clearly, we still have everything to learn about why these animals live so long and are so resistant to cancer.

Having the *Heterocephalus* genome sequence will facilitate the ability of researchers to perform experiments by allowing researchers to employ the broad array of molecular biology tools necessary to study a complex process like aging. It will allow researchers to study gene expression profiles across species, as has been done before (Fraser et al., 2005), some of which may be related to species different in longevity. For example, rodent cells express low levels of DDB2 when compared to human cells and overexpression of DDB2 in mice protects against UV-induced cancer (Alekseev et al., 2005). Moreover, recent studies suggest a large effect of sequence divergence in microarrays, even in closely related species (Gilad et al., 2005). Hence, species-specific comparisons will be necessary, which require knowledge of *Heterocephalus* coding sequences. In addition to gene expression studies, having the full genome sequence will allow researchers to gain insights into the evolutionary forces shaping *Heterocephalus* and will provide targets for experimentation, for example by constructing transgenic mice with genes or pathways found through computational studies using the *Heterocephalus* genome sequence. Having the full genome sequence will also help researchers study regulatory regions, perhaps coupled to gene expression studies, to gain new insights about the regulation of aging and age-related diseases, as has been previously done (Lu et al., 2004).

There is a vast body of behavioral research on *Cebus capucinus* (Fragaszy et al., 2004; Perry and Mason, in press) as well as studies of physiology (Panger, 1998), biochemistry (Tanioka et al., 1986), and genetics (Couturier et al., 1983; Hiramatsu et al., 2005). Some animals are found at research institutions. Because they are easier to manage, the closely-related *Cebus apella* is more widely used as a disease model for human conditions and some colonies exist (Bergeron et al., 2000; Palfi et al., 2000; Garcez et al., 2002; Malik et al., 2004; Madsen et al., 2006). It is unlikely that *Cebus* monkeys will soon become a major model of human disease, yet having the genome sequence of *Cebus capucinus* will foster and aid in research in the whole genus since it should be easy to use the *Cebus capucinus* genome sequence to develop tools (such as PCR-based methods, microarrays, and immunohistology technologies) for research on other *Cebus* species. Furthermore, studying the evolutionary forces shaping *Cebus* will, as in *Heterocephalus*, allow researchers to identify candidate longevity genes that can be used for experimentation. Although longitudinal aging studies in *Cebus* (or in any of the proposed organisms) are difficult, biological studies such as studies of physiology, biochemistry, cell biology, behavior, and genetics are certainly feasible and they can used in conjunction with studies in the much shorter-lived *Callithrix*.

Because *Cebus apella* is more used for biomedical research—although not as widely used as rhesus monkeys (*Macaca mulatta*) or *Callithrix*—the case can be made that perhaps *Cebus apella*, rather than *Cebus capucinus*, is a better target organism for sequencing. Among all species of the *Cebus* genus, *Cebus capucinus* has the longest recorded longevity. Other members of the *Cebus* genus have been reported to live over 40 years, but none are known to live more than 50 years. Although *Cebus apella* are commonly kept in zoos and wildlife parks, their record longevity is 46 and there are at least two (probably three) specimens of *Cebus capucinus* with a longer longevity (Weigl, 2005), so it seems likely that *Cebus capucinus* are longer-lived. For this reason, we think *Cebus capucinus* has the highest probability of providing researchers with clues about the evolution of longevity in primates and about the genetic basis of aging and age-related diseases. Nonetheless, should it be deemed more relevant for biomedical research, we would not oppose replacing *Cebus capucinus* by *Cebus apella* as a target organism for sequencing. Because the *Cebus* genus is long-lived (Judge and Carey, 2000; Weigl, 2005), we think that sequencing *Cebus apella* would also be extremely useful.

Though there have been some studies of behavior, physiology, and genetics in *Balaena mysticetus* (Rooney et al., 2001; Werth, 2004), this organism is not and will not be used as a biomedical model organism. In fact, one of the reasons why we advocate the genome sequence of *Balaena* is our almost total inability to conduct in vivo studies in *Balaena*. The best way to gather new insights into *Balaena*'s physiology, biochemistry, and genetics is through comparative genomics which can then help guide experimentation in more traditional model systems such as in vitro models and short-lived model organisms. Moreover, because *Balaena* outlives humans, obtaining its genome sequence is a first, but necessary, step in uncovering clues about the genetic and molecular mechanisms that allow some animals to live longer than humans. For example, we would like to study the evolution of DNA repair genes in *Balaena* which may help us understand how these animals evade cancer. *Balaena* cell lines also exist, namely those derived by Dr. John Wise. Though the use of these requires a federal permit (see below), in vitro studies of, for example, DNA repair mechanisms would benefit from the molecular biology tools, like microarrays and proteomics, made possible by sequencing the genome.

