The manifestations and interdependence of social and physiological aging in wild female Assamese macaques

*(Macaca assamensis)*

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Summary

Aging is a multifaceted process which increases the probability of death, and although there can be only one outcome, the ways to get there are diverse. Accumulating evidence of aging in natural animal populations has shed light on this diversity, both across and within species, but we still have little knowledge of the opportunities, constraints and trade-offs faced by individuals as they go through this ultimate life-history stage. Specifically, it remains unclear which individual traits exhibit signs of aging, how individuals may adjust their behavior in response to physiological decay, and how, together, these changes may ultimately contribute to whole-organism aging. As such, investigating age-related changes in social behavior and physiological functions is imperative to reach a more holistic understanding of aging.

A key issue in addressing social and physiological aging is the strong association between sociality and health. Social bonds, social integration, and exposure to social adversity are important predictors of health and lifespan across mammals. As some of the physiological mechanisms underlying the connections between sociality and health are uncovered, it becomes especially clear that aging of one component may influence aging in the other. Yet, the interdependences between sociality and physiology have seldom been addressed in the study of aging, due partly to a lack of detailed data on social behavior in natural populations of long-lived species. Another difficulty arises from the need to assess physiology via markers measured from non-invasively collected samples. Finally, although aging is an individual-level process, demographic and cohort effects can influence the patterns of aging uncovered in cross-sectional studies, which calls for a longitudinal approach.

In this thesis, I investigate age-related changes in social and physiological traits in wild female Assamese macaques (Macaca assamensis) to address key questions at the crossroad of aging, sociality, and health in natural animal populations: Does social behavior exhibit age-related changes? Which physiological systems, at the interface with sociality, should be investigated for age-related changes? Does social aging influence the manifestations of aging in physiological traits, and vice versa? Can biomarkers of energetic state provide means of non-invasively investigating the physiological constraints on social behavior under field conditions? To investigate these issues in the female Assamese macaques, I use extensive behavioral data, and a review of the mechanisms linking sociality and health in non-human primates, after which I focus on the HPA axis and the gut microbiome as key physiological systems. Finally, I validated the non-invasive measurement of markers of metabolic activity in macaques to offer perspective to explore the causal path from physiology towards sociality.
My results show that female Assamese macaques experience both social and physiological aging. Females decreased their social engagement and reduced the size of their grooming network with advancing age. This phenomenon could not be explained by a lack of opportunities to interact and was not associated with a greater tendency to focus on preferred partners with age. In addition to within-individual age effects, cross-sectional aging dynamics were influenced by demographic processes consistent with the selective disappearance of poorly socially connected individuals. Together, these results highlight the contribution of within-individual and demographic processes to group-level social aging and do not support increasing social selectivity as the primary driver of social disengagement in this population.

For physiological traits, I find trait-specific aging patterns, across and within traits. HPA axis activity assessed from fecal glucocorticoid concentrations was not associated with age, neither before nor after accounting for the strength of individuals’ strong bonds. The taxonomic diversity of the gut bacterial community was not associated with age, and age was not a consistent predictor of gut bacterial composition across individuals. However, the gut bacterial composition exhibited a personal signature which became less stable and increasingly personalized with age. A decrease in the social transmission of gut bacteria may have contributed to, but was not sufficient to explain, the age-related patterns uncovered here.

The validation of markers of metabolic activity was conducted via a food restriction experiment in captive macaques and assessed urinary concentrations of triiodothyronine and cortisol. Both hormones exhibited the predicted change with a decrease in triiodothyronine and an increase in cortisol during the restriction phase of the experiment. Furthermore, variation in urinary triiodothyronine concentrations correlated positively with variation in body mass. Concentrations of urinary triiodothyronine were reasonably robust to many issues associated with collection of samples under field conditions, making it a suitable non-invasive marker of energetic state in macaques.

In conclusion, in female Assamese macaques, age is associated with progressive social disengagement and modifications in several aspects of the gut bacterial communities, whereas glucocorticoid concentrations appear constant over adulthood. The characterization of consistent aging patterns across individuals in a population is a necessary step in understanding the opportunities, constraints, and potential trade-offs that individuals face. As such, my thesis contributes to the growing field of biogerontology. Future research should investigate how individuals differ from the average trajectories and consider potential age-specific optima, in a diversity of systems, to advance our understanding of the links between sociality, health, and aging.
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Chapter 1

General introduction

Demographics changes are foreseen as major societal challenges within the next decades, as the population is rapidly getting older. With increasing life-expectancy, two billion people are predicted to be over 60 in 2050 (United Nations, 2017), creating the need for care and medical assistance on a scale not yet experienced. The lengthening of lifespan underlying this demographic revolution is not matched by an equal increase in healthspan, the number of years of life free from diseases (Partridge et al., 2018), and is associated with increasing loneliness among the elders (Müller and Ellwardt, 2022), which urges to address the physiological, psychological, and social dimensions of aging.

Finding a clear definition for aging is however not straightforward, and consequently, there is a long tradition of debates on the best way to characterize pathological, normal, as opposed to successful aging (Bulterijts et al., 2015; Rowe and Kahn, 1987; Stowe and Cooney, 2015). In any case, there should be an agreement on the phenotypic traits to investigate for age-related changes. One solution offered to us to broaden our thinking is to consider aging in its entire diversity by moving beyond humans to adopt a comparative perspective.

Indeed, our very conception of aging is focused on humans and model organisms for human aging (Mikuła-Pietrasik et al., 2021). To better understand the variation in life-history and aging trajectories from an evolutionary standpoint, we need interdisciplinary approaches studying long-lived species, ideally under natural conditions. To investigate the link between sociality, health, and aging, the study of a range of species with complex social systems will be imperative. In this thesis, I investigate social and physiological manifestations of aging in a wild population of Assamese macaques (*Macaca assamensis*). In this first chapter, I briefly present the concept of aging from an evolutionary perspective and illustrate how aging translates into decreasing survival and reproduction. Then, I introduce the manifestations of aging in other traits because we know much less here, which is why my work focuses on age-related changes in social behavior, hormonal systems, and the gut microbiome. In the third part, I emphasize on the interdependence between sociality and physiology tied through the sociality-health nexus, and how it relates to aging. In the last part, I detail the aims and approaches followed in this thesis.
1.1 Evolution of aging

Aging, or senescence, is the deterioration of physiological functions that leads to an increased probability of death and a decrease in reproductive success with advancing age (Kirkwood and Austad, 2000; Maklakov and Chapman, 2019). The phenomenon is so ubiquitous in our immediate environment that it may seem inevitable, yet aging represents an evolutionary paradox. As natural selection favors the traits of those organisms that contribute the most offspring to the next generation, aging should be selected against. The key to this paradox is that the strength of natural selection weakens with age, because deleterious traits that preferentially affect the fitness of individuals with the least reproductive value are not strongly selected against (Haldane, 1941; Hamilton, 1966; Medawar, 1952; Williams, 1957). Williams (1957) was the first to suggest an explanation for the decrease in reproductive value with age, which for him was that external age-independent causes of mortality inevitably reduce the probability that any organism will rear offspring over time. Several non-mutually exclusive mechanistic theories have emerged from this understanding, suggesting that age-related declines result from the accumulation of deleterious mutations (Mutation Accumulation: Medawar, 1952), from pleiotropic alleles that are beneficial in early life but detrimental in late life (Antagonistic Pleiotropy: Williams, 1957), and from trade-offs between investment in soma repair and reproductive effort (Disposable Soma: Kirkwood, 1977). Although reassessment of Hamilton’s mathematical formulation of aging (Hamilton, 1966) has demonstrated that the decline in reproductive value is not due to extrinsic mortality, as postulated by Williams, but to the decreasing proportion of older age classes in the population over time (Moorad et al., 2019; Wensink et al., 2017; Williams et al., 2006), early contributions provided the evolutionary foundation for what has since become biogerontology.

Research on laboratory model organisms has led to major advances in our understanding of the molecular and cellular drivers of aging, including for example genomic instability, telomere attrition, and loss of proteostasis (López-Otín et al., 2013). Experiments on fruit flies (Drosophila melanogaster), nematode worms (Caenorhabditis elegans), yeast (Saccharomyces cerevisiae), and mice (Mus musculus) have demonstrated that lifespan can be extended by the mutation of single genes, caloric restriction, and drugs like metformin (Fontana and Partridge, 2015; López-Otín et al., 2013; Mikulà-Pietrasík et al., 2021). At the same time, several long-held assumptions have been challenged by accumulating evidence. For example, the concept of oxidative damage as a fundamental cause for aging is slowly being abandoned (Gladyshev, 2014; Selman et al., 2012; Speakman et al., 2015), the decoupling of reproductive performance and survival in fruit flies and nematodes is forcing nuances in the Disposable Soma theory (Cohen et al., 2020; Maklakov and Chapman, 2019), and positive
correlations between early and late-life performance support the notion of ‘positive pleiotropy’ (Maklakov et al., 2015).

The early emphasis placed on the contribution of extrinsic mortality to the declining strength of natural selection led Medawar and his successors to assume that only humans and domestic animals would senesce, whereas wild animals would seldom reach this stage (Kirkwood and Austad, 2000; Medawar, 1952). Possibly because of the focus on model organisms and their contribution to our understanding of human aging, the diversity of aging has only recently been recognized thanks to a more comparative perspective (Cohen, 2018; Jones et al., 2014). Evidence that some organisms do not age, even among those with distinct germ lines and soma, such as turtles and tortoises (da Silva et al., 2022), has questioned the rationale underlying the theories of aging (Jones et al., 2014) and contributed to nuance the contribution of extrinsic mortality to aging. The burst in the field of aging in non-model animal systems at the turn of the century was quickly followed by increasing research on aging in wild vertebrate populations (Nussey et al., 2013), and more recently plants (Gaillard and Lemaître, 2020; Roach and Carey, 2014). The original formulation predicting shorter lifespan and earlier onset of senescence under high (random) extrinsic mortality (broadly summarized by a ‘fast pace of life’ (Healy et al., 2019; Jones et al., 2008) has been extended to integrate how demographics and individual traits, such as population density (Abrams, 1993), condition-dependent mortality (Williams et al., 2006; Williams and Day, 2003), and the counterbalancing force of increasing fecundity with age (da Silva et al., 2022; Gaillard and Lemaître, 2020; Jones et al., 2014) interact to shape patterns of senescence in naturally occurring populations (Ronget et al., 2017).

Of course, it is important to bear in mind that beyond average aging trajectories, organisms exhibit immense heterogeneity (Alpert et al., 2019; Badal et al., 2020; Johnson et al., 2022), which allows to investigate sources of deviations between an individual’s expected (i.e., chronological) and realized (i.e., biological) age (Arneson et al., 2022; Hannum et al., 2013; Horvath, 2013; Johnson et al., 2022). Although biological age may closely depict changes occurring within each individual, assessing biological age is technically complex and still out of reach for most research especially in wildlife (Arneson et al., 2022), so chronological age is used throughout the literature reviewed below and in the present work. Having outlined the current consensus on the evolutionary roots of aging and the diversity of patterns, it is necessary to ask which are the broadly conserved manifestations of aging at the population and individual levels.

1.2 Manifestations of aging

In the following section, I will present how aging manifests itself with regards to mortality and reproduction, the two concepts most widely studied, but I will also highlight the need to consider aging
in other traits, both across and within species if we are to answer some of the most pressing challenges in the field of biogerontology (Gaillard and Lemaître, 2020).

1.2.1 Aging in life-history traits

Actuarial senescence is the most common operational definition of aging which focuses on describing the increase in mortality with age (Gaillard and Lemaître, 2020; Monaghan et al., 2008). The phenomenon is found in organisms as phylogenetically distant as bacteria, insects and mammals (Jones et al., 2014, 2008), but exhibit marked variation between and within taxa. Comparisons can only be made using common metrics, and in practice, actuarial senescence has been studied either through lifespan or age-dependent mortality rates (Ronget and Gaillard, 2020). Lifespan expresses the pace of aging and allows to compare short-lived to long-lived species, whereas age-specific mortality expresses the shape of aging and is modeled by continuous statistical functions such as the Gompertz or the Weinbull laws (Ronget and Gaillard, 2020). Maximum lifespan, life expectancy at birth (i.e., mean age at death reached by individuals in the population), or 90% longevity (i.e., age at which 90% of the individuals have died) are all metrics for the pace of aging, each of which has advantages and shortcomings (Ronget and Gaillard, 2020). Maximum lifespan is currently available for a wide range of taxa and is nowadays easily accessible in public databases (e.g., AnAge, De Magalhães and Costa, 2009), but has been criticized for its sensitivity to outliers (Ronget and Gaillard, 2020). Life expectancy and 90% longevity are more robust metrics but require more detailed data on wild populations and call for long-term field research (Clutton-Brock and Sheldon, 2010).

Together, these metrics have revealed a number of insights, like the fact that lifespan and aging rate may differ between sexes (Bronikowski et al., 2022; Clutton-Brock and Isvaran, 2007; da Silva et al., 2022), with females living longer and aging more slowly than males in most (but not all) mammals (Lemaître et al., 2020b), including humans (Austad, 2006; Colchero et al., 2016); that some species exhibit no signs of aging, irrespective of their lifespan (da Silva et al., 2022; Reinke et al., 2022); and that the rate of aging is slower in species protected from extrinsic mortality, either by physical shields (e.g., turtles) or specific habitats, namely aerial (e.g., birds and bats), underground (e.g., mole rats), or arboreal (e.g., primates) (Reinke et al., 2022; Ricklefs, 2010; although see Moorad et al., 2019). Whether disparities between taxa or sexes result from differences in genetic content or from local environmental conditions is actively debated (Austad and Fischer, 2016; Bronikowski et al., 2022; Gaillard and Lemaître, 2020; Lemaître et al., 2020b). For example, across vertebrates, shorter male lifespan and faster aging appear to be mostly confined to polygynous species where males are under strong sexual selection (Clutton-Brock and Isvaran, 2007; Tidière et al., 2015; but see Lemaître et al., 2020b). However, this finding may not be a universal pattern, as shown by the example that in roe
deer (*Capreolus capreolus*), a polygynous ungulate, longer female lifespan is not observed in all populations (Garratt et al., 2015), highlighting the role of local environmental pressures in shaping actuarial senescence.

Due to the direct relationship between reproduction and fitness, reproductive aging is often investigated alongside the link between aging and mortality (Nussey et al., 2013), also in light of a human-focus interest on post-reproductive lifespan (Croft et al., 2015). Fertility (Aubry et al., 2009; Martin and Festa-Bianchet, 2011), miscarriage (Fogel et al., 2023), litter or clutch size (St. Lawrence et al., 2022; Tidière et al., 2018), interbirth interval (Campos et al., 2022; Hoffman et al., 2010), probability to wean offspring (Catry Paulo et al., 2006), and offspring survival to maturity (Bouwhuis et al., 2009; Campos et al., 2022; Hoffman et al., 2010) have all been found to decline with age in taxa as distantly related as meerkats (*Suricata suricatta*) (Sharp and Clutton-Brock, 2010), kitiwakes (*Rissa tridactyla*) (Aubry et al., 2009), albatrosses (*Thalassarche chrysostoma*) (Catry Paulo et al., 2006), non-human primates (Campos et al., 2022; Hoffman et al., 2010; Tidière et al., 2018), yellow-bellied marmots (*Marmota flaviventer*) (St. Lawrence et al., 2022), and bighorn sheep (*Ovis canadensis*) (Martin and Festa-Bianchet, 2011). The decline in reproductive success with aging is found in both sexes, for example in wandering albatrosses (*Diomedea exulans*), where father’s age is associated with lower fledging success (Lecomte et al., 2010), and ruffed lemurs (*Varecia sp.*), where mother’s age is negatively correlated to offspring survival (Tidière et al., 2018). The deterioration in reproductive function is not necessarily linear with age but may exhibit accelerated decline in the last years of life, or may vary independently of chronological age, rather decreasing with respect to an individual age at death (Martin and Festa-Bianchet, 2011; Reed et al., 2008). Actuarial and reproductive senescence may also appear to be decoupled if individuals increase their terminal investment in reproduction at the end of life (Duffield et al., 2017; Hoffman et al., 2010). Overall, reproductive senescence occurs in the majority of free- or semi-free ranging female mammals and birds studied to date (Vágási et al., 2021).

To summarize, evidence of actuarial and reproductive senescence in non-human animal populations has contributed to bridge some of the ultimate gaps between aging, fitness, and natural selection. Often studies have investigated survival or reproduction, and combining them is a first important step, but ultimately it is necessary to investigate the other traits underlying an organism’s ability to survive challenges, mobilize energy, and invest in reproduction as they may be themselves subjected to aging.
Chapter 1

1.2.2 Manifestations of aging in other traits

“[...] while we now have fairly accurate knowledge of demographic senescence patterns in the wild, at least in mammals and birds, we know much less about age-specific changes in other biological parameters associated with physiology, life history or behaviour.” Gaillard and Lemaître 2020, p.5

When studying aging, we must bear in mind that survival results from a mixture of extrinsic and intrinsic mortality, and that both are likely to depend, at least to some extent, on the performance of many morphological, physiological, and behavioral traits. Similarly, the declining reproductive performance observed across a wide range of taxa calls for an investigation of somatic functions over age, while acknowledging that decoupled reproductive and somatic aging exists, for example among species with extended post-reproductive lifespan (Alberts et al., 2013; Croft et al., 2015). Furthermore, the preconception that aging occurs synchronously within individuals is being challenged by both conceptual and empirical work (Fay et al., 2021; Hayward et al., 2015; Moorad and Ravindran, 2022), allowing to explore patterns of aging in various traits to infer on the strength of selection pressure applied. Finally, if the aim is to learn from comparative research to inform human medicine, then healthspan will be better approximated by other systems than by comparing taxa based solely on the shape of actuarial and reproductive senescence. Some of the best documented traits to date are physiology and sociality (Gaillard and Lemaître, 2020; Nussey et al., 2013; Partridge et al., 2018; van den Beld et al., 2018).