Strategic issues in acquiring new sequence data

The demand for the new sequence data

As reflected in the list of scientists supporting this proposal (Appendix I), we expect that a large community of researchers working on aging and age-related diseases will employ the genome sequence of *Heterocephalus*, *Cebus*, and *Balaena* in their work. Even researchers that will not directly employ these resources will benefit from them, for example, by being able to study in short-lived model organisms any homologs of candidate longevity genes that are revealed by evolutionary genomic studies using the proposed resources. Moreover, the genome sequence of *Heterocephalus* will provide an arsenal of molecular biology tools for biogerontologists to study these animals in vivo and in vitro. Researchers studying *Heterocephalus*, *Cebus*, and *Balaena* will also considerable benefit from this proposal and have enthusiastically supported it. We also anticipate that having the genome sequence for these long-lived species will encourage many in the large community of researchers working on the broad area of genome sciences to focus on the evolution of longevity and aging. Overall, this proposal will not only benefit gerontologists but it will attract other researchers to study the evolution and genetics of aging and age-related diseases. Lastly, the resources proposed in this white paper will aid the work (e.g., monitoring the genetic diversity and population dynamics) of conservation researchers and ecologists working with wild populations of *Cebus* and *Balaena*, the latter listed as an endangered species by the USFWS (http://ecos.fws.gov/speciesProfile/SpeciesReport.do?spcode=A02N).

Suitability of the organisms for experimentation

As detailed above, *Heterocephalus* is already being used as a model for biomedical research (a few colonies exist already) and is easy to breed in captivity, though mutants are not available at present. Having its genome, however, would allow researchers to create transgenic mice with candidate *Heterocephalus* genes. *Cebus capucinus* is not a major model of biomedical research but *Cebus apella* is moderately used as a model. *Balaena mysticetus* is not and will likely never be an organism suitable for biomedical experiments.

Essence of longevity-associated genomic information and the rationale for the complete sequence of the organisms

Although the focus of this proposal is on *Cebus capucinus*, *Balaena mysticetus*, and *Heterocephalus glaber*, it is equally clear to us that the genome sequence from several additional species will likely be necessary to fully understand the evolution of longevity in mammals. Ideally, we would like to obtain the genome sequence of several long-lived organisms (Holmes, 2004; de Magalhaes, 2006). Our choices, however, reflect not only the longevity of the proposed organisms but how they dovetail with existing sequencing efforts. For example, several species of birds are also long-lived for their body size, but were not included in this proposal because with the current emphasis of sequencing efforts on mammals, and on primates in particular, the analysis and data-mining of mammalian genomes is considerably easier and more likely to produce important findings. Nonetheless, other organisms—like some of the abovementioned primates, long-lived birds, and organisms that appear to live longer than humans (reviewed in Finch, 1990; de Magalhaes, 2006)—would also be informative about aging and longevity. For the time being, however, we think the proposed organisms are those that have the highest probability of providing important biological insights about longevity and aging.

As mentioned above, we know very little about why different species age at different rates. The hypothesis behind this white paper is that the genomic information regulating longevity, or at least sequence-level features that can later be experimentally tested in more traditional models, can be detected with pairs of similar species with diverging lifespans and studies in the long-lived *Heterocephalus*. We know almost nothing about the genetic sequences that determine longevity and disease-resistance in different species, however, which makes it nearly impossible to predict how many genomes will be necessary to identify such information. The way we do not know whether protein-coding, RNA-encoding, regulatory, or even some genomic feature determines rate of aging highlights the need for complete genome sequencing in order to study the evolution of longevity and gather insights about the genomic information determining disease susceptibility in different species. Moreover, important genes may be missing from EST and cDNA libraries because, for example, they are expressed at very low levels or not expressed at all in normal conditions. Overall, the value of finished sequence is well-established not only for microarrays but for many applications such as PCR-based methods and studies of proteins, as highlighted in the recent sequencing of the rhesus macaque genome (Gibbs et al., 2007).

The cost of sequencing the genome and the state of readiness of the organisms' DNA for sequencing

One of us (Dr. Rochelle Buffenstein) can provide the necessary materials (e.g., tissue samples) for genome sequencing of *Heterocephalus*. Dr. Odile Petit has agreed to provide *Cebus capucinus* samples for genome sequencing. Dr. Philip Morin has agreed to provide us with *Balaena* DNA sources for sequencing. Other *Balaena* tissue materials might be available due to the annual hunts by Alaskan natives. Because *Balaena* is an endangered species, however, the use of its materials (including tissues, cells, and DNA) requires a federal permit that may take up to 18 months to obtain. One alternative suggested by Dr. Philip Morin is to extend one existing permit specifically for genome sequencing, which should not take more than a few weeks.