1.2.2.a Physiological aging

One of the most studied physiological markers of aging is body mass because variations in body mass provide important cues about the organism’s state of energy balance, and loss of body mass has been associated with lower fertility and higher mortality risk (Denryter et al., 2022; Gaillard et al., 2000; Kroeger et al., 2018; Nussey et al., 2011). Age-related loss of body mass is documented in birds (Catry Paulo et al., 2006), ungulates (Nussey et al., 2011), primates (Altmann et al., 2010; Emery Thompson et al., 2020b; Hämläinen et al., 2014), yellow-bellied marmots (Kroeger et al., 2021), and can be gradual or accelerated preceding death (Kroeger et al., 2021; Nussey et al., 2011). Decreases in fat and muscle may be caused by reduced feeding ability owing to tooth wear, declining physical strength in aerial and arboreal species, or hunting skills (Altmann et al., 2010; Catry Paulo et al., 2006; Frankish et al., 2020; Hämläinen et al., 2015a; Lecomte et al., 2010; MacNulty et al., 2009; Pelletier and Festa-Bianchet, 2004). However, old individuals can overcome age-related deterioration in more favorable environments (Kroeger et al., 2021, 2018) or by investing relatively more resources towards the maintenance of body mass (Kroeger et al., 2021; Nussey et al., 2011). At least among mammals and birds, the general trend points to decreasing body mass post sexual maturity being modulated by the
ability of old individuals to prioritize energy allocation to body condition. Despite its value, measurement of body mass is not possible in all situations (e.g., research on large mammals), so biomarkers allowing more direct assessment of energy balance could be particularly informative. Biomarkers of energy mobilization, energy intake and metabolic activity, such as thyroid hormones, are especially promising (Behringer et al., 2018; Girard-Buttoz et al., 2011; Heistermann et al., 2006) and could contribute to assess age-related changes in body condition, although this requires to first establish their link to energy balance and their suitability to conditions associated with field research.

More recently, other age-related physiological changes have also been documented, encompassing a wide range of immunological, hormonal, and microbiome systems. Multidimensional physiological data aggregated into a single metric have demonstrated a common pattern of physiological dysregulation with advancing age in humans and several captive non-human primates (Cohen et al., 2013; Dansereau et al., 2019; Kraft et al., 2020). The changes underlying this dysregulation are especially pronounced among immune cells, with a decline of immune activity referred to as immunosenescence, translating into reduced innate immunity through decreased phagocytosis and natural killer cell activity (Blacher et al., 2022; Linehan and Fitzgerald, 2015), altered inter-cellular chemokine signaling (Blacher et al., 2022; Metcalf et al., 2020), depletion in the pool of naïve lymphocytes associated to an accumulation of deficient clones (Alpert et al., 2019; Linehan and Fitzgerald, 2015), and overall heightened pro-inflammatory profiles (Franceschi et al., 2017; Müller et al., 2017; Watowich et al., 2022). Among hormonal systems, age is associated with marked changes in glucose homeostasis, insulin resistance, and the hypothalamic-pituitary-peripheral organ axes (Clegg et al., 2013; van den Beld et al., 2018). Strikingly, the microorganisms communities residing in the mouth, gut, genitalia, and on the skin exhibit decreasing diversity and stability as humans age (Badal et al., 2020; Bana and Cabreiro, 2019; Bosco and Noti, 2021; Ghosh et al., 2022; O’Toole and Jeffery, 2015) even though they are not part of the soma per se.

These findings are just some examples of the profound physiological changes occurring with age and which contribute to age-related delayed wound healing, increased risk for several types of cancer and cardiovascular diseases, and greater frailty (i.e., general decline in health and disease resistance) (Clegg et al., 2013; Lemaître et al., 2020a; Linehan and Fitzgerald, 2015). The adaptive value of some of those processes are, however, still debated. For example, some have argued that age-related increase in inflammation, negatively coined as inflammaging (Franceschi et al., 2017), may be the most efficient defense against pathogens in a context of depleted humoral immunity (Fulop et al., 2016). Broadening the scope to other species will likely reveal informative on the correlates of physiological changes with advancing age. As such, evidence of immunosenescence in wildlife is accumulating (Cheynel et al., 2017; Nussey et al., 2013, 2012; Peters et al., 2019). However, most investigations have been cross-
sectional with the risk that selective disappearance of individuals with poor immune defenses biases profiles measured at old age when survival co-varies with immune functions (Nussey et al., 2008; van de Pol and Wright, 2009). One noticeable longitudinal study found that Soay sheep (Ovis aries) exhibit a gradual decline in antibody titers with age before a sharp drop predicting death in the following winter (Froy et al., 2019), providing rare evidence for a fitness correlate of immunosenescence in the wild. On the contrary, longitudinal immune profiles in common terns (Sterna hirundo) did not match expected immunosenescence (Bichet et al., 2022).

Investigating physiological aging in free-ranging animal populations requires the use of non-invasive methods whenever handling or blood sampling raises practical or ethical concerns, which limits the range of available biomarkers. In this context, the study of hormonal profiles and gut microbiome appear especially promising. The development of methods to measure hormones from urine and feces has opened up many possibilities for the investigation of physiology in wildlife (Higham, 2016). Validated measurements of steroid hormones (Heistermann et al., 2004) and biomarkers of immune activation (Behringer et al., 2017; Heistermann and Higham, 2015) are beginning to provide insights into reproductive (Altmann et al., 2010) and immunological (Cooper et al., 2022; Dibakou et al., 2020; Müller et al., 2017) aging.

Recently, the gut microbiome has become a major target in both human and animal aging research. The gut microbiome consists of the community of *Bacteria*, microbial eukaryotes, *Archaean*, and viruses residing in the gastrointestinal tract and engaging in continuous interactions with each other and the host (Björk et al., 2019; Miller et al., 2018). This ecosystem’s composition is highly dynamic, shaped by competitive and cooperative microbial interactions (Coyte et al., 2015), as well as host-microbes interactions. The gut microbiome affects host health by conditioning immune responses, metabolic processes, and neurological functions, while host dynamics in turn impact microbial communities (Björk et al., 2019; Ezenwa et al., 2012; Miller et al., 2018; Sherwin et al., 2019). Disrupting this often mutualistic relationship can lead to dysbiosis, which is increasingly implicated in metabolic, digestive, and age-related diseases (Askarova et al., 2020; Badal et al., 2020; Bana and Cabreiro, 2019; Ghosh et al., 2020, 2022; Popkes and Valenzano, 2020; Ragonnaud and Biragyn, 2021; P. Smith et al., 2017). Consequently, gut microbiome health has been introduced as a new hallmark of aging (López-Otín et al., 2023; Schmauck-Medina et al., 2022), with potential as an early diagnostic tool for frailty (Jackson et al., 2016; Renson et al., 2020). As an example, ‘gut clocks’ gathering information on microbiome diversity, taxonomic and metabolic composition outperform epigenetic clocks in detecting age-related diseases and frailty (Ratiner et al., 2022). A less diverse and less stable gut bacterial composition, as well as enriched energy-related pathways have been described in older human patients (Badal et al., 2020; Bana and Cabreiro, 2019; Ghosh et al., 2022). However, the relationship between age and the
gut microbiome depends on cultural, environmental, and lifestyle variables which prohibits the generalization of patterns in WEIRD panels (de la Cuesta-Zuluaga et al., 2019; Lloyd-Price et al., 2016; Yatsunenko et al., 2012). Furthermore, “dysbiosis” has a broad definition that encompasses various scenarios, including the shift in dominant microbial groups, the microbial composition seen in sick patients, or any differences in the abundance of specific microbial taxa between a healthy state and a state of interest (Berg et al., 2020; Hooks and O’Malley, 2017). Finally, it remains unclear whether aging of the host per se is the cause for these changes, as gut bacterial composition is extremely sensitive to lifestyle-linked factors (often) associated with aging (e.g., reduced caloric intake, medication use).

Fecal samples can be used to non-invasively assess gut microbiome communities, making them suitable for wild animal research. Investigations in natural populations have revealed that gut microbial composition is influenced by seasonal cycles, diet, sex, and host genetics (Grieneisen et al., 2020; Hicks et al., 2018; Huang et al., 2022; Murillo et al., 2022a; Trosvik et al., 2018), and undergoes significant changes during early development (Baniel et al., 2022; Petrullo et al., 2021). The influence of host aging has received comparatively little attention (Björk et al., 2019). Furthermore, investigations have focused mostly on gut microbial diversity and composition similarity across hosts (Janiak et al., 2021; Perofsky et al., 2017; Reese et al., 2021), which only provide partial insight into this dynamic and complex ecosystem. Which features of the gut microbiome exhibit aging and whether these changes are conserved across species, are central questions left unanswered.

To summarize, the sheer number of physiological traits associated with senescence imply that it is very unlikely that a single consistent pattern of physiological senescence will ever emerge. Nevertheless, one take-home message from wildlife research is that life under natural conditions is compatible with the slow deterioration of, at least some, physiological functions. Which of these functions are under the most pressure from natural selection is still unclear (Hayward et al., 2015; Moorad and Ravindran, 2022). Although not necessarily fatal, declining physiological traits nevertheless impose constraints which must be mitigated, and it is therefore important to understand how individuals cope with these changes.

1.2.2.b Social aging

Behavior plays a key role in allowing individuals to adapt to physiological changes, by altering their activity budget (Corr, 2003; Frankish et al., 2020; Rathke and Fischer, 2021; Veenema et al., 1997), roaming strategies (Frankish et al., 2020; Froy et al., 2018), and diet selection (Galbany et al., 2011; Logan and Sanson, 2002; Venkataraman et al., 2014), which in turn may contribute to age-structured spatial distribution at the population level (Albery et al., 2022; Frankish et al., 2020; Froy et al., 2018; Lecomte et al., 2010). As such, behavioral changes represent another trait that ‘ages’. These changes
Chapter 1

not only concern behavior centered on self, but further extend to social interactions in group living species. Flexible behavioral strategies can then serve to mitigate decreasing physical competitive ability (Fischer et al., 2004), as seen in aging male non-human primates, who may switch to more opportunistic mating strategies, invest in affiliative interactions with females as an alternative to costly mate-guarding activity (Silk et al., 2020), provide more parental care (Silk et al., 2020), or engage increasingly in coalitions with other males (Berghänel et al., 2011; Rathke et al., 2017).

A striking feature of social behavior among aging humans is a general tendency to decrease social engagement with advancing age, which may translate into reduced time spent social, decreasing social network size, or both (Cornwell et al., 2021; Lang et al., 1998; Lang and Carstensen, 1994; Mor-Barak and Miller, 1991; Müller and Ellwardt, 2022; Röhr et al., 2020; Zheng and Chen, 2020). Several competing theories, mostly coming from psychological research rooted in human social psychology, have attributed this decline in turn to increasing lack of interest in the social world (Cumming and Henry, 1961), increasing frailty (reviewed in Müller and Ellwardt, 2022), and gradual shift towards more emotionally-rewarding relationships (Charles and Carstensen, 2010), and suggested models for successful aging based on the maintenance of physical activity, social engagement, or the achievement of age-specific goals (Antonucci et al., 2014; Carstensen, 2019; Cumming and Henry, 1961; Freund et al., 2021; Rowe and Kahn, 1997, 1987). Recently, evidence of social withdrawal among non-human mammals has broadened our appreciation of social aging (Machanda and Rosati, 2020; Siracusa et al., 2022a), offering an opportunity to test the involvement of cognitive and psychological mechanisms in social withdrawal (e.g., increasing social selectivity, decreased motivation) (Almeling et al., 2016; Nakamichi, 2003, 1984; Rathke et al., 2022; Rathke and Fischer, 2021, 2020; Rosati et al., 2018, 2016; Rosati and Santos, 2017), and to anchor social aging in broader life-history theories (e.g., kin selection, competitive exclusion) (Albery et al., 2022; Kroeger et al., 2021; Siracusa et al., 2022b). On the downside, most studies on social aging are cross-sectional or fail to account for potential cohort effects, which emphasizes the need to investigate social aging with longitudinal approaches (Nussey et al., 2008; Rohrer and Murayama, 2023).

It is becoming clear that ‘aging’ refers to a collection of biological changes which increase the risk of mortality. Which traits show aging, and which do not? Which trait-specific declines best translate into whole-organism aging? These are unresolved questions that would require, as a first step, finer characterizations of aging in a variety of traits. Captive animals have provided critical insights into physiological aging, that need to be confirmed in their wild counterparts using validated non-invasive markers of energetic state and inflammation, and by investigating the gut microbiome. The importance of social behavior in mediating age-related constraints is becoming increasingly clear, calling for a more
holistic view of aging. Investigating these issues in natural populations will provide a better picture of the trade-offs associated with the type of environment in which aging patterns have evolved.

1.3 Interdependences in aging systems

Above, I briefly described how some of the changes in behavioral and physiological traits that occur with age ultimately contribute to declining performance in survival and reproduction. Although I presented those systems independently, evidence points to their interdependence, which suggests that aging in one system may precede and promote aging in another. Notably, physiological functions are major building blocks of an organism’s health state, which has been repeatedly linked to sociality in animals, including humans (Kappeler et al., 2015; Ostner and Schülke, 2018; Snyder-Mackler et al., 2020). As sociality and physiology are interdependent, i.e., social behavior impacts physiology and vice versa, determining the independent influence of age on sociality and age on physiology can be challenging. I will discuss the current state of knowledge on these complex links in the following section.

1.3.1 Sociality and health

A first important consequence of group living is heightened exposure to pathogens (Kappeler et al., 2015). Body contact and mouth-to-hand interactions, such as grooming, promote the acquisition of parasites from social partners, and consequently, more socially connected and more central individuals in a network face higher parasitism (Briard and Ezenwa, 2021). Even in the absence of direct contact, individuals can become infected by picking up parasites from contaminated soil (Friant et al., 2016; Müller-Klein et al., 2019). This implies that the markers of immune and endocrine activity discussed above will be partly influenced by the social position of individuals through the link between sociality and parasitism (Defolie et al., 2020). Social transmission of microorganisms applies not only to viral and gastrointestinal parasites, but also to gut microbes (Sarkar et al., 2020). Shared environments and close interactions facilitate the horizontal transmission of microbes (Ezenwa et al., 2012; Sarkar et al., 2020; Sherwin et al., 2019), which homogenizes microbial compositions within populations (Björk et al., 2022; Perofsky et al., 2017; Rudolph et al., 2022), especially between strongly connected individuals (Murillo et al., 2022b; Raulo et al., 2021; Sarkar et al., 2020). Therefore, age-related changes in gut microbiome composition must integrate age-related changes in social behavior.

The frequency of social contact and number of social partners are quantitative aspects of sociality influencing physiology through the sociality-parasitism link, but qualitative aspects are also important. In humans, competitive, agonistic, and more broadly stressful, interactions elicit the activation of immune and neuroendocrine processes (Cohen et al., 2012; Hostinar et al., 2014). Experience of social
adversity translates into low-grade inflammation, increased prevalence of cardiovascular, endocrine, cancer and gastrointestinal diseases (Bergmann et al., 2014; Cole et al., 2007; Fuligni et al., 2009; Guarner and Rubio-Ruiz, 2015; Kiecolt-Glaser et al., 2010; Miller et al., 2011; Rohleder, 2014; Snyder-Mackler et al., 2020; Tamashiro et al., 2011). Strong evidence for the causal influence of sociality on these biological processes has been provided by experiments with animal models (Adams et al., 1985; Capitanio et al., 1998; Cohen et al., 1997; Hawkley and Capitanio, 2015; Shively et al., 2009, 1997; but see Zane et al., 2021). For example, experimentally manipulated low dominance rank predicted dampened anti-viral response in favor of a pro-inflammatory immune phenotype in female rhesus macaques (*Macaca mulatta*) (Snyder-Mackler et al., 2016), and greater susceptibility to cold infections in male long-tailed macaques (*Macaca fascicularis*) (Cohen et al., 1997). Conversely, a positive social environment provided by the presence of strongly bonded partners has a beneficial effect on individual health and disease resistance (Ezenwa et al., 2016b), due to a subjective feeling of integration and an effective partner support (Cohen et al., 2015, 1992; Cohen and Wills, 1985; Donovan et al., 2018; Hennessy et al., 2009; Hostinar et al., 2014; Young et al., 2014). Therefore, assessing aging of neuroendocrine functions requires integrating different aspects of sociality.

Investigations of the links between sociality and neuroendocrine activity in wildlife have relied largely on the non-invasive measurements of glucocorticoids since they are excreted unchanged or as metabolites in several matrices (e.g., saliva, feathers, hair, feces, and urine), some of which can be collected non-invasively. Activation of the hypothalamic-pituitary-adrenal axis triggers the secretion of glucocorticoids (GCs) involved in maintaining homeostasis in response to predictable (e.g., seasonal migration) and unpredictable (e.g., predator attack, major injury) events, the latter being commonly referred to as stressors (McEwen and Wingfield, 2003; Romero et al., 2009). GC concentrations are often elevated after agonistic interactions (Wittig et al., 2016; Young et al., 2014) or at times of heightened competition (Petrullo et al., 2022a; Sadoughi et al., 2021b), and are lowered by friendly interactions (Wittig et al., 2016) and in those benefiting from greater social support (Brent et al., 2011; Fürtbauer et al., 2014), illustrating the value of measuring GCs concentrations to investigate the physiological correlates of sociality in wildlife (Beehner and Bergman, 2017).

A critical difference between humans (and animal models) and wildlife may be the consequences of repeated HPA axis activation. In humans and animal models, repeated elevations of GCs outside of the physiological range are thought to cause wear and tear on the organism (Allostatic Load: McEwen and Wingfield, 2003; Reactive Scope: Romero et al., 2009), lowering the capacity to cope with stressor over time and leading to deleterious outcomes (Charmandari et al., 2005; Sapolsky, 2004; Sapolsky et al., 2002). Whether the social activation of HPA axis in natural living environments is associated to some of the chronic stress effects seen in humans is strongly debated (Beehner and Bergman, 2017; Bonier
et al., 2009a; Dickens and Romero, 2013; MacDougall-Shackleton et al., 2019; Schoenle et al., 2021; Scott et al., 2012; Vitousek et al., 2019b).