The exact genome sizes of *Heterocephalus*, *Cebus*, and *Balaena* are not known but are expected to be around the 3,000 Mb, as reported for other—respectively—rodents, primates, and cetaceans (http://www.genome.gov/19516773). We are not aware of any unusual biological feature posing challenges for genome sequencing. We propose the complete sequencing of *Heterocephalus glaber* and *Cebus capucinus* and the low coverage sequencing of *Balaena mysticetus* using a whole genome shotgun strategy including paired sequence reads from large-insert clones for long-range continuity as judged cost-effective at the time. The cost of sequencing is estimated to be around $15-20M for complete sequencing and $5-7M for low coverage sequencing.

We have contacted Dr. Eric Lander of the Broad Institute who has expressed a strong interest in pursuing this project and carrying out the sequencing of these organisms at the Broad Institute.

Other sources of funding and previous NHGRI proposals

No other sources of funding are currently being sought. To the best of our knowledge, no formal request for whole genome sequencing has been made for any of the organisms in this proposal.

Concluding remarks

Because significant research on *Heterocephalus* is already underway, we think priority should be given to sequencing its genome. Due to its usefulness to annotate the human genome, we think a higher priority and coverage should be given to *Cebus* relative to *Balaena*. Because of the inability to do experiments in *Balaena*, a lower coverage is proposed, though it can be increased if it is later deemed necessary and cost-effective.

In conclusion, the longevity of a species is a major indicator of its disease susceptibility. As more organisms are sequenced, longevity should also be considered when choosing new target organisms so researchers are able to study the genetic basis of disease susceptibility, which will help guide experimentation. We think the complete sequencing of *Heterocephalus glaber* and *Cebus capucinus* and the low coverage sequencing of *Balaena mysticetus* complement the current crop of organisms being sequenced and provide pairs of closely-related species with different lifespans. These resources will be a major step forward in helping establish *Heterocephalus* as the first long-lived model of biomedical research and providing researchers with the tools to investigate the evolution of longevity in mammals and ultimately help understand the genetic basis of human disease with an emphasis on age-related diseases.

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Appendix I

The following scientists, mostly biogerontologists but also evolutionary biologists and mammalogists working on the three proposed organisms, support the proposal. A selection of the support letters is provided as a separate file in Appendix II.

Name	Institution
Bada, Jeffrey	University of California, San Diego
Baker, C. Scott	Oregon State University
Barja, Gustavo	Complutense University
Bennett, Nigel	University of Pretoria
Botkin, Daniel	University of California, Santa Barbara
Brand, Martin	Medical Research Council Dunn Human Nutrition Unit
Bronikowski, Anne	Iowa State University
Brunet, Anne	Stanford University
Burda, Hynek	University of Duisburg-Essen
Burhans, William	Roswell Park Cancer Institute
Campisi, Judith	Lawrence Berkeley National Laboratory
Carey, James	University of California, Davis
Casper, Diana	Montefiore Medical Center
Coles, L. Stephen	University of California, Los Angeles
Demetrius, Lloyd	Harvard University
Downs, Colleen	University of KwaZulu-Natal
Erwin, Joseph	Foundation for Comparative and Conservation Biology
Estep, Preston	Longenity, Inc.
Faragher, Richard	University of Brighton
Faulkes, Christopher	Queen Mary, University of London
Finkel, Toren	National Heart, Lung, and Blood Institute
Fragaszy, Dorothy	University of Georgia
Fraifeld, Vadim	Ben Gurion University of the Negev
Gems, David	University College London
Goldman, Bruce	University of Connecticut
Gorbunova, Vera	University of Rochester
Gros-Louis, Julie	Indiana University
Guerin, John	Centenarian Species and Rockfish Project
Hayflick, Leonard	University of California, San Francisco
Heguy, Adriana	Memorial Sloan Kettering Cancer Center
Holmes, Donna	Washington State University
Hornsby, Peter	University of Texas Health Science Center at San Antonio
Hulbert, A. J.	University of Wollongong
Janson, Charles	University of Montana
Jepsen, Karl	Mount Sinai School of Medicine
Kapahi, Pankaj	Buck Institute for Age Research
Kawamura, Shoji	University of Tokyo
Kenyon, Cynthia	University of California, San Francisco
Khalyavkin, Alexander	Russian Academy of Sciences
Kirkwood, Tom	Newcastle University
Koski, William	LGL Limited Environmental Research Associates
Larson, John	University of Illinois at Chicago
Lee, Phyllis	University of Stirling
Lee, Siu	Cornell University

Lee, Virginia	University of Pennsylvania
Levitt, Jonathan	City College of New York
Lewin, Gary	Max Delbrück Center for Molecular Medicine
Macieira-Coelho, Alvaro	French National Institute of Health
Mate, Bruce	Oregon State University
McCarter, Roger	Pennsylvania State University
Moradas-Ferreira, Pedro	University of Porto
Morin, Phillip	Southwest Fisheries Science Center
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