This debate is important for our understanding of the relationship between age and GCs. In humans and animal models, age-related changes in GCs are thought to result from the gradual accumulation of damage (Glucocorticoid Cascade Hypothesis: Sapolsky et al., 2002). Studies have documented several age-related changes in the diurnal rhythm, baseline, and stress-induced levels of GCs (Dodt et al., 1991; Ferrari et al., 2001; Goncharova et al., 2019; Goncharova and Lapin, 2002; Gust et al., 2000, 2000; Nathanielsz et al., 2020; Otte et al., 2005; Reul et al., 1991, 1991; Sapolsky, 1992a; Touitou et al., 1983; Van Cauter et al., 1996, 1996; Yang et al., 2017), some stronger in women than in men (Born et al., 1995; Otte et al., 2005; Van Cauter et al., 1996). Nevertheless, the picture is far from clear, with many examples reporting no changes or changes in only some aspects of GC physiology (Born et al., 1995; Dodt et al., 1991; Goncharova et al., 2019; Goncharova and Lapin, 2002; Harris and Saltzman, 2013; Nathanielsz et al., 2020; Yang et al., 2017; Zambrano et al., 2015), and most studies contrasting profiles between two groups rather than evaluating changes over as much of adulthood as possible. In the wild, the relationship between age and GCs has been considered in relation to GC-mediated trade-offs between reproduction and stress-responsiveness in birds (Heidinger et al., 2010; Lendvai et al., 2015; Schoenle et al., 2021; Vitousek et al., 2019b). Individuals are thought to down-regulate HPA axis activity or sensitivity to GCs when the value of current reproduction is higher than future reproduction (Angelier et al., 2007; Elliott et al., 2014). In mammals, which typically display a higher rate of physiological aging than birds (Ricklefs, 2010), no clear pattern has yet emerged, with increased (Altmann et al., 2010; Campos et al., 2021; Charpentier et al., 2018; Emery Thompson et al., 2020a; Pavitt et al., 2016, 2015), decreased (Maestripieri and Georgiev, 2016), or unchanged GCs (Gesquiere et al., 2011) with age. Crucially, only three studies accounted for social traits (Campos et al., 2021; Charpentier et al., 2018; Emery Thompson et al., 2020a), and none tried to disentangle changes that may arise from demographics rather than aging processes.

In summary, social relationships have multiple and complex influences on physiology, resulting in important inter-individual differences in health. All of these mechanisms likely contribute to the association between greater social support and lower mortality risks in humans and other animals (Archie et al., 2014; Holt-Lunstad et al., 2015; Kajokaite et al., 2022; Maestripieri and Georgiev, 2016; Marescot et al., 2018; Marmot and Sapolsky, 2014; Ostner and Schülke, 2018; Silk et al., 2010; Snyder-Mackler et al., 2020; Thompson, 2019). Despite an overall clear benefit of sociality, notable exceptions point to context dependence, suggesting that relationships imposed by habitat saturation or unstable bonds are detrimental to survival (Blumstein et al., 2018; Thompson and Cords, 2018). These links between sociality and physiology are also scrutinized in explaining differences in lifespan not only
within but also between species, as comparative data indicate that eusocial insects and social mammals live longer than their asocial counterparts (Zhu et al., 2023; but see Lucas and Keller, 2020 for critical discussion across species).

However, whether the benefits and shortcomings of sociality are experienced uniformly across the lifespan is unknown. To date, few studies have attempted to contrast the contribution of social network size to survival or health across life stages. One study in humans found social network size to impact the health of the young and the elderly more compared to middle-aged subjects (Yang et al., 2016), whereas another study in humans and one in macaques found the opposite (Brent et al., 2017; Holt-Lunstad et al., 2015). Age-dependent benefits of sociality can arise in cross-sectional studies if those reaching old ages are especially robust, or if benefits of sociality earlier in life determine the susceptibility to hazards later on (De Nys et al., 2017). To make progress, it is necessary to investigate age-related changes in sociality in longitudinal studies across more diverse contexts, and where possible, to account for age-specific optima when studying sociality-physiology dynamics.

1.3.2 Physiology and sociality

The previous section illustrates how social stimuli trigger neuroendocrine responses and may influence vital physiological functions. Evidence demonstrates that neuroendocrine responses can in turn play a strong role in modulating behavior (Raulo and Dantzer, 2018). Elevations in GCs appear to inhibit proactive social behavior while promoting pair-bonding and cooperation among group members (Raulo and Dantzer, 2018). Another example of reversed causality between physiology and sociality is the influence of skin and gland microbial communities on the host's olfactory signaling with conspecifics, susceptible to affect recognition and bonding (Ezenwa et al., 2012; Müller-Klein et al., 2019; Poirotte et al., 2017; Sherwin et al., 2019).

Physiological conditions that lead to deteriorating health or poorer physical condition may impact social integration and social status, or cause individuals to withdraw from their social environment (Kröger et al., 2015). However, little effort has been devoted to the causal influence of frailty on sociality, with mixed results. In humans, physical decline appears to have less impact on the social lives of older people compared to cognitive decline, because subjects effectively shift the composition of their network to family members or receive increasing support from extended networks, while opportunities to compensate for the loss of distant network ties seems absent when cognition declines (Aartsen et al., 2004; Cornwell, 2009; Li and Zhang, 2015). However, others have found no influence of subjective, physiological, or cognitive health on social network size (Mor-Barak and Miller, 1991). In other mammals, declining locomotor skills, sensory system acuity, cognitive functions, and the physiological changes reviewed above, are all thought to contribute to decreasing social engagement.
with age (Machanda and Rosati, 2020; Siracusa et al., 2022a), although direct causal evidence is lacking.

The links within the sociality-physiology-health nexus are complex and possibly include self-reinforcing loops, with individuals having access to poorer social resources experiencing more rapid declining health, which in turn results in further social withdrawal. To improve our understanding of how the causes and effects of the social environment on individual health transcend into variation in aging processes, and how health and aging in turn impact social behavior, long-term, holistic studies of social animals under natural conditions that include detailed information on life-history, social environment, physiology, immune function, and frailty will be imperative.

1.4 This thesis

1.4.1 Study species

Assamese macaques (*Macaca assamensis*) are cercopithecine primates native to South and South-East Asia (Schülke et al., 2011; Thierry, 2007), and the population studied at the Phu Khieo Wildlife Sanctuary (PKWS) in Thailand inhabits the species’ southern-eastern range. Assamese macaques are primarily arboreal, exhibit female philopatry with male dispersal, and live in female-bonded multi-male-multi-female social groups (Ostner et al., 2013; Richter et al., 2016; Schülke et al., 2011). Female social structure is organized into matrilines of adjacently ranked kin, with mostly stable dominance relationships across and within matrilines. Juvenile and subadult males emigrate from their natal group between roughly three to seven years of age and migrate repeatedly throughout their life. Dominance among males is achieved according to competitive ability, which may rely on the combination of individual quality and the formation of alliances (Schülke et al., 2010). Due to male dispersal, knowledge about female life-history trajectories is more complete, and ages in females can be estimated with more certainty, which is why this thesis focuses on female aging.

Assamese macaques are seasonal breeders with the vast majority of births occurring over three months, and a maximum spread from the first to the last birth of six months (Fürtbauer et al., 2010; Touitou et al., 2021a). Female fertility is concealed and receptivity is synchronized, contributing to a low paternity skew (Sukmak et al., 2014). Gestation lasts on average 164 days and interbirth intervals are bimodally distributed around one and two years (Fürtbauer et al., 2010). This pattern arises because females reproducing early in a birth season are more likely to resume cyclicity and conceive at the end of the next mating season, while females giving birth late in the birth season skip the next mating season. Female Assamese macaques are relaxed-income breeders relying on energy stored in a season of high food abundance several months before their fertile periods (Touitou et al., 2021a).
Lactation is timed to periods of high food availability, during which conception success is enhanced by food availability (Heesen et al., 2013), and nutritional requirements of gestation are met by behavioral, dietary, and physiological adaptations (Touitou et al., 2021a). Females’ first live birth coincides with growth cessation around six years of age (Anzà et al., 2022), and therefore first conception marks the transition to adulthood for this thesis.

Adult males and females form differentiated and lasting social bonds within and across sexes (Haunhorst et al., 2016; Kalbitz et al., 2016; Macdonald, 2014) which are known to be beneficial. In males, same-sex bonds enhanced coalitionary support (Schülke et al., 2010) and both same- and opposite-sex bonds increase male siring success (Ostner et al., 2013; Schülke et al., 2010). In females, female-female bonds provide increased feeding tolerance (Heesen et al., 2014), while male-female bonds are associated with higher levels of male support and increased foraging efficiency (Haunhorst et al., 2017). Therefore, females may be under strong pressure to maintain social investment into old age. Power asymmetries, owing to dominance rank among females, govern access to male partners (Haunhorst et al., 2020) and to feeding sites (Heesen et al., 2015, 2014), which lower-ranking females mitigate by adopting flexible feeding strategies (Heesen et al., 2015, 2014, 2013) rather than trading grooming for rank-related benefits (Macdonald et al., 2013).

The population has been followed since 2005, and for this thesis, I included data on physiology and social behavior collected from 2010 to 2020, spanning about half of the average adulthood of female macaques (Brent et al., 2017; Johnson and Kapsalis, 1995). Although very few individuals have complete life records, combined cross-sectional and longitudinal analyses offer an opportunity to address central issues surrounding social and physiological aging over a timespan associated with noticeable physical aging within individual (Figure 1).

Figure 1. Physical aging in a female Assamese macaque from 2006 to 2020 (left to right).
1.4.2 Aims and hypotheses

The general aim of this thesis was to investigate the social and physiological manifestations of aging in wild female Assamese macaques, and by doing so, contribute to pressing questions about the prevalence and characterization of aging across traits in a long-lived species (Figure 2). To address these issues, I collected behavioral data on adult female-female social interactions, and incorporated data from the long-term project at PKWS in my analysis. To investigate physiological aging non-invasively, I collected fecal samples to sequence bacterial 16S rRNA genes to reconstruct the composition of individual gut bacterial communities, validated the measurement of hormonal markers for the assessment of physiological response to energy shortage, and continued the long-term collection of fecal samples for GC metabolites analysis.

After this first chapter, introducing essential notions and pressing questions in the study of aging, I divided my investigations into five chapters. In doing so, special attention was paid to considering the interdependence between sociality and physiology. Although the work presented cannot directly test causal relationships, hypotheses are inevitably formulated following causal pathways. The first four chapters presented follow a progression from sociality towards physiology, while the fifth chapter offers perspective to explore the reverse path from physiology towards sociality.

To start on the social end of the sociality-health nexus, Chapter 2 of this thesis focuses on age-related changes in social relationships among adult females. In non-human animals, sociality is a loose term describing the position of an individual within its social environment inferred from spatial associations, affiliative, and agonistic interactions among group members (Hinde, 1976; Silk and Kappeler, 2017). Group living animals can form differentiated relationships between pairs of individuals creating group-level social network structures and dynamics. Social status and affiliative relationships are the most commonly studied dimensions of sociality, and the second is further subdivided into integration assessed by the number of ties, and bonding assessing relationships quality and arranged into strong bonds and weak ties (Schülke et al., 2022; Silk et al., 2018). Evidence from captive non-human primate populations suggests that individuals decrease the size of their social network with age but may at the same time partially offset the costs of social isolation by increasing selectivity for strongly bonded partners (Almeling et al., 2016). The benefits of sociality, and more specifically female-female relationships, identified in this population may be decisive to cope with external pressures and thereby constrain the social expression of aging. The aim of this study was to investigate whether females manifest social aging under natural predation and environmental pressures, and to test whether changes would be consistent with theories of social selectivity.
Moving along the sociality-health nexus, Chapter 3 of this thesis reviews the many links between sociality and health with a focus on primates. The aim is to provide a comprehensive summary of the mechanisms best documented and to point to innovative research perspectives, some of which are addressed further in this thesis.

Chapter 4 focuses on one hormonal system, the HPA axis. Previous investigations have shown that reproductive state, climatic conditions, and social interactions correlate with GC concentrations in female Assamese macaques (Fürtbauer et al., 2014; Touitou et al., 2021b). However, it remains unclear whether GC profiles shift across the lifetime, and whether those changes could be attributed to endocrinological aging or be rather mediated by social aging. In a longitudinal analysis of the long-term hormonal database, I investigate the relationship between age and GCs concentrations measured in feces (fGCs). The first aim is to test for an increase in fGCs consistent with the ‘Glucocorticoid Cascade Hypothesis’, while accounting for circannual variations in fGCs dictated by climatic conditions and the influence of reproductive state. To disentangle the influence of age from the potential confounding effects of social decline (cf. Chapter 2), I examine whether any association between age and fGCs persists after controlling for social bonding and dominance status.

Chapter 5 investigates age-related modifications in the composition of gut bacterial communities using a set of complementary metrics based on data collected over one and a half years and analyzed cross-sectionally. The gut microbiome undergoes profound modifications with age in humans and is scrutinized for its contribution to both healthy and pathological aging. Whether those modifications are features of a widely shared transitions from adulthood to late-life or merely the consequence of modern human lifestyle is unknown because age-related changes in the gut microbiome are strikingly understudied in other natural mammalian populations. Thereby, I test the often-cited loss of gut bacterial diversity with age, the causal relationship between diversity and stability, and the age-structuring of gut bacterial composition. I further investigate whether sociality mediates changes in gut microbiome to assess the interdependence between social and physiological aging.

After exploring aging via the sociality-health nexus from sociality to physiology, Chapter 6 opens on the perspective to explore the influence of physiological aging on social aging. In this introductory chapter, I highlighted the need for biomarkers of energetic status and body condition, so Chapter 6 is a contribution to the development of non-invasive markers of metabolic response. Urine samples originally collected for the validation of C-peptide in captive macaques (Girard-Buttoz et al., 2011) were analyzed for concentrations of triiodothyronine and cortisol. Both hormones contribute to trade-offs between energy mobilization and allocation, but their profiles have not been simultaneously
investigated under controlled conditions nor linked to variation in body mass, which are necessary steps prior to their application in field research as biomarkers of aging.

**Figure 2.** Overview of the physiological and social traits investigated for age-related changes in this thesis. Traits are in bold, components belonging to one trait are in plain text, and those bridging between two traits are in italic. Created in BioRender.com.
Chapter 2

Social network shrinking is explained by active and passive effects but not increasing selectivity with age in wild macaques

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Abstract

Evidence of social disengagement, network narrowing, and social selectivity with advancing age in several non-human animals challenges our understanding of the causes of social ageing. Natural animal populations are needed to test whether social ageing and selectivity occur under natural predation and extrinsic mortality pressures, and longitudinal studies are particularly valuable to disentangle the contribution of within-individual ageing from the demographic processes that shape social ageing at the population level. Data on wild Assamese macaques (Macaca assamensis) were collected between 2013 and 2020 at the Phu Khieo Wildlife Sanctuary, Thailand. We investigated the social behavior of 61 adult females observed for 13,270 hours to test several mechanistic hypotheses of social ageing and evaluated the consistency between patterns from mixed-longitudinal and within-individual analyses. With advancing age, females reduced the size of their social network, which could not be explained by an overall increase in the time spent alone, but by an age-related decline in mostly active, but also passive, behavior, best demonstrated by within-individual analyses. A selective tendency to approach preferred partners was maintained into old age but did not increase. Our results contribute to our understanding of the driver of social ageing in natural animal populations and suggest that social disengagement and selectivity follow independent trajectories during ageing.

Keywords

senescence, social behavior, social selectivity, primate, longitudinal study, wild population
Introduction

The world’s human population is ageing rapidly, reaching an estimated 2 billion people over the age of 60 by 2050 (United Nations, 2017). This challenge to societal organizations and health care systems, can be better met by promoting healthier ageing. One of the keys to healthy ageing may lie in the positive influence of strong social integration on health (Evans et al., 2019; Snyder-Mackler et al., 2020). Concerns therefore arise from research showing that people reduce the size of their social network and their level of social engagement with age (Müller and Ellwardt, 2022). The causes and consequences of this reduction in social engagement are still actively debated (Evans et al., 2019; Müller and Ellwardt, 2022).

Several causal models for reduced social engagement are anchored in the framework of age-related losses (e.g., activity and social exchange theories, reviewed in (Müller and Ellwardt, 2022)). Accordingly, individuals experiencing frailty, sickness, or loss of social value with age are unable to maintain their social engagement due to physiological constraints, exclusion by younger generations, or diminished self-perception. Although in one case physiological decline translates into social decline while in the other exclusion hampers social engagement, in both cases, the reduction in network size and in time spent socially engaged is considered detrimental to wellbeing. However, reduced network size may also result from the tendency of older people to actively focus on preferred partners and positive relationships (e.g., socioemotional selectivity and convoy model theories, (Antonucci et al., 2014; Charles and Carstensen, 2010)). Recently, evidence is accumulating for decreasing social engagement in non-human animals (Albery et al., 2022; Hrdy and Hrdy, 1976; Nakamichi, 1991; Picq, 1992; Rathke and Fischer, 2021; Thompson González et al., 2021; Veenema et al., 1997) and particularly for social selectivity among non-human primates (Almeling et al., 2016; Rosati et al., 2020; Schino and Pinzaglia, 2018; Siracusa et al., 2022b).

The social selectivity in human self-reports can be translated into several predictions for observational studies quantifying animal interaction networks (figure 1). Differences arise from a need to test social features relevant to the study species, and because socioemotional selectivity theory remains vague about how exactly selectivity is expressed at the interaction level. Older individuals may focus their interactions on preferred partners and maintain their overall level of social engagement (Rosati et al., 2020; Siracusa et al., 2022b), with engagement in preferred partners compensating for the loss of more distant relationships (figure 1A). Alternatively, individuals may reduce their overall engagement (figure 1B), while maintaining a constant level of engagement with preferred partners (Lang and Carstensen, 1994; Veenema et al., 1997), possibly due to a loss of interest in less preferred partners. Finally, they may decrease their engagement in all relationships, but less so with preferred partners (figure 1C).
Social aging (Lang et al., 1998). While increasing selectivity would account for a reduction in network size in the first and second scenarios, the third suggests that another level of constraint is at play.

**Figure 1. Social selectivity with age can manifest in different ways.** (A) Social engagement does not change with advancing age, but individuals increasingly prioritize their preferred partners leading to a reduction in network size. (B) Social engagement decreases with advancing age but reduction in network size allows individuals to maintain constant engagement with their preferred partners. (C) Social engagement decreases with age imposing a reduction in network size and engagement with preferred partners, which may still be prioritized relatively to the other potential partners.

Although the socioemotional selectivity hypothesis focusses on the motivation of the ageing individual in explaining changes in social network and engagement, these changes could also result from several other mechanisms. First, since spatial proximity is a prerequisite for close range interactions, age-structured spatial distribution may account for age-related social patterns (Albery et al., 2022). Individuals who spend relatively more time alone will have fewer opportunities for social engagement, and this may arise from differences in either social or non-social traits (e.g., travelling more slowly, foraging in a different area, or spending more time on the ground). Therefore, it is important to test whether spatial segregation could drive social interactions on lower scales. Second, individuals may engage in fewer interactions if they experience avoidance or exclusion by others. Crucially, active behaviors typically viewed as actor-driven, such as lower rates of approaching others, may also stem from exclusion. Therefore, actor-centered measures should be complemented with metrics that focus on the partners. Third, older individuals may be unable, or lack the motivation, to socialize leading to a general disengagement from social interactions across partners in the absence of increasing focus on preferred partners, despite preserved opportunities to do so. Distinguishing between the different hypotheses is a necessary first step in better understanding the drivers and consequences of social ageing. As no behavior in isolation provides necessary support for one or the other mechanism, investigations should aim to identify the most consistent pattern and a set of minimum necessary conditions.
Age-associated changes in a sample population can result from within-individual ageing or from the selective disappearance of individuals at a younger age based on the feature investigated or other correlated traits (Hämäläinen et al., 2014). Selective disappearance effects could be strong enough to mask or bias within-individual trajectories leading to erroneous conclusions about ageing (Hämäläinen et al., 2014). As sociality often influences health and survival (Ostner and Schülke, 2018; Snyder-Mackler et al., 2020), less socially integrated individuals could be more likely to be missing from a cohort, particularly among older individuals, creating an apparent association between age and social traits. In the wild, biases due to selective disappearance are of even greater concern, because predation and fatal accidents are more likely to affect individuals in poorer body condition or more peripheral to the group. Captive and food-provisioned populations, which are less likely subject to selective disappearance have provided compelling evidence for social ageing across (Machanda and Rosati, 2020) and within individuals (Siracusa et al., 2022b) although patterns may be exacerbated by extreme lifespan and comorbidities (e.g., obesity) that are absent in the wild. Longitudinal data can address this issue by partitioning variance that occurs within individuals versus across individuals (van de Pol and Wright, 2009), and thus provide a more accurate picture of ageing than purely cross-sectional (i.e., one observation per subject over a given age span) or mixed-longitudinal (i.e., consisting of repeated observations over a limited age range for each individual) studies. In this context, longitudinal data on wild animal populations are particularly relevant to determine the occurrence, drivers, and consequences of social ageing.

Here, we investigated social ageing in a long-term study population of Assamese macaques (Macaca assamensis) living in their natural environment at Phu Khieo Wildlife Sanctuary in Thailand (De Moor et al., 2020). We focused on females, the philopatric sex in macaques, for which longitudinal social data and more precise age estimates were available. In this population, close female-female affiliative relationships provide benefits such as enhanced tolerance at feeding sites and access to spatially more central positions that may be associated with reduced predation risk (Heesen et al., 2015; Ostner and Schülke, 2018). In the wild, females must travel daily with their group and experience multiple energetic challenges throughout the year and throughout their lives (Touitou et al., 2021b, 2021a), which may exacerbate physiological and physical constraints on sociality compared to captive populations. In this context, two competing predictions can be made. If dynamics in the wild parallel findings from captive populations, we predict that females will reduce their active social engagement in favor of increasing social selectivity with age. On the contrary, if social integration is closely linked to survival, individuals will exhibit no signs of social ageing, and patterns at the population level will suggest an increase in social traits due to selective disappearance. Using data on female-female interactions collected over eight years across five social groups, we first aimed to exclude the
possibility that (a) spatial segregation would explain age-related changes of closer range interactions by testing for an increase with age in the time females spent alone. From there on, we tested three hypotheses for social ageing using directed social behaviors to weight the contribution of the partner’s and the ageing subject’s behavior. If older females are being excluded, we would expect (b) a decrease with age in the frequency of being approached by others, the number of partners grooming the female, and the time spent receiving grooming. Older females may drive age-related social changes either by becoming increasingly selective or by disengaging from social interactions more generally. In both cases, we would first predict (c) females to actively groom a decreasing number of social partners. Increasing or constant time spent grooming with the remaining partners would be consistent with increasing selectivity (figure 1A,B). In case of decreasing time spent engaging, contrasting the slopes for preferred and all partners will tell whether increased selectivity is observed (figure 1C). In the absence of evidence for selectivity, the concomitant decrease in the number of partners and the time spent actively interacting with them would suggest social disengagement, which would be further supported by a decrease in the frequency at which females approached others. For all analyses, we report both mixed-longitudinal and within-individual trends, and (d) discuss evidence for inconsistencies.

Methods

Study site and study subjects

We studied a population of Assamese macaques living in their natural environment at Phu Khieo Wildlife Sanctuary (PKWS, 16°05’-35’N, 101°20’-55’E, >1600 km²), part of the more than 6500km² Western Isaan Forest Complex in Northeastern Thailand (Touitou et al., 2021b). These Assamese macaques are seasonal breeders living in hill evergreen forests, with a mating season spanning over the dry season from October to February and births spread from March to August with a peak in mid-May (Fürtbauer et al., 2010).

Each study group was composed of adult females, their immature offspring, and several adult and subadult males for a total group size between 28 and 98 macaques, with fluctuations resulting from male immigr- and emigration, births, deaths, and group splits. This study focused on adult females, with females considered adult from the mating season of their first conception (usually at 5.5 years of age) (Anzà et al., 2022). Year of birth was either known from witnessed birth or inferred by experienced members of the research team based on morphological comparison with individuals of known age. The age distribution had a median[IQR] of 10[7–16] years and a range of 4 to 30, consistent with the
low survival probability beyond 25 and the maximum documented lifespan in semi-free captive populations of other macaque species (Brent et al., 2017; Johnson and Kapsalis, 1995).

Behavioral data collection

Data were collected on two groups between January 2013–September 2014, and three groups from January 2015–July 2019, October 2019–March 2020, and October 2020–March 2021 (figure S1). Observations were assigned to the mating or birth season of the corresponding year, creating a season-year time unit (e.g., data collected from October 2019 through February 2020 belonged to the season-year ‘mating 2019’).

During 40min continuous focal animal protocols (Altmann, 1974) we recorded all instances of dyadic grooming interactions between adult females along with the identity of the actor and the receiver. Approaches within 1.5m of another adult female were also recorded, with the identity of the initiator and receiver. Dyadic agonistic interactions (including aggressions or spontaneous submissions) were recorded during focal animal protocols and ad libitum. Over the study period, 61 adult females were observed for a total of 13,270 hours (median[IQR] = 216[85–332] hours per female, figure S2). Females were observed up to 14 season-years (10[4–13] season-years per female), for 24[19–32] hours per season-year, and per season-year we observed 39[33–44] females.

In addition, from January 2015 through March 2021 the identity of group members within 5m of the focal individual were recorded instantaneously every 10min during focal animal protocols. A total of 81,969 such instantaneous data points were recorded during 21,722 focal animal protocols (median[IQR] = 5[2–5] scans per focal follow) from 58 females. For the proximity dataset, females were observed 10[6–11] season-years.

Sociality metrics

Grooming out- and instrength, out- and indegree

From the adult female social network with tie weight reflecting the duration of directed grooming interactions in a group-season-year, we determined two dyad level metrics reflecting grooming given to and received from a specific adult female partner and four node level metrics of directed grooming behavior (out- and instrength, out- and indegree (Sosa et al., 2021)) (see also Sosa et al., 2021 for an introduction to social network terminology). Strength and degree are direct sociality measures which have been extensively scrutinized for their link to fitness in primates and other mammals (Ellis et al., 2019; Ostner and Schülke, 2018) and pertain to different social strategies (Schülke et al., 2022).
Grooming given to a partner was determined as the time (sec/hour) grooming a female partner divided by dyadic observation time (i.e., divided by the sum of observation time for each member of the dyad during which the other member of the dyad was also present and adult). Similarly, grooming received from a partner was determined as the time receiving grooming from that partner divided by dyadic observation time. The resulting dyad-level metrics indicate the strength of directed ties for every pair of females in a group. Individual-level grooming outstrength (given) and instrength (received) were determined as the sum of all female dyadic outgoing and ingoing weighted ties, respectively, allowing to compare females according to their overall engagement. We determined outdegree and indegree (figure S3) as the number of unique female partners an individual groomed and was groomed by, irrespective of the duration of the interactions.

Selective approach based on tie strength

To test for increasing selectivity with advancing age, we assessed whether females increasingly approached those partners they shared a stronger tie with. We calculated a dyadic sociality index (DSI) for every pair of females in a group based on the time spent in 1.5m proximity, time spent grooming, the frequency of approach given, and the frequency of grooming given (Silk et al., 2013), irrespective of which partner initiated the interaction. The value for each behavior in a pair was corrected for dyadic observation time and further divided by the average of all dyadic measures in the group for that behavior (cf. equation in supplementary material). For each season-year, the index was calculated based on interactions in all previous season-years, so we refer to it as a cumulated DSI. If females are selective, we expected that greater cumulated DSI would predict longer, or more frequent, interactions directed towards the partner in the next season-year, and if females are increasingly selective this association between past and future interactions to strengthen with age.

Approaching and being approached by other adult females

The number of times a focal female approached or was approached by another adult female into 1.5m was determined per season-year. For one female in one season-year, focal data were collected over only one day. This female-season-year was excluded from the analysis. Females directed approximately 1.0 approaches/hour (median[IQR] = 20[11–35] approaches per season-year of roughly 25h of observations) and received 1.3 approaches/hour (27[18–44] approaches per season-year).

Proportion time alone

For each instantaneous proximity record, we determined whether the focal female was within 5m proximity of at least one other adult female. The one female-season-year excluded from the approaches was also excluded here for the same reason. Out of 81,969 data points, 62,842 were
recorded with no other female in proximity. With a median[IQR] of 168[134–230] data points collected per female-season-year, the proportion of records spent alone was 0.77[0.71–0.83] per female.

**Control variables**

To control for social status, dominance Elo scores were determined from winner-looser agonistic interactions (Neumann et al., 2011), and female’s Elo score at the end of each season-year or on the last day observed were extracted (see supplementary material for details). To account for possible effects of reproductive state, females giving birth in a given birth season were defined as reproductively active during the preceding mating season and the respective birth season (coded as a binary variable with reference set to not reproductively active). About half of the females were reproductively active per season-year (median[IQR] = 0.56[0.45 – 0.67]). Finally, we calculated the number of available partners in each season-year-group as the (number of females in the group – 1, 11[9 – 14]).

**Statistical analysis**

Response variable and model structures are detailed below. Full model formulae and sample sizes can be found in the supplementary material. Shortened model results for the effect of within-individual age are presented in the main text, while detailed results and mixed-longitudinal model results are provided in tables S1–10.

**Grooming outstrength and grooming instrength**

The influence of age on outstrength and instrength in the grooming network was modelled with Gaussian error distribution. To improve homogeneity of the distribution of residuals, we applied a square root transformation on the response variables.

**Outdegree and indegree**

The number of partners an individual was observed grooming and being groomed by, i.e., degree (Sosa et al., 2021), is not adequately modelled with a Poisson or Normal error distribution because it is biased by sampling effort and reaches an upper limit imposed by group size. To overcome this limitation, we modelled degree as a binomial probability to observe a directed tie from any actor towards any receiver over age with binomial error distribution including an offset for the log of dyadic observation time.

**Selective approach based on tie strength**
We first aimed to investigate selectivity by testing whether cumulated DSI would be associated with how long females groomed each partner in the next season. However, as the number of partners and time spent giving grooming sharply decreased with age (cf. Results), data lacked resolution. Therefore, we used the number of approaches given to each partner, a more frequent behavior even at old ages, as the response. Approach directed towards a given partner was modelled with a negative binomial error distribution including an offset for dyadic observation time in the season-year.

Approaching and being approached by other adult females

The tendency of females to engage in social interactions, irrespective of partner identity, was modelled as the count of approaches towards and by others, respectively, with a negative binomial error distribution including an offset for the log of female’s observation time.

Proportion time alone

The influence of age on the proportion of instantaneous data points an individual was observed alone was modelled with a beta error distribution weighted for the number of records during a given female-season-year. To allow convergence, group was included as a fixed and not a random effect.

General statistical considerations

All analyses were conducted with Generalized Linear Mixed Models in R with RStudio (R Core Team, 2021 version 4.0.4; RStudio Team, 2022 version 2022.07.02+576). All models included the covariate female age as the key predictor and we furthermore included fixed effects of Elo score as well as reproductive status and season and their two-way interactions, differentiating females who did or did not conceive, did or did not give birth in each mating or birth season, respectively, to control for their effects. The (number of females in the group – 1) was also included as a fixed effects to account for variation in the availability of partners across season-years. The interaction of actor’s age and cumulated DSI was included in the model assessing selective approach. To avoid pseudo-replication, models of grooming outstrength, grooming instrength, approaching others, being approached by others, and time alone included female identity, group, and season-year as random intercept effects. Models on grooming outdegree, grooming indegree, and selective approach, included the identity of the actor, the identity of the receiver, the identity of the dyad, group, and season-year as random intercept effects. Covariates were z-transformed, and factors were dummy coded and centered when included as random slopes. Within and average individual age terms were scaled by mean-centering and then dividing by the standard deviation of raw age to facilitate model convergence and comparisons across models. For all models, we started from a maximal random slopes structure best suited to maintain type 1 error rate at the nominal 5% level (Barr et al., 2013). When model
convergence failed, we inspected the parameters for the correlations among random intercepts and slopes and removed seemingly unidentifiable correlations close to +1 or -1 (Matuschek et al., 2017). Furthermore, we removed random slopes with the lowest estimated variance sequentially until a model converged.

Assessing result robustness to uncertainty about age

Studying wild animal populations implies a degree of uncertainty regarding several individual attributes. As age is central to the present study, we evaluated whether results were robust to possible errors regarding the date of birth assigned to individuals by assigning to each female a symmetric range of possible years around our best estimated year of birth (see supplementary material for details). Results showed that limited uncertainty regarding females’ year of birth would not compromise the conclusions of the present study (figure S6).

Investigating selective disappearance and its possible influence on the relationship between age and sociality

All individuals are subject to age-related changes in physiology, cognition, and possibly social behavior. Within-individual ageing represents changes in the response as an individual grows older, whereas between-individual ageing represents the association between age and the response at the level of the sample population under study. In a situation where individuals’ death is not random but statistically associated to the response variable (i.e., selective disappearance, with or without causality), a between-individual effect is measured even without within-individual ageing. For example, more social individuals may be better buffered against external or intrinsic sources of mortality (e.g., predation), resulting in the ‘selective disappearance’ of less social individuals at an earlier age. Within-individual centering is advocated to remove this confound, because it separates the within and between-individual effect of age into two independent variables (van de Pol and Verhulst, 2006; van de Pol and Wright, 2009; Westneat et al., 2020) (see supplementary material for details). Eleven females died during the study (median[IQR] = 21[15–27] years old).

To investigate whether selective disappearance influenced the relationship between age and sociality, we reran all models after applying within-individual centering. To identify the slopes of within and between-individual ageing, a first set of models included within-individual age and average age. A second set of models included the raw age and the average age to assess the significance of the difference between the within and between-individual slopes, indicated by the p-value of the average age term (see supplementary material for details).
Results

(a) Spatial segregation as a possible driver of changes in social interactions with advancing age

Regardless of age, females were typically observed without another adult female within 5m of them. The proportion of time spent alone showed a marginal 3% increase in mixed-longitudinal analyses. This was not consistent within-individual, which we express for a female on average 15 years old and ageing from 11 to 19 over the eight-year study period. The predicted proportion time alone showed a small 8% decrease within-individual, associated with large uncertainty (table 1 and S1, figure 2A,B). This suggests that with advancing age, females did not spend more time alone, spatially segregated without any partner to interact with. This condition being fulfilled, we next investigated whether passive and active social behaviors changed with age.

Table 1. Estimated slopes for the effect of increasing age within and between individuals on the social behavior investigated (see supplementary table S2-10 for full model results). Hypotheses tested are increasing spatial segregation, exclusion by partners, increasing social selectivity, and disengagement.

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Response</th>
<th>Within-individual effect</th>
<th>Between-individual effect</th>
<th>Slope difference</th>
<th>Supported</th>
</tr>
</thead>
<tbody>
<tr>
<td>spatial segregation</td>
<td>proportion time alone</td>
<td>-0.291 ±0.187</td>
<td>-0.623</td>
<td>0.049</td>
<td>0.127</td>
</tr>
<tr>
<td>exclusion</td>
<td>being approached</td>
<td>0.317 ±0.092</td>
<td>0.163</td>
<td>0.495</td>
<td>0.003</td>
</tr>
<tr>
<td>exclusion</td>
<td>grooming instrength</td>
<td>-0.720 ±0.453</td>
<td>-1.623</td>
<td>-0.193</td>
<td>-0.592</td>
</tr>
<tr>
<td>exclusion</td>
<td>grooming indegree</td>
<td>-0.661 ±0.232</td>
<td>-1.067</td>
<td>-0.217</td>
<td>0.008</td>
</tr>
<tr>
<td>disengagement/selectivity</td>
<td>grooming outdegree</td>
<td>-0.937 ±0.204</td>
<td>-1.349</td>
<td>-0.516</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>disengagement/selectivity</td>
<td>grooming outstrength</td>
<td>-2.564 ±0.509</td>
<td>-3.590</td>
<td>-1.582</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>increased selectivity</td>
<td>selective approach</td>
<td>-0.130 ±0.147</td>
<td>-0.427</td>
<td>-0.172</td>
<td>0.375</td>
</tr>
<tr>
<td>disengagement</td>
<td>approaching others</td>
<td>-0.414 ±0.139</td>
<td>-0.647</td>
<td>-0.158</td>
<td>0.004</td>
</tr>
</tbody>
</table>

The significance of the difference between within and between-individual age slopes is indicated by the p-value of the estimate of average individual age in a model including both raw and average individual age terms and suggests selective disappearance.

(b) Contribution of social exclusion to patterns of social ageing

The mixed-longitudinal and within-individual analyses revealed that age was associated with a higher tendency to be approached, with a predicted increase of 54% approaches received per hour from 11 to 19 years (table 1 and S1, figure 2C,D). Age did not affect the time females spent receiving grooming from female partners (instrength, figure 2D,E). Finally, the number of partners grooming the subject (indegree) appeared to be relatively constant in the mixed-longitudinal analysis, with females being
groomed by an average of six partners. However, this result was not representative of the clear 43% decrease in indegree within individuals (figure 2E,F). To place this in a socially relevant context, figure 2G,H depicts this probability also as a count of partners (i.e., degree) by multiplying age-specific probabilities for each tie by 12, which is the median female group size in this study. Therefore, at least one aspect of passive sociality, here the number of partners grooming the subject, decreased with advancing age, whereas the patterns across all three passive behaviors investigated provided only mixed evidence for the social exclusion of aging females.

Figure 2. (A) Time spent alone slightly increased with age in the mixed-longitudinal analysis (B) and did not change within-individual. The possibility that older individuals were being excluded was not supported by (C,D) the increasing frequency of being approach, and (E,F) the absence of change in grooming received. In mixed-longitudinal analysis, (G) the number of partners grooming the subject (indegree) remained relatively unchanged which contrasted with (H) a sharp decrease within-individual. Degree is modelled as the probability to observe a directed tie between a female and every other female in her social group (left axis). To facilitate interpretation, this probability is expressed on the right axis as a count of partners for a group size of 12 adult females with equal observation time. Age is expressed in years in the upper row and frequency is given per hour.

(c) Testing increasing selectivity and increasing disengagement with advancing age

To disentangle scenarios of social selectivity, it is necessary to consider the number of partners a female is actively interacting with, the overall engagement across all partners, and interactions
directed to preferred partners (scenarios A-C; figure 1). Older females were less likely to groom other females, leading to a marked 61% decrease in the probability to observe a specific outgoing tie for a female ageing from 11 to 19 years of age (table 1-S1). Grooming outstrength, a measure of total engagement in grooming given, was also negatively correlated with age, showing a reduction by 48% (figure 3C,D). These patterns were consistent between mixed-longitudinal and within-individual analyses and indicated that network narrowing, i.e., the reduction in grooming partners, was not compensated by increased investment in remaining partners, which is incompatible with scenario A of selectivity (figure 1).

We modelled the frequency at which a female approached each partner in a season-year as a function of her age and cumulated previous DSI with the partner. In the mixed-longitudinal model, the slope for age was negative, the slope for cumulated previous DSI positive, and the interaction of the two not significant (table S1). Therefore, females were selective as they approached those partners more with whom they had shared a stronger tie in the past (figure G), an effect that was not moderated by age. The model differentiating between within- and between-individual effect corroborated these results; previous relationship strength was a positive predictor of approaching a partner that was not affected by age changes within the individual (table S8). In summary, females exhibited a decreasing frequency of approaches towards partners, even those with whom they had a higher cumulative DSI. Despite this decrease, they maintained a selective inclination towards stronger ties, thereby refuting scenario B in favor of scenario C. We note that effects of cumulative DSI were quite small and conclude that these time series analyses provided some evidence for relatively constant, but not increasing, selectivity across the lifespan.

These findings indicate that the decrease in both the number of partners groomed and the time spent grooming did not align with a concurrent tendency to focus on a few specific partners. Instead, it appeared that females actively withdrew from social interactions. This hypothesis was further supported by the net reduction in the frequency with which females approached others, irrespective of the partner’s identity (table 1-S1). Data showed that a female on average 15 years old would have decreased approaches towards others from 1 to 0.56 per hour over eight years (figure 3E,F).
Figure 3. Age was associated with (A,B) a reduction in the number of partners females groomed (outdegree). Degree is modelled as the probability to observe a directed tie between a female and every other female in her social group (left axis). To facilitate interpretation, this probability is expressed on the right axis as a count of partners for a group size of 12 adult females with equal observation time. (C,D) Grooming outstrength, i.e., total grooming given across all partners, decreased with age. (E,F) The frequency with which females approached others decreased with age. (G) The frequency of approaches directed at specific partners increased with increasing cumulated dyadic sociality index estimated based on past interactions. (H) Nineteen-year-old female Assamese macaque from the study population. Age is expressed in years in the upper row and frequencies are given per hour.

(d) Comparison of within-individual and between-individual effects

Most conclusions drawn from mixed-longitudinal analyses were confirmed at the individual level. Yet, mixed-longitudinal effects tended to underestimate age-related changes in social behaviour, and in some cases, diverged from within-individual dynamics. These inconsistencies are better understood when comparing within-individual and between-individual effects. For grooming outdegree, grooming outstrength, and approaching others, both within- and between-individual slopes were significant and negative, but the between-individual effect sizes were much smaller than the within-individual effects (table 1). More surprisingly, within-individual ageing was associated with a decline in the number of partners grooming the subject, in contrast to a tendency to increase across individuals. This suggests that the between-individual effect masked the decrease experienced by individuals with age when the
pattern was investigated mixed-longitudinally without differentiating within and between-individual effects. These results together suggest that individuals with fewer grooming partners, grooming and approaching others less, i.e., less social, are underrepresented at older ages. We note, however, that the significant difference in the slope of within and between-individual effects on the frequency of being approached by others would suggest the opposite, namely that more social individuals died at an earlier age (although the between-individual effect for this variable was far from significant). Finally, within- and between-individual slopes did not differ for selective approach, instrength, and time spent alone, although here again between effects were small compared to within-individual changes.

Discussion

This study provides evidence for social ageing among females of a wild population of Assamese macaques. By investigating changes in several social behaviors with age, we tested four hypotheses on drivers of social ageing. With advancing age, females did not spend more time alone isolated from others, providing no support for increasing spatial segregation with age. Yet, as females aged, they approached others less frequently, groomed fewer partners, and spent less time grooming others. Females were socially selective by approaching more often those partners they shared a stronger tie with, and this tendency was maintained, but not increasingly expressed, into old age. Finally, females were not less frequently approached or groomed by others, but received grooming from increasingly fewer partners as they aged, consistent with some aspects of social exclusion. Together, these results provide evidence for the contribution of both active and passive processes in the reduction of a female’s social network and engagement with advancing age. Furthermore, age effects were weaker between than within individuals, and consequently, age-related changes identified by mixed-longitudinal analyses did not always match true ageing effects. Here, we first discuss what could explain such active and passive social changes, then what these results bring to comparative ageing in light of the social selectivity hypothesis, before ending with a consideration of the implications of studying ageing longitudinally.

Changes experienced with advancing age are not limited to the social domains and most evidence in wildlife shows alterations in body condition and reproductive function (Gaillard and Lemaître, 2020; Siracusa et al., 2022a). For example, ageing females may modify their activity budget, reducing social time to free time for resting and feeding (Nakamichi, 1991; Pavelka, 1990; Veenema et al., 1997). An increase in time spent resting could explain a decrease in time spent actively grooming others and in the number of partners groomed but would not be sufficient to explain a decrease in the number of partners grooming the ageing subject as recipients of grooming are resting. Additionally, changes in the activity budget may be reflected in different spatial distributions (Emery Thompson et al., 2020b).
Although ageing was not associated with increasing time spent alone, older females may remain within 5m proximity to others while engaging in other activities, like feeding, without initiating grooming interactions. Indeed, age was associated with a tendency to approach others into 1.5m less often, suggesting that females were less motivated to interact with those around them or that they found themselves close to others when the context was not favorable to grooming.

Active social behavior may also decrease if older individuals experience a decline in the ability to digest food and extract energy due to reduced gut integrity, disruption of gut microbiome communities, or infection with macroparasites (e.g., helminths) (Mitchell et al., 2017; Sadoughi et al., 2022a). Such gastrointestinal disorders may further influence passive social behavior if group members detect signs of infections (e.g., lethargy or modified fecal odors) and avoid infected conspecifics, as shown in mandrills and Barbary macaques (Müller-Klein et al., 2019; Poirotte et al., 2017). In this population of female Assamese macaques, age is associated with an increased abundance in the gut of several pro-inflammatory bacterial taxa and the oldest females have a lower stability of gut bacterial composition (Sadoughi et al., 2022b), which could impact gut health and contribute to both the active and passive decrease in grooming partners. The linear relationships between age and the social traits observed here do not exclude the possibility of an additional age-independent deterioration among the oldest which could more closely match physiological decline. For example, body mass decreases linearly with age to a point where it drops more rapidly before death in ungulates and yellow-bellied marmots (Kroeger et al., 2021; Nussey et al., 2011). We did not test for non-linear effects and a larger sample size of subjects at old ages is needed to test whether non-human primates experience accelerated social decline in late life.

Social disengagement could also arise from external, rather than internal frailty-related, constraints, if ageing females find themselves in increasingly unfavorable social environments. Non-human primates often show a preference to interact with close kin (De Moor et al., 2020; Siracusa et al., 2022b; Veenema et al., 1997) or individuals of similar age (Rosati et al., 2020; Veenema et al., 1997). The availability of such partners will depend on stochastic demographic events like group splits or the death of same-aged individuals which then constrains the ability to focus on preferred partners. However, current evidence does not suggest that kin availability decreases with age in cercopithecines (Ellis et al., 2022; Silk et al., 2006), and we did not examine the influence of the death of a close partner because, with the exception of spousal death in humans (Wrzus et al., 2013), affiliative networks in humans (Cornwell et al., 2021), non-human primates (Fedurek et al., 2022; Siracusa et al., 2022b), and ungulates (Albery et al., 2022) appear to be resilient to partner loss. In summary, investigations of within-individual changes in non-social behavior and health appear as the most fruitful follow-up to pinpoint the causes for active and passive social ageing in this population.
Although frailty and declining motivation may contribute to decreasing social engagement with age, prevailing socioemotional (SES) theories of ageing tend to emphasize increasing selectivity in partner choice as a driver of age-related network narrowing (Antonucci et al., 2014; Charles and Carstensen, 2010; Cornwell et al., 2021; Müller and Ellwardt, 2022). According to SES theories, among humans changes in network composition and size result from greater priority given to maintaining emotionally positive relationships over acquiring new information, leading subject to discard less valued relationships. Evidence for a greater investment in two-sided rather than one-sided grooming partners (Rosati et al., 2020), reduced interest in non-social compared to social information (Almeling et al., 2016), and an increase in the proportion of kin in the network in non-human primates suggest that preferential interest may shift with age in other species too. How could such a shift account for the reduction in social engagement and network size with age in several species (Albery et al., 2022; Almeling et al., 2016; Rathke and Fischer, 2021; Schino and Pinzaglia, 2018) remained unclear, especially because similar social patterns are expected irrespective of whether social selectivity or general decline is assumed (figure 1). We have proposed several scenarios of selectivity, with two (A and B) considering selectivity as a driver of network narrowing and reduction in overall time spent social. Contributing to answering this question, our findings corroborate the reduction in network size and social engagement with age, and further provide evidence that these changes occur within individuals, emphasizing the social dimension of ageing in wild populations. However, selectivity did not account for this decline, which echoes previous evidence of concomitant decrease in grooming given and preserved selective interest in cues from preferred partners in another primate (Almeling et al., 2016), and contrasts with patterns more closely related to scenario A (Rosati et al., 2020; Siracusa et al., 2022a). In the latter two cases, overall time spent social did not decrease with age. However, this would likely not explain the differences between findings, as the scenarios proposed in figure 1 decouple overall time spent social from the expression of selectivity. Taken together, these results suggest that selectivity and engagement may be two social dimensions that remain independent into old age at a time when other constraints lead the ageing subject to disengage from most social interactions.

One of the aims of the social selectivity hypothesis of ageing was to suggest a counter narrative to previous theories considering social disengagement as a detrimental process. Older individuals may not experience detrimental consequences of social disengagement if partners continue investing in them. In Assamese macaques, remaining partners appeared to compensate for the decrease in the number of partners grooming the subject, resulting in mostly constant grooming received. The overall time spent grooming from combined active and passive processes nevertheless decreased with age (Figure S6). Although decreasing social engagement with age may be described with loaded terms such
as “decline” or “disengagement”, the consequences of such disengagement may be beneficial, which has not been conclusively tested yet. There is repeated evidence that the effect of social bonds and integration on physiology and health is modulated by individual dominance status (Brent et al., 2011), reproductive state (Crockford et al., 2008), sex or dominance status of the social partner (Fürtbauer et al., 2014; Sapolsky, 1992b), or by context-dependencies, such as timing to mating season or group-level social stability (Sapolsky, 2005). Similarly, the social or emotional benefits derived from social interactions could differ across the lifespan, resulting in several age-specific optima. This hypothesis has only rarely been directly addressed, and results are inconclusive (Brent et al., 2017; Holt-Lunstad et al., 2015; Yang et al., 2016). Describing how social bond strength, social status, and broader social integration vary for specific age-classes will be a necessary first step requiring large enough cohorts at different ages. It is further important to formulate hypotheses regarding which social system or environmental conditions should favor increasing, or conversely decreasing, benefits of sociality with age.

Critical to the study of ageing in wild populations, the comparison of mixed-longitudinal and within-individual effects of age revealed that changes within-individual were partially confounded by demographic processes at the population level. For example, the decrease in the number of partners grooming the subjects was only discovered using a longitudinal analysis. The difference between the within-individual (i.e., senescence) and between-individual (i.e., demographic) effects of age on social traits would suggest selective disappearance of females that groomed and were groomed by fewer grooming partners and groomed overall less. In other free-ranging populations selective disappearance was demonstrated for lighter individuals in ungulates (Nussey et al., 2011) and mouse lemurs (Hämäläinen et al., 2014), poorer breeders in great tits (Bouwhuis et al., 2009), and subordinates in meerkats (Cram et al., 2018), but not in rhesus macaques (Siracusa et al., 2022b) and wolves (MacNulty et al., 2009). It has to be noted though that in the Assamese macaques between-individual effects were generally small perhaps because sampling was limited across the within-individual age range (Westneat et al., 2020). The oldest subjects were already mid-aged adults at the beginning of the study, which implies that age at the start and at the end of the study are not independent. A more direct test of selective disappearance is achieved if all individuals enter the study at the same age and are followed until death (Nussey et al., 2008). More confidence can be placed into the estimates of the within-individual slopes which are robust to model choices (Westneat et al., 2020).

What could explain the disappearance at younger ages of females with fewer grooming partners, approaching others less, but being approached more? Social integration has been positively linked to survival in mammals (Kappeler et al., 2015; Silk et al., 2010; Snyder-Mackler et al., 2020), in part mediated by enhanced access to food resources (Kajokaite et al., 2022), more central positions within
groups associated with lower predation-related mortality (Cram et al., 2018; Hrdy and Hrdy, 1976; van Noordwijk and van Schaik, 1987), resistance to disease (Sadoughi et al., 2022a), and buffering against adverse social and abiotic environments (Archie et al., 2014; Campos et al., 2021; Young et al., 2014). In this population, females mitigate intra-group competition by distancing themselves when feeding, which may come at the expense of an increased predation risk (Heesen et al., 2015). However, time spent alone only increased slightly across individuals with advancing age, suggesting that those more frequently observed alone were also on average older, inconsistent with increased mortality from predation at the periphery. Females also navigate feeding competition by preferentially attending food patches with females with whom they frequently groom (Heesen et al., 2014), which could lead to sociality-dependent energy intake and survival of those with better body condition (Hämäläinen et al., 2014), whereas frequency of being approached could increase if females are more frequently displaced during those feeding bouts. This is entirely speculative, and we would therefore predict the value of these social bonds to be especially high during gestation and late lactation when females increase feeding to meet their energetic requirements (Touitou et al., 2021a), which could be tested by assessing the relationship between social integration and timing of death in the population.

The possible coexistence of senescence and selective disappearance in shaping age-sociality dynamics is critical for interpreting the results of previous mixed-longitudinal studies. Active engagement in social interactions, measured either as grooming given or a combination of grooming and approaching others, was found to decrease [15,8,10–12,17,14,females: 13 and 82] or remain stable [male chimpanzees: 13,16] in several non-human primates with age. Passive engagement was found to increase [one of two social groups: 10, males: 82], decrease [12,17,females: 82,males: 13,one of two social groups: 10], or stay stable [15,16,females: 13]. Finally, studies reporting undirected measures of social engagement found a decrease (Archie et al., 2014; Nakamichi, 1991; Veenema et al., 1997) (or a decreasing trend (Silk, 1994)) or no change (Borries and Koenig, 2008; Pavelka, 1990; van Noordwijk and van Schaik, 1987) with age. Combining all metrics (and counting sexes from the same study separately), 12 out of 17 studies report at least one negative relationship between social engagement and age. Under the (extreme) assumption of no social senescence, the negative correlation between age and social engagement in four wild and eight captive studies would be caused by the selective disappearance of highly socially engaged individuals. Alternatively, assuming some degree of senescence, the absence of a relationship between age and sociality in three wild and two captive populations could have arisen from the selective disappearance of poorly connected individuals. Although speculative, it seems more plausible that unaccounted selective disappearance contributes to discrepancies between studies by masking social senescence.
In conclusion, this study highlights that social ageing occurs in wild primates and, in conjunction with demographic effects, contributes to age-related variation in social behavior. As females aged, they engaged less frequently in social interactions which led to a narrowing of their social network despite evidence for selectivity in partner choice. Possibly because of such disengagement, fewer partners interacted with females as they grew older. This effect was partially confounded by demographic trends, compatible with selective disappearance, further highlighting the need to account for demographic effects, a critical point in both animal and human biogerontology research (Bouwhuis et al., 2009; Cornwell et al., 2021; Lang et al., 1998). Complete life-course trajectories are almost impossible to collect in humans, and mixed-longitudinal studies are biased by selective mortality and sensitive to self-perception and memory recollection biases when surveys are used (Lang et al., 1998; Müller and Ellwardt, 2022). In this context, longitudinal data collected through systematic direct observation of animal populations represent a valuable contribution to social ageing research.

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**Supplementary material**

**Supplementary methods**

**Cumulated dyadic sociality index**

A dyadic sociality index (Silk et al., 2013) was calculated for every pair of females in a group in each season-year based on the time spent in 1.5m proximity, time spent grooming, the frequency of
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approach given, and the frequency of grooming given, irrespective of which partner initiated the interaction, across all previous season-years.

\[
cumulated \text{DSI}_{xy} = \sum_{i=1}^{d} \frac{f_{i\text{xy}}}{\bar{f}_{iz}}
\]

with \(d\) being the number of behaviors contributing to the index, here four, \(f_{i\text{xy}}\) being the rate of behavior \(i\) for dyad \(xy\) (i.e., count or time spent interacting divided by dyadic observation time), and \(\bar{f}_{iz}\) being the average rate for the behavior \(i\) across all pairs \(xy\) from group \(z\).

Control variables

Dominance Elo scores

Based on the direction of submission and aggression recorded during focal follows and \textit{ad libitum}, we determined the winner of each dyadic female-female conflict (including spontaneous submission, Ostner et al., 2008) and computed group-specific Elo scores with the package Elo_rating version 0.46.11 (functions elo_seq and elo_plot with \(k = 100\) and a starting value of 1,000) (Neumann et al., 2011). Undecided conflicts (8% of records) were counted as half a win for each participant following the package’s default setting. Female’s Elo score at the end of a season (30\textsuperscript{th} of September for the previous birth and 1\textsuperscript{st} of March for the previous mating season) or on the last day observed (four females who died or disappeared during a season) were extracted. The correlation between female age and Elo score was low (Pearson’s rho = 0.24; Kendall’s tau-b = 0.13; \(n = 517\), \(p < 0.001\), package stats version 4.0.4 (R Core Team, 2021), figure S4).

Reproductive status

Macaques do not exhibit menopause, but may experience extended interbirth intervals with advancing age (Nakamichi, 1991; van Noordwijk and van Schaik, 1987). If reproductive status influences sociality, the effect of age on sociality may be partially mediated by or confounded with reproductive status (Thompson González et al., 2021). To account for possible effects of reproductive states, females giving birth were defined as reproductively active in the given birth and preceding mating season (coded as a binary variable with reference level set to not reproductively active). About half of the females were reproductively active per season-year (median\[Q1–Q3\] = 0.56[0.45 – 0.67]).

Statistical analysis

The package \textit{lme4} version 1.1-30 (Bates et al., 2015) was used for models with an error distribution being Gaussian (function \textit{lmer}), binomial (function \textit{glmer}, family argument set to binomial), or negative
binomial (function glmer.nb). Beta models were fitted with the package glmmTMB version 1.1.4 (Brooks et al., 2017) (function glmmTMB). We fitted Gaussian models with identity link, binomial and beta model with a logit link, and negative binomial models with a log link function (McCullagh and Nelder, 1989). P-values on individual terms in Gaussian models were obtained using the Satterthwaite approximation implemented in the package lmerTest version 3.1-3 (Kuznetsova et al., 2017), and for other models with the function drop1. The 95% confidence intervals of model estimates were determined by means parametric bootstrapping (N = 1,000 bootstraps; package glmmTMB function simulate; package lme4 function bootMer).

Assessing models for issues or violations of assumptions

There were no issues with or violations of assumptions in any of the models. All models were of good stability as assessed by excluding levels of random factors one at a time and comparing the estimates of models fitted to the derived subsets with those derived for the full dataset. Furthermore, in none of the negative binomial and beta models the response variable was overdispersed given the model (dispersion parameter: binomial models: grooming outdegree = 0.79; grooming indegree = 0.77; negative binomial models: approaches towards others = 0.94; approaches by others = 0.99; beta models; time alone = 1.12, selective approach = 0.90). None of the Gaussian models revealed a deviation from the assumptions of normally distributed and homogeneous residuals (packages performance version 0.9.2 function check_model, and DHARMA version 0.4.5 (Hartig, 2022; Lüdecke et al., 2021)), and collinearity also was no issue in any of the models (vif function in package car version 3.1-0 (Fox and Weisberg, 2018), max VIF = 2.28).

Model sample sizes

<table>
<thead>
<tr>
<th>Model</th>
<th>No. females</th>
<th>No. female-season-years</th>
<th>No. dyads (when applicable)</th>
<th>No. social groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grooming outstrength</td>
<td>61</td>
<td>518</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Grooming instrength</td>
<td>61</td>
<td>518</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Grooming outdegree</td>
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<td>7004</td>
<td>539</td>
<td>5</td>
</tr>
<tr>
<td>Grooming indegree</td>
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<td>7004</td>
<td>539</td>
<td>5</td>
</tr>
<tr>
<td>Selective approach</td>
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<td>5926</td>
<td>470</td>
<td>5</td>
</tr>
<tr>
<td>Approaching others</td>
<td>61</td>
<td>517</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Being approach by others</td>
<td>61</td>
<td>517</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Proportion time alone</td>
<td>58</td>
<td>451</td>
<td>-</td>
<td>4</td>
</tr>
</tbody>
</table>
Model pseudocode formulae

The pseudocodes below follow the syntax of the package *lme4* (Bates et al., 2015). For clarity, $A * B$ means the two main effects and their interactions, the character $|$ stands in front of the random effects factor, and $||$ means that correlation between random slopes and the random intercept were excluded within a given random effects factor. The model formulae presented include the raw age term used in mixed-longitudinal analyses, which was replaced by a within-individual centered age term and a between-individual age term in longitudinal analyses.

**Grooming outstrength:**

```r
lme4::lmer(sqrt(outstrength) ~ age actor + Elo score actor + reproductive state actor * season + nfemale +
            (1 + age actor + Elo score actor + reproductive state actor + season + nfemale | actor) +
            (1 + age actor + Elo score actor + reproductive state actor * season | group) +
            (1 + age actor + Elo score actor + reproductive state actor | season_year),
            REML = FALSE,
            control = lmerControl(optimizer="bobyqa", optCtrl = list(maxfun=100000)))
```

**Grooming instrength:**

```r
lme4::lmer(sqrt(instrength) ~ age receiver + Elo score receiver + reproductive state receiver * season + nfemale +
            (1 + age receiver + Elo score receiver + reproductive state receiver + season + nfemale | receiver) +
            (1 + age receiver + Elo score receiver + reproductive state receiver * season | group) +
            (1 + age receiver + Elo score receiver + reproductive state receiver | season_year),
            REML = FALSE,
            control = lmerControl(optimizer="bobyqa", optCtrl = list(maxfun=100000)))
```

**Grooming outdegree:**

```r
lme4::glmer(degree ~ age actor + Elo score actor + reproductive state actor * season + nfemale +
            offset(log(dyads_obs_time/3600)) +
            (1 + age actor + Elo score actor + reproductive state actor + season + nfemale | actor) +
            (1 + age actor + Elo score actor + reproductive state actor + season | group) +
            (1 + age actor + Elo score actor + reproductive state actor + season | dyad) +
            (1 + age actor + Elo score actor + reproductive state actor + season + nfemale | receiver) +
            (1 + age actor + Elo score actor + reproductive state actor | season_year),
```
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family = binomial,
control = glmerControl(optimizer = "bobyqa", optCtrl = list(maxfun=100000000))

**Grooming indegree:**

lme4::glmer(degree ~ age receiver + Elo score receiver + reproductive state receiver * season + nfemale +
offset(log(dyads_obs_time/3600)) +
(1 + age receiver + Elo score receiver + reproductive state receiver * season + nfemale | | receiver) +
(1 age receiver + Elo score receiver + reproductive state receiver * season | | group) +
(1 + age receiver + Elo score receiver + reproductive state receiver * season | | dyad) +
(1 + age receiver + Elo score receiver + reproductive state receiver * season + nfemale | actor) +
(1 + age receiver + Elo score receiver + reproductive state receiver | | season_year),
family = binomial,
control = glmerControl(optimizer = "bobyqa", optCtrl = list(maxfun=100000000))

**Selective approach:**

glmmTMB::glmmTMB(approach towards partner ~ age actor * cumuDSI previous season + Elo score actor +
reproductive state actor * season + nfemale +
offset(log(dyads_obs_time/3600)) +
(1 + age actor + cumuDSI previous season + Elo score actor + reproductive state actor + season + nfemale +
reproductive state actor + season | | actor) +
(1 + age actor + cumuDSI previous season + Elo score actor + reproductive state actor + season + nfemale +
receiver) +
(1 + age actor + cumuDSI previous season + Elo score actor + reproductive state actor + season + dyad) +
(1 + age actor + cumuDSI previous season + Elo score actor + reproductive state actor + season | | group) +
(1 + age actor + cumuDSI previous season + Elo score actor + reproductive state actor | | season_year),
family = nbinom2(link = "log"))

**Approaching others:**

lme4::glmer.nb(Approaching others ~ age + season * reproductive state + Elo score + nfemale +
offset(log(lobs_time/3600)) +
(1 + age + season + reproductive state + Elo score + nfemale | female) +
(1 + age + season * reproductive state + Elo score | | group) +
(1 + age + reproductive state + Elo score | season_year),
control = glmerControl(optCtrl = list(maxfun=100000)))

**Being approached by others:**

lme4::glmer.nb(Being approached by others ~ age + season * reproductive state + Elo score + nfemale +
offset(log(obs_time/3600)) +
(1 + age + season + reproductive state + Elo score + nfemale | female) +
(1 + age + season * reproductive state + Elo score || group) +
(1 + age + reproductive state + Elo score | season_year),
control = glmerControl(optCtrl = list(maxfun=100000)))

**Proportion time alone:**

glmmTMB::glmmTMB(Proportion time alone ~ age + season * reproductive state + Elo score + group + female +
(1 + age + Elo score + reproductive state * season + nfemale || female) +
(1 + age + Elo score + group.ms2 + group.mst + group.sst + reproductive state || season_year),
family = beta_family,
weights = (data$weight/sum(data$weight))*nrow(data),
control = glmmTMBControl(optimizer = "nlminb", optCtrl = list(iter.max=10000, eval.max=100000)))

The weighting term was defined such that $weight = \frac{x}{X} \times \pi$ with $x$ total number of records during a given female-season-year, $X$ the sum of $x$ across all female-season-years, and $\pi$ total sample size.

**Assessing result robustness to uncertainty about age**

Studying wild animal populations implies a degree of uncertainty regarding several individual attributes. As age is central to the present study, we tested whether results were robust to possible error regarding the date of birth assigned to individuals. We assigned to each female a symmetric range of possible years around our best estimated year of birth. Among the 61 females, 12 had an uncertainty of several years, 10 had uncertainty of one year, and 39 had an age known within a few days or weeks. For each model, year of birth was randomly sampled from a normal distribution with a mean set to the best estimated year and a standard deviation of $sd = \frac{\text{max-min}}{4}$. The procedure was repeated 1,000 times and the respective full model was fitted to each data set obtained. The distribution of the estimated coefficients for the slope of age was inspected for evidence of large variability. Results
showed that limited uncertainty regarding females’ year of birth would not compromise the conclusions of the present study (figure S6).

Estimating within and between–individual ageing

All individuals are subject to age-related changes in physiology, cognition, and possibly social behavior. In standard linear mixed models, the influence of age (expressed as a fixed effect) on the response results from a combination of within and between-individual processes. Within-individual ageing represents changes in the response as an individual grows older, whereas between-individual ageing represents the association between age and the response at the level of the sample population under study. In a situation where individuals’ death is not random but statistically associated to the response variable (i.e., selective disappearance, with or without causality), a between-individual effect is measured even without within-individual ageing. For example, more social individuals may be better buffered against external or intrinsic sources of mortality (e.g., predation), resulting in the ‘selective disappearance’ of less social individuals at an earlier age. Within-individual centering is advocated to remove this confound, because it separates the within and between-individual effect of age into two independent variables (van de Pol and Verhulst, 2006; van de Pol and Wright, 2009; Westneat et al., 2020).

Within-individual age centering involves subtracting the mean age at which the individual was observed from the age associated to each data point. Equation (1) including a fixed effect for age turns into (2).

\[ y_{ij} = \beta_0 + \beta_1 age_{ij} + u_{0j} + e_{0ij} \]  

(1)

with \(age_{ij}\) being the age at measurement \(i\) for individual \(j\), a population intercept \(\beta_0\), the slope for age \(\beta_1\), an individual random intercept \(u_{0j}\) and residual error \(e_{0ij}\).

\[ y_{ij} = \beta_0 + \beta_\omega(age_{ij} - \bar{age}_j) + \beta_B \bar{age}_j + u_{0j} + e_{0ij} \]  

(2)

with \(\bar{age}_j\) being the mean age for individual \(j\), \(\beta_\omega\) being the within-individual effect, and \(\beta_B\) being the between–individual effect of \(age\) on \(y\).

However, this procedure is not a one fits all solution (Fay et al., 2022; van de Pol and Wright, 2009; Westneat et al., 2020). First, within and between-individual estimates are only accurate if the (unknown) underlying biological process actually matches the assumed mathematical relation (Westneat et al., 2020). Second, when the direction of the two slopes differs, the between-individual estimate is biased towards the within-individual estimate (Westneat et al., 2020). Finally, it has already been shown that the accuracy of \(\beta_B\) diminishes when within-individual variance is high (van de Pol and
Verhulst, 2006), but it remains unclear how many within-individual measurements are needed to accurately estimate $\beta_\omega$. In this study, individuals were observed on average over 10 season-years. Whether or not this allows to effectively partition the effects of age is unknown given the complexity associated with any natural animal system (cf. figure S7, which shows that in the present case the models are in good agreement with the observed data, and therefore capture the observed behaviors well). Therefore, we chose to present results in the main text based on the pooled effect of age (equation 1). To investigate the potential mismatch between within and between-individual ageing effects, we also report results using within-individual centering (equation 2).

Whenever the two effects (within- and between-individual age) appear to act in opposite direction, or in the same direction with fairly different magnitude, it suggests that selective disappearance of highly or poorly social individuals contributes to the age-associated social changes observed. Testing for a significant difference between the slopes is achieved by modifying equation (2) into

$$y_{ij} = \beta_0 + \beta_\omega age_{ij} + (\beta_B - \beta_\omega) \bar{age}_j + u_{0j} + e_{0ij}$$  \hspace{1cm} (3)

with the slope associated to the between-individual effect $\bar{age}_j$ now expressing the difference between the slopes of the within and between-individual effect in (2) (equation 3).
Supplementary figures

Figure S1. Season-years for which the study females were observed, with continuous sampling represented by lines connecting observations. Age is displayed with a color gradient, and females are ordered by increasing mean age on the y-axis.

Figure S2. Hours of focal observation time per female per season-year. Females are ordered on the x-axis by ascending median focal observation time. Boxes represent the interquartile range (IQ), which contains the middle 50% of the records. Vertical lines extend from the upper and lower edges of the
box to the highest and lowest values which are no greater than 1.5 times the IQ range, and a line across the box indicates the median. Circles represent outliers.

Figure S3. Number of unique partners a female (A) groomed (outdegree) and (B) was groomed by (indegree). Observations correspond to a count over a season-year. Females are ordered on the x-axis by ascending median of the response. Boxes represent the interquartile range (IQ), which contains the middle 50% of the records. Vertical lines extend from the upper and lower edges of the box to the highest and lowest values which are no greater than 1.5 times the IQ range, and a line across the box indicates the median. Circles represent outliers.
Figure S4. Estimated relationship between age and Elo score obtained from a linear mixed model with Gaussian error distribution including female identity, group, and season-year to account for non-independence among data. Age is expressed in years.

Figure S5. Distribution of the estimated slopes for age accounting for uncertainty regarding true individual’s age. Female age was drawn from a normal distribution with mean = estimated age and standard deviation = (range of uncertainty/4). Random sampling was performed 1,000 times. The
respective response variable is specified as the title following denomination in Table 1. Estimated slopes are far from approaching zero for all models providing adequate confidence in the results.

Figure S6. Undirected grooming strength decreased with age in both (A) mixed-longitudinal and (B) within-individual analyses. Undirected grooming strength is calculated from summing for each female all undirected dyadic ties to her grooming partners, with each dyadic tie calculated as the duration of grooming given and received corrected for dyadic observation time of the grooming partners.
Supplementary tables

Table S1. Mixed-longitudinal estimated slopes for the social behaviors investigated over age. Age was z-transformed in all models. Detailed model results including estimated slopes for the covariates Elo score and number of females, and the interaction between reproductive status and season are presented in Tables S1-S9. Hypotheses tested are increasing spatial segregation, increasing social selectivity, disengagement, and exclusion by partners.

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Response</th>
<th>$\beta_{age} \pm SE$</th>
<th>CI$_{lower}$</th>
<th>CI$_{upper}$</th>
<th>P$_{(age)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>spatial segregation</td>
<td>proportion time alone</td>
<td>0.052 ±0.025</td>
<td>0.005</td>
<td>0.099</td>
<td>0.040</td>
</tr>
<tr>
<td>exclusion</td>
<td>being approached</td>
<td>0.061 ±0.027</td>
<td>0.011</td>
<td>0.110</td>
<td>0.032</td>
</tr>
<tr>
<td>exclusion</td>
<td>grooming instrength</td>
<td>0.273 ±0.283</td>
<td>-0.371</td>
<td>0.846</td>
<td>0.475</td>
</tr>
<tr>
<td>disengagement/ selectivity</td>
<td>grooming outdegree</td>
<td>-0.402 ±0.059</td>
<td>-0.520</td>
<td>-0.292</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>disengagement/ selectivity</td>
<td>grooming outstrength</td>
<td>-1.465 ±0.184</td>
<td>-1.843</td>
<td>-1.072</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>increased selectivity</td>
<td>selective approach</td>
<td>-0.103 ±0.038</td>
<td>-0.177</td>
<td>-0.027</td>
<td>0.010</td>
</tr>
<tr>
<td>disengagement</td>
<td>approaching others</td>
<td>-0.161 ±0.040</td>
<td>-0.224</td>
<td>-0.083</td>
<td>0.002</td>
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</table>
Table S2. Detailed results for the model of proportion time spent alone. (A) Estimates for slopes with standard error, 95% confidence interval, test statistic, degrees of freedom, and p-values, are provided for fixed effects alongside (B) the estimated standard deviations for random effects intercepts and random slopes. Reference levels were set as reproductive status = not reproductively active, season = mating and group = mot.

<table>
<thead>
<tr>
<th>A Fixed term</th>
<th>Est.</th>
<th>SE</th>
<th>CI_lower</th>
<th>CI_upper</th>
<th>Df</th>
<th>Test stat.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>intercept</td>
<td>1.336</td>
<td>0.113</td>
<td>1.103</td>
<td>1.555</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>within-individual age</td>
<td>-0.291</td>
<td>0.187</td>
<td>-0.623</td>
<td>0.049</td>
<td>1</td>
<td>2.334</td>
<td>0.127</td>
</tr>
<tr>
<td>between-individual age</td>
<td>0.083</td>
<td>0.028</td>
<td>0.023</td>
<td>0.140</td>
<td>1</td>
<td>7.979</td>
<td>0.005</td>
</tr>
<tr>
<td>Elo score</td>
<td>-0.121</td>
<td>0.026</td>
<td>-0.173</td>
<td>-0.072</td>
<td>1</td>
<td>18.192</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>season</td>
<td>-0.154</td>
<td>0.146</td>
<td>-0.454</td>
<td>0.120</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>reproductive status</td>
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<td>0.174</td>
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<td>-</td>
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<td>season * reproductive status</td>
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<td>-0.346</td>
<td>0.105</td>
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<td>1.112</td>
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</tr>
<tr>
<td>group ms2</td>
<td>-0.264</td>
<td>0.158</td>
<td>-0.539</td>
<td>0.009</td>
<td>3</td>
<td>5.415</td>
<td>0.144</td>
</tr>
<tr>
<td>group mst</td>
<td>0.070</td>
<td>0.172</td>
<td>-0.272</td>
<td>0.402</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>group sst</td>
<td>-0.209</td>
<td>0.108</td>
<td>-0.409</td>
<td>-0.013</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

| B Grouping factor Random intercept or slope SD |
|-----------------------------------------------|-------|
| female                                       | intercept | 0.111 |
| female                                       | Elo score | 0.079 |
| female                                       | season    | 0.077 |
| female                                       | reproductive status | 0.042 |
| female                                       | nfemale   | <0.001 |
| season-year                                  | intercept | 0.214 |
| season-year                                  | Elo score | 0.001 |
| season-year                                  | reproductive status | 0.092 |
| season-year                                  | group ms2 | <0.001 |
| season-year                                  | group mst | 0.144 |
| season-year                                  | group sst | 0.147 |

Within and between-individual age were standardized by mean centering and divided by the standard deviation of raw age. Within-individual age mean = 0, between-individual age mean = 11.88, raw age sd = 6.
Table S3. Detailed results for the model of being approached by others. (A) Estimates for slopes with standard error, 95% confidence interval, test statistic, degrees of freedom, and p-values, are provided for fixed effects alongside (B) the estimated standard deviations for random effects intercepts and random slopes. Reference levels were set as reproductive status = not reproductively active and season = mating.

<table>
<thead>
<tr>
<th>A</th>
<th>Fixed term</th>
<th>Est.</th>
<th>SE</th>
<th>CI lower</th>
<th>CI upper</th>
<th>Df</th>
<th>Test stat.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
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<td>intercept</td>
<td>0.141</td>
<td>0.085</td>
<td>-0.018</td>
<td>0.282</td>
<td></td>
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<td>-</td>
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<tr>
<td></td>
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<td>0.317</td>
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<td>0.163</td>
<td>0.495</td>
<td>1</td>
<td>8.559</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
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<td>0.027</td>
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<td>1.263</td>
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<tr>
<td></td>
<td>Elo score</td>
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<td>-0.102</td>
<td>0.000</td>
<td>1</td>
<td>3.371</td>
<td>0.066</td>
</tr>
<tr>
<td></td>
<td>season</td>
<td>0.011</td>
<td>0.120</td>
<td>-0.177</td>
<td>0.223</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>reproductive status</td>
<td>-0.195</td>
<td>0.071</td>
<td>-0.314</td>
<td>-0.064</td>
<td></td>
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<td>-</td>
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<tr>
<td></td>
<td>season * reproductive status</td>
<td>0.568</td>
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<td>1</td>
<td>0.003</td>
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</table>

<table>
<thead>
<tr>
<th>B</th>
<th>Grouping factor</th>
<th>Random intercept or slope</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>female</td>
<td>intercept</td>
<td>0.137</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>Elo score</td>
<td>0.065</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>season</td>
<td>0.077</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>reproductive status</td>
<td>0.039</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>nfemale</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td>group</td>
<td>intercept</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>group</td>
<td>Elo score</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>group</td>
<td>season</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>group</td>
<td>reproductive status</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>group</td>
<td>season * reproductive status</td>
<td>0.131</td>
</tr>
<tr>
<td></td>
<td>season-year</td>
<td>intercept</td>
<td>0.132</td>
</tr>
<tr>
<td></td>
<td>season-year</td>
<td>Elo score</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>season-year</td>
<td>reproductive status</td>
<td>0.119</td>
</tr>
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</table>

Within and between-individual age were standardized by mean centering and divided by the standard deviation of raw age. Within-individual age mean = 0, between-individual age mean = 11.88, raw age sd = 5.85.
Table S4. Detailed results for the model of instrength. (A) Estimates for slopes with standard error, 95% confidence interval, test statistic, degrees of freedom, and p-values, are provided for fixed effects alongside (B) the estimated standard deviations for random effects intercepts and random slopes. Reference levels were set as reproductive status = not reproductively active and season = mating.

**A**

<table>
<thead>
<tr>
<th>Fixed term</th>
<th>Est.</th>
<th>SE</th>
<th>CI_lower</th>
<th>CI_upper</th>
<th>Df</th>
<th>Test stat.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>intercept</td>
<td>7.615</td>
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<td>6.852</td>
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<td>0.222</td>
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<td>season</td>
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</tr>
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<td>-1.599</td>
<td>0.035</td>
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<td>2.059</td>
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<td>0.051</td>
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</table>

**B**

<table>
<thead>
<tr>
<th>Grouping factor</th>
<th>Random intercept or slope</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>receiver</td>
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<tr>
<td>receiver</td>
<td>season</td>
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</tr>
<tr>
<td>receiver</td>
<td>reproductive status</td>
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</tr>
<tr>
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<td>nfemale</td>
<td>0.479</td>
</tr>
<tr>
<td>group</td>
<td>intercept</td>
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</tr>
<tr>
<td>group</td>
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</tr>
<tr>
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<td>group</td>
<td>reproductive status</td>
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</tr>
<tr>
<td>group</td>
<td>season * reproductive status</td>
<td>0.362</td>
</tr>
<tr>
<td>season-year</td>
<td>intercept</td>
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</tr>
<tr>
<td>season-year</td>
<td>Elo score</td>
<td>0.113</td>
</tr>
<tr>
<td>season-year</td>
<td>reproductive status</td>
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</table>

Within and between-individual age were standardized by mean centering and divided by the standard deviation of raw age. Within-individual age mean = 0, between-individual age mean = 11.86, raw age sd = 5.85.
Table S5. Detailed results for the model of indegree. (A) Estimates for slopes with standard error, 95% confidence interval, test statistic, degrees of freedom, and p-values, are provided for fixed effects alongside (B) the estimated standard deviations for random effects intercepts and random slopes. Reference levels were set as reproductive status = not reproductively active and season = mating.

### A

<table>
<thead>
<tr>
<th>Fixed term</th>
<th>Est.</th>
<th>SE</th>
<th>CI lower</th>
<th>CI upper</th>
<th>Df</th>
<th>Test stat.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
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<td>-4.757</td>
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<td>-</td>
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<td>-0.578</td>
<td>-0.182</td>
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<td>10.531</td>
<td>0.001</td>
</tr>
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</table>

### B

<table>
<thead>
<tr>
<th>Grouping factor</th>
<th>Random intercept or slope</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>actor</td>
<td>intercept</td>
<td>0.326</td>
</tr>
<tr>
<td>actor</td>
<td>Elo score</td>
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</tr>
<tr>
<td>actor</td>
<td>season</td>
<td>0.133</td>
</tr>
<tr>
<td>actor</td>
<td>reproductive status</td>
<td>0.363</td>
</tr>
<tr>
<td>actor</td>
<td>season * reproductive status</td>
<td>0.534</td>
</tr>
<tr>
<td>actor</td>
<td>nfemale</td>
<td>0.233</td>
</tr>
<tr>
<td>receiver</td>
<td>intercept</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>receiver</td>
<td>Elo score</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>receiver</td>
<td>season</td>
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<td>receiver</td>
<td>reproductive status</td>
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<tr>
<td>receiver</td>
<td>season * reproductive status</td>
<td>&lt;0.001</td>
</tr>
<tr>
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<td>nfemale</td>
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</tr>
<tr>
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<td>intercept</td>
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</tr>
<tr>
<td>dyad</td>
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</tr>
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</tr>
<tr>
<td>dyad</td>
<td>season * reproductive status</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>group</td>
<td>intercept</td>
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</tr>
<tr>
<td>group</td>
<td>Elo score</td>
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</tr>
<tr>
<td>group</td>
<td>season</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>group</td>
<td>reproductive status</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>group</td>
<td>season * reproductive status</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>season-year</td>
<td>intercept</td>
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</tr>
<tr>
<td>season-year</td>
<td>Elo score</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>season-year</td>
<td>reproductive status</td>
<td>0.141</td>
</tr>
</tbody>
</table>

Within and between-individual age were standardized by mean centering and divided by the standard deviation of raw age. Within-individual age mean = 0, between-individual age mean = 11.70, raw age sd = 5.71.
Table S6. Detailed results for the model of grooming outdegree. (A) Estimates for slopes with standard error, 95% confidence interval, test statistic, degrees of freedom, and p-values, are provided for fixed effects alongside (B) the estimated standard deviations for random effects intercepts and random slopes. Reference levels were set as reproductive status = not reproductively active and season = mating.

### A

<table>
<thead>
<tr>
<th>Fixed term</th>
<th>Est.</th>
<th>SE</th>
<th>Clower</th>
<th>Clower</th>
<th>Df</th>
<th>Test stat.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>intercept</td>
<td>-4.657</td>
<td>0.162</td>
<td>-4.933</td>
<td>-4.348</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
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<td>-0.937</td>
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<td>-0.210</td>
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<td>-0.063</td>
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<td>0.626</td>
</tr>
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<td>0.184</td>
<td>0.088</td>
<td>0.783</td>
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### B

<table>
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<th>Grouping factor</th>
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<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
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<td>&lt;0.001</td>
</tr>
<tr>
<td>actor</td>
<td>reproductive status</td>
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</tr>
<tr>
<td>actor</td>
<td>season * reproductive status</td>
<td>0.399</td>
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<td>actor</td>
<td>nfemale</td>
<td>0.108</td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td>receiver</td>
<td>Elo score</td>
<td>&lt;0.001</td>
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<tr>
<td>receiver</td>
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<td>dyad</td>
<td>intercept</td>
<td>1.141</td>
</tr>
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<td>dyad</td>
<td>Elo score</td>
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</tr>
<tr>
<td>dyad</td>
<td>season</td>
<td>0.663</td>
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<td>dyad</td>
<td>reproductive status</td>
<td>0.177</td>
</tr>
<tr>
<td>dyad</td>
<td>season * reproductive status</td>
<td>&lt;0.001</td>
</tr>
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<td>intercept</td>
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</tr>
<tr>
<td>group</td>
<td>Elo score</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>group</td>
<td>season</td>
<td>0.052</td>
</tr>
<tr>
<td>group</td>
<td>reproductive status</td>
<td>0.121</td>
</tr>
<tr>
<td>group</td>
<td>season * reproductive status</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>season-year</td>
<td>intercept</td>
<td>0.231</td>
</tr>
<tr>
<td>season-year</td>
<td>Elo score</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>season-year</td>
<td>reproductive status</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Within and between-individual age were standardized by mean centering and divided by the standard deviation of raw age. Within-individual age mean = 0, between-individual age mean = 11.70, raw age sd = 5.71.
Table S7. Detailed results for the model of grooming outstrength. (A) Estimates for slopes with standard error, 95% confidence interval, test statistic, degrees of freedom, and p-values, are provided for fixed effects alongside (B) the estimated standard deviations for random effects intercepts and random slopes. Reference levels were set as reproductive status = not reproductively active and season = mating.

### A

<table>
<thead>
<tr>
<th>Fixed term</th>
<th>Est.</th>
<th>SE</th>
<th>CI_{lower}</th>
<th>CI_{upper}</th>
<th>Df</th>
<th>Test stat.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>intercept</td>
<td>6.239</td>
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<td>5.537</td>
<td>6.893</td>
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<tr>
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<td>-2.564</td>
<td>0.509</td>
<td>-3.590</td>
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<tr>
<td>between-individual age</td>
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<td>-1.527</td>
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<td>59.527</td>
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<td>4.829</td>
<td>0.944</td>
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<tr>
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<td>0.809</td>
<td>2.023</td>
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<td>0.390</td>
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<td>440.961</td>
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### B

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<th>SD</th>
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<td>Elo score</td>
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<tr>
<td>actor</td>
<td>season</td>
<td>0.753</td>
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<td>actor</td>
<td>reproductive status</td>
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<tr>
<td>actor</td>
<td>nfemale</td>
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</tr>
<tr>
<td>group</td>
<td>intercept</td>
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<td>group</td>
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<td>season-year</td>
<td>intercept</td>
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</tr>
<tr>
<td>season-year</td>
<td>Elo score</td>
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</tr>
<tr>
<td>season-year</td>
<td>reproductive status</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Within and between-individual age were standardized by mean centering and divided by the standard deviation of raw age. Within-individual age mean = 0, between-individual age mean = 11.86, raw age sd = 5.85.
Table S8. Detailed results for the model on selective approach. The interaction of age and cumulated DSI was non-significant and removed from the model (estimate ±SE = 0.017 ±0.015, p = 0.244). (A) Estimates for slopes with standard error, 95% confidence interval, test statistic, degrees of freedom, and p-values, are provided for fixed effects alongside (B) the estimated standard deviations for random effects intercepts and random slopes. Reference levels were set as reproductive status = not reproductively active and season = mating.

<table>
<thead>
<tr>
<th>A</th>
<th>Fixed term</th>
<th>Est.</th>
<th>SE</th>
<th>CI_lower</th>
<th>CI_upper</th>
<th>Df</th>
<th>Test stat.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>intercept</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>within-individual age</td>
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<td>0.147</td>
<td>-0.427</td>
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<td>0.786</td>
<td>0.377</td>
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</tr>
<tr>
<td>between-individual age</td>
<td>-0.085</td>
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<td>-0.163</td>
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<td>4.737</td>
<td>0.023</td>
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<tr>
<td>cumuDSI previous season</td>
<td>0.090</td>
<td>0.019</td>
<td>0.053</td>
<td>0.130</td>
<td>1</td>
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<td>Elo score</td>
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<td>0.263</td>
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<td>0.232</td>
<td>-</td>
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<td>-0.031</td>
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<td>season * reproductive status</td>
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<table>
<thead>
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<th>Grouping factor</th>
<th>Random intercept or slope</th>
<th>SD</th>
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<td>cumuDSI previous season</td>
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<td>actor</td>
<td>Elo score</td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td>actor</td>
<td>season</td>
<td></td>
<td>0.081</td>
</tr>
<tr>
<td>actor</td>
<td>reproductive status</td>
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<td>0.122</td>
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<tr>
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<td>season</td>
<td></td>
<td>0.133</td>
</tr>
<tr>
<td>receiver</td>
<td>reproductive status</td>
<td></td>
<td>0.060</td>
</tr>
<tr>
<td>receiver</td>
<td>nfemale</td>
<td></td>
<td>0.127</td>
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</tr>
<tr>
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<td>cumuDSI previous season</td>
<td></td>
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</tr>
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<td>Elo score</td>
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</tr>
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<td>0.189</td>
</tr>
<tr>
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<td>cumuDSI previous season</td>
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Table S9. Detailed results for the model of approaching others. (A) Estimates for slopes with standard error, 95% confidence interval, test statistic, degrees of freedom, and p-values, are provided for fixed effects alongside (B) the estimated standard deviations for random effects intercepts and random slopes. Reference levels were set as reproductive status = not reproductively active and season = mating.

<table>
<thead>
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<th>A</th>
<th>Fixed term</th>
<th>Est.</th>
<th>SE</th>
<th>CLower</th>
<th>CIupper</th>
<th>Df</th>
<th>Test stat.</th>
<th>P</th>
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<tbody>
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<td>-</td>
<td>-</td>
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<tr>
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<td>8.254</td>
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</tr>
<tr>
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<td>between-individual age</td>
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<td>-0.148</td>
<td>-0.019</td>
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<td>4.332</td>
<td>0.037</td>
</tr>
<tr>
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<td>Elo score</td>
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<td>0.037</td>
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<td>-</td>
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<tr>
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<td>0.089</td>
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<table>
<thead>
<tr>
<th>B</th>
<th>Grouping factor</th>
<th>Random intercept or slope</th>
<th>SD</th>
</tr>
</thead>
<tbody>
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<td></td>
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<td>intercept</td>
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</tr>
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<td>Elo score</td>
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</tr>
<tr>
<td></td>
<td>female</td>
<td>season</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>reproductive status</td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>group</td>
<td>season</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>group</td>
<td>reproductive status</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>group</td>
<td>season * reproductive status</td>
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<tr>
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<td>season-year</td>
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<td>season-year</td>
<td>reproductive status</td>
<td>0.136</td>
</tr>
</tbody>
</table>

Within and between-individual age were standardized by mean centering and divided by the standard deviation of raw age. Within-individual age mean = 0, between-individual age mean = 11.88, raw age sd = 5.85.

Table S10. Assessing the difference in within and between-individual age estimated effects on the social behavior investigated. Models are based on equation (3) including the main term for age and individual average-age. The estimated slope and significance test assigned to the between-age effect represents the difference in the slopes of the within and between-individual effects, allowing to test for selective disappearance. Raw age was z-transformed and between-individual age mean-centered and scaled to the standard deviation of raw age in all models.

<table>
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<th>Hypothesis</th>
<th>Response</th>
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<th>P</th>
<th>$\beta_{average age} \pm SE$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>spatial segregation</td>
<td>proportion time alone</td>
<td>-0.281 ±0.181</td>
<td>0.127</td>
<td>0.374 ±0.204</td>
<td>0.072</td>
</tr>
<tr>
<td>exclusion</td>
<td>being approached</td>
<td>0.317 ±0.093</td>
<td>0.003</td>
<td>-0.287 ±0.105</td>
<td>0.013</td>
</tr>
<tr>
<td>exclusion</td>
<td>grooming instrength</td>
<td>-0.720 ±0.453</td>
<td>0.668</td>
<td>1.257 ±0.515</td>
<td>0.253</td>
</tr>
<tr>
<td>exclusion</td>
<td>grooming indegree</td>
<td>-0.661 ±0.232</td>
<td>0.008</td>
<td>0.804 ±0.258</td>
<td>0.004</td>
</tr>
<tr>
<td>disengagement/ selectivity</td>
<td>grooming outdegree</td>
<td>-0.937 ±0.204</td>
<td>&lt;0.001</td>
<td>0.626 ±0.227</td>
<td>0.007</td>
</tr>
<tr>
<td>disengagement/ selectivity</td>
<td>grooming outstrength</td>
<td>-2.564 ±0.509</td>
<td>&lt;0.001</td>
<td>1.402 ±0.586</td>
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</tr>
<tr>
<td>increased selectivity</td>
<td>selective approach</td>
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<td>0.375</td>
<td>0.045 ±0.166</td>
<td>0.786</td>
</tr>
<tr>
<td>disengagement</td>
<td>approaching others</td>
<td>-0.414 ±0.139</td>
<td>0.004</td>
<td>0.332 ±0.157</td>
<td>0.036</td>
</tr>
</tbody>
</table>
Chapter 3

Parasites in a social world: Lessons from primates

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Animal Behavior and Parasitism (2022)

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Abstract

Social behavior and parasitism interconnect at all levels of sociality, from the community to the population and from the group down to the individual. This chapter explores key findings on the parasite-related costs and benefits of sociality, focusing on primates. The research spans across social networks, dominance and affiliative relationships, and individual behavior and physiology, highlighting established links between primate sociality and parasitism and identifying important gaps for future research. Given the use of nuanced conceptual frameworks and new analytical methods, combined with experimental studies and growing empirical data from long-term field projects, primates are a particularly exciting and helpful taxon for studying sociality-parasite interactions.

Keywords

social behavior, disease ecology, social structure, anti-parasite behavior, social network, glucocorticoids, exposure, susceptibility, transmission, health
Chapter 4

Investigation of glucocorticoid dynamics during aging in female Assamese macaques living in their natural habitat

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Manuscript in preparation
Abstract

Aging is often associated with dysfunction of the HPA axis in humans, thought to result from the mismatch between the classic vertebrate stress response and the constant social challenges arising in human daily life. This mismatch is thought to be shared with other non-human primates, and possibly other highly social species, navigating complex social interactions. Aging of the HPA axis in natural populations of non-human primates has been little studied, and the contribution of social factors to potential age-related changes is poorly understood. Non-invasive assessment of fecal glucocorticoid concentrations and social dynamics in wild female Assamese macaques (*Macaca assamensis*) over several years provided no evidence for age-related changes in endocrine function and did not implicate sociality as a mediator between age and HPA axis activity. These findings suggest that context-dependence, rather than a universal primate specificity, may be important in explaining age-related changes in HPA axis.

Keywords

senescence, HPA axis, cortisol, physiological aging, sociality, primate
Introduction

Glucocorticoids (GC) exert an extraordinary range of effects on the metabolism, reproductive system, immune function and behavior of vertebrates, and thereby contribute to important life-history events (e.g., growth and reproduction) (Crespi et al., 2013; Landys et al., 2006). The association between dysfunction of the HPA axis reactivity (whether blunted or enhanced) or HPA axis activation (relatively short pulses versus prolonged activation) and diseases has been demonstrated in humans and laboratory animal models (Charmandari et al., 2005; Sapolsky et al., 2000). Such findings have often been at odds with the scant evidence for detrimental effects of GCs in natural populations of birds and mammals (Beehner and Bergman, 2017; Bonier et al., 2009a; Schoenle et al., 2021). The rise in circulating concentrations of GCs in the face of experienced or anticipated stressors is influenced by the nature of the challenge. The greater the frequency, intensity, and the lower the predictability and level of control on the stressor, the stronger and more persistent the elevation in GCs (Sapolsky, 2021; Schoenle et al., 2018). Furthermore, the response orchestrated by GCs is predicted to be beneficial only if the downstream physiological adaptations effectively allow the organism to escape the challenge (Schoenle et al., 2018). A mismatch occurs when the stressor is unavoidable or must be dealt with by means not regulated by GCs. This mismatch is often cited when discussing the long-term consequences of social competition and hierarchies on the stress physiology of primates, including humans (Sapolsky, 2021). Individuals navigating unstable hierarchies, losing stable relationships, or experiencing harassment from dominants may exhibit prolonged HPA axis, potentially contributing to reduced survival and reproductive success (Abbott et al., 2003; Creel, 2001; Creel et al., 1996; Sapolsky, 2005; Scott et al., 2012; Thompson and Cords, 2018).

The manifestations of a lifetime of exposure to chronic social challenges are especially expected in old age because of the cumulative nature of the damage caused by repeated elevations of GCs on the brain and peripheral organs (Mizoguchi et al., 2003; Sapolsky et al., 2002). Loss of sensitivity to GC feedback results in a chronic elevation of baseline GCs, heightened responsiveness to acute stressors, and a blunted diurnal cyclicity with age (Born et al., 1995; Dodt et al., 1991; Ferrari et al., 2001; Goncharova et al., 2019; Goncharova and Lapin, 2002; Gust et al., 2000; Nathanielsz et al., 2020; Otte et al., 2005; Reul et al., 1991; Sapolsky, 1992b; Touitou et al., 1983; Van Cauter et al., 1996; Yang et al., 2017). However, very little data is available to corroborate the dysregulation of HPA activity outside of humans and captive non-human primates. In one population of yellow baboons, age was associated with reduced negative feedback and increased concentrations of baseline GCs, further manifested in increased fecal GCs (fGCs) in females but not males (Altmann et al., 2010; Sapolsky and Altmann, 1991). In a wild chimpanzee community urinary cortisol concentrations increased with age and diurnal
rhythmicity appeared blunted, although there was no evidence that the changes were mediated by sociosexual factors (Emery Thompson et al., 2020a).

We set out to investigate the relationship between age and fGCs in a long-term monitored population of Assamese macaques (*Macaca assamensis*). We focused on females for whom more precise age estimates were available and for whom we have previously documented a gradual social disengagement with progressing age (Chapter 2 of this thesis, submitted). Specifically, females decreased their active engagement in grooming interactions with age, which resulted in a decrease of the strength of the bonds with their top partners. As strongly bonded partners are important source of support in female cercopithecines (Ellis et al., 2019; Ostner and Schülke, 2018; Thompson, 2019), decreasing strength of the bonds with top partners could results in females facing higher level of adversity with age. In the present study, we investigate the influence of age and bond strength with the top partners, while controlling for environmental variables, to assess the contribution of social dynamics to patterns of fGCS over the life course. We predicted that an increase in fGCS with age, robust to intermittent changes in reproductive activity and sociality, would support an intrinsic aging of the HPA axis.

**Methods**

The study was carried out at the Phu Khieo Wildlife Sanctuary (PKWS, 16°05’–35’N, 101°20’–55’E, 1573 km²), in northeastern Thailand, where a population of wild Assamese macaques fully habituated to human observers has been studied since 2005. Ambient temperature at the study site was automatically recorded by data loggers, allowing daily minimum temperatures to be computed. Fruit abundance at the study site was measured from tree species abundance in 45 botanical plots covering 21ha and monthly phenology scores from more than 650 trees representing important fruit sources (Heesen et al., 2013) and we extrapolated daily scores between consecutive monthly records. Temperature and fruit abundance were necessary here because GCs are secreted to promote catabolic activity during energy challenging conditions associated with low temperature or low energy intake (Sadoughi et al., 2021a; Touitou et al., 2021b).

Behavioral data and fecal samples were collected from adult females intermittently from 2010 to 2020. Years were divided into a mating (October to February) and a non-mating (March to September) seasons, giving unique season-year time periods. Females were observed for a mean of 6.0±2.9 season-years, with a range of to one to 11. Females were considered adult from the mating season of first conception (usually at 5.5 years of age) (Anzà et al., 2022) with ages known from witnessed births or inferred from comparisons with individuals of known age. Average (±SD) age was 11.9±5.5 years old, for a range of 4 to 29, covering most of the reported lifespan in populations of semi-free ranging
Aging HPA axis

macaques (Brent et al., 2017; Johnson and Kapsalis, 1995). A detailed description of the behavioral protocol can be found in Chapter 2 of this thesis (Sadoughi et al. (submitted)). In brief, we followed groups from dawn to dusk using 40min continuous focal animal protocols (Altmann, 1974) to record all instances of dyadic grooming interactions between adult females along with the identity of the actor and the receiver. Dyadic agonistic interactions were collected during focal animal protocols and *ad libitum*, with the identity of the winner and loser. Based on 9404 hours of focal observation (27±9.3 hours per female per season-year), we calculated individual-level social metrics per season-year. The time spent grooming (both given and received) between two females was divided by dyadic observation time (i.e., divided by the sum of observation time for each member of the dyad during which the other member of the dyad was also present and adult). The resulting dyad metrics indicate the strength of ties for every pair of females in a group. For each female, we summed the three highest dyadic values to obtain an individual-level bond strength with the top partners. Dominance between adult females from the same group was determined from the direction of submission (e.g., silent bared teeth display, give ground) and aggression (e.g., open-mouth threat, chase), and computed as Elo scores with the package Elo_rating (functions elo_seq and elo_plot with k=100 and a starting value of 1,000) (Neumann et al., 2011). Undecided conflicts were counted as half a win for each participant following package default setting. Dominance relationships are highly stable within groups (function stab_elo, stability > 0.99) so we only extracted female’s Elo scores at the end of each season (30th of September for the birth and 1st of March for the mating season) or on the last day observed (four females who died or disappeared during a season) and assigned the scores to all samples collected in the respective season.

Fecal samples were collected opportunistically throughout the day from clearly identified females and processed according to established protocols for storage and extraction in the field (Ostner et al., 2008; Shutt et al., 2012, 2007). Samples were shipped to the German Primate Center in Germany and kept frozen at -20°C until assayed using an in-house enzyme immunoassay validated for the measurement of 11β–hydroxyetiocholanolone a metabolite of GC in macaques (Heistermann et al., 2006). All samples were run in duplicates, and samples with intra-assay coefficients of variation (CVs) >10% between duplicates analyzed again. Average intra-assay CVs across assay plates based on high and low-concentration quality controls (QCs) were 9.1% and 11.5% respectively, while inter-assay CVs calculated across assay plates were 11.6% for high and 22.8% for low QCs. For each sample, we also determined whether the female was gestating, lactating, or non-gestating non-lactating (NGNL). Onset of gestation was determined *a posteriori* by subtracting the average gestation length (i.e., 164 days, Fürtbauer et al., 2010) from the date of parturition. If the exact date of parturition was unknown, we assigned the date of birth based on the last day we saw the mother not carrying an infant and on infant
morbidity, and when the uncertainty was greater than two months (due to short interruptions in data collection), so we chose the peak of the birth season (15th of May, Fürtbauer et al., 2010; unpublished data) as birth date.

Analyses included two Linear Mixed Models (LMMs) in R with RStudio (R Core Team, 2021 version 4.0.4; RStudio Team, 2022 version 2022.07.02+576) using the package lme4 (Bates et al., 2015). Covariates were z-transformed, and factors were dummy coded and centered when included as random slopes. The first model included the covariate for age, separated into within- and between-individual age terms (van de Pol and Verhulst, 2006; van de Pol and Wright, 2009) to disentangle individual aging from population level effect (e.g., selective disappearance). The factor term reproductive state (three levels), and the linear terms for daily fruit availability, daily minimum temperature, and the polynomial of daily minimum temperature were included as control variables because energy metabolic activity varies with reproductive state (Fürtbauer et al., 2014; Touitou et al., 2021b), and because caloric intake and temperature were found to correlate with urinary GCs in the population (Touitou et al., 2021b). The second model added to the first model the grooming strength to the female’s top three adult female partners in the group, and dominance Elo score. Female ID, date of sample collection, and season-year were included as random effects terms in both models. The random slopes for within-individual age, Elo score, reproductive state, fruit availability and minimum temperature within ID and season-year, and for between-individual age within season-year were also included to maintain type I error rate at 5% and avoid false positive (Barr et al., 2013). The correlation of random intercept and slope were excluded from the second model to ease converge. Both models were of good stability as assessed by excluding levels of random effects factors one at a time and comparing the model estimates of models fitted to the derived subsets with those derived for the full dataset. The distribution of residuals followed a normal distribution and was homogeneous (packages performance function check_model), and collinearity was not an issue (vif function in package car, max VIF=3 excluding polynomial term, 3.2 when including it). The 95% confidence intervals of model estimates were determined by means of parametric bootstrapping (N=100 bootstraps; package glmmTMB function simulate). To avoid false positives when testing several predictors at once, we compared with Chi-square tests the first model to a model lacking within- and between-age terms, and the second model to a model lacking grooming strength and Elo scores. When full-reduced model comparisons were statistically significant, p-values on individual terms were obtained using the Satterthwaite approximation in the package lmerTest (Kuznetsova et al., 2017). P-values <0.05 were considered significant. Sample size was 2,752 samples.
Results

Daily minimum temperature ranged from 4.2 to 23.4°C (mean±SD=18.3±3.4°C), and daily fruit availability from 2.9 to 79.3 (28.5±17.6). Seven hundred thirty samples were collected from gestating females, 1358 from lactating females, and 664 from NGNL females, with one to 102 samples per female (47.5±27.0) with on average 7.9±3.9 samples per female per season-year. Concentrations of fGCs ranged from 21.0 to 3204.0 ng/g (mean±SD=180.3±145.1 ng/g).

The full-null model comparison for the model including only age and environmental control variables was not significant ($\chi^2=1.664$, Df=2, p=0.435), providing no evidence for modifications of HPA axis activity during individual aging, nor support for a correlation of GCs and age at the population level (table 1A). As aging is associated with changes in social engagement, and as the support from closely bonded partners has been repeatedly linked to GCs dynamics in non-human primates, we included female’s bond strength to her top partners and dominance status. The full-null model comparison was again not significant ($\chi^2=3.789$, Df=2, p=0.150) (table 1B).

Table 1. Effects of age on the concentration of glucocorticoid metabolites (11β-hydroxyetiocholanolone) in feces (A) before and (B) after accounting for social factors. Covariates were z-transformed. P-values are not shown as full-reduced model comparisons were not significant.

<table>
<thead>
<tr>
<th>Fixed term</th>
<th>Estimate</th>
<th>SE</th>
<th>95% CI_lower</th>
<th>95% CI_upper</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>intercept</td>
<td>5.23</td>
<td>0.08</td>
<td>5.08</td>
<td>5.38</td>
</tr>
<tr>
<td>within-age</td>
<td>0.13</td>
<td>0.09</td>
<td>-0.03</td>
<td>0.32</td>
</tr>
<tr>
<td>between-age</td>
<td>-0.02</td>
<td>0.03</td>
<td>-0.08</td>
<td>0.04</td>
</tr>
<tr>
<td>reproductive state (lactating)</td>
<td>-0.17</td>
<td>0.05</td>
<td>-0.27</td>
<td>-0.06</td>
</tr>
<tr>
<td>reproductive state (NGNL)</td>
<td>-0.07</td>
<td>0.06</td>
<td>-0.19</td>
<td>0.05</td>
</tr>
<tr>
<td>group (mst)</td>
<td>-0.16</td>
<td>0.07</td>
<td>-0.31</td>
<td>-0.01</td>
</tr>
<tr>
<td>group (oth)</td>
<td>0.01</td>
<td>0.08</td>
<td>-0.16</td>
<td>0.16</td>
</tr>
<tr>
<td>group (sst)</td>
<td>0.03</td>
<td>0.09</td>
<td>-0.18</td>
<td>0.20</td>
</tr>
<tr>
<td>group (stu)</td>
<td>-0.22</td>
<td>0.15</td>
<td>-0.55</td>
<td>0.18</td>
</tr>
<tr>
<td>daily fruit availability</td>
<td>0.16</td>
<td>0.12</td>
<td>-0.07</td>
<td>0.35</td>
</tr>
<tr>
<td>daily min temperature</td>
<td>0.00</td>
<td>0.03</td>
<td>-0.08</td>
<td>0.07</td>
</tr>
<tr>
<td>daily min temperature$^2$</td>
<td>-0.01</td>
<td>0.01</td>
<td>-0.03</td>
<td>0.02</td>
</tr>
</tbody>
</table>
### Table B

<table>
<thead>
<tr>
<th>Fixed term</th>
<th>Estimate</th>
<th>SE</th>
<th>95% CI lower</th>
<th>95% CI upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>intercept</td>
<td>5.20</td>
<td>0.08</td>
<td>5.02</td>
<td>5.36</td>
</tr>
<tr>
<td>within-age</td>
<td>0.09</td>
<td>0.09</td>
<td>-0.10</td>
<td>0.22</td>
</tr>
<tr>
<td>between-age</td>
<td>-0.01</td>
<td>0.03</td>
<td>-0.07</td>
<td>0.05</td>
</tr>
<tr>
<td>bond strength top partners</td>
<td>0.00</td>
<td>0.02</td>
<td>-0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>Elo score</td>
<td>-0.06</td>
<td>0.03</td>
<td>-0.12</td>
<td>0.01</td>
</tr>
<tr>
<td>reproductive state (lactating)</td>
<td>-0.14</td>
<td>0.06</td>
<td>-0.23</td>
<td>-0.05</td>
</tr>
<tr>
<td>reproductive state (NGNL)</td>
<td>-0.06</td>
<td>0.05</td>
<td>-0.16</td>
<td>0.03</td>
</tr>
<tr>
<td>group (mst)</td>
<td>-0.13</td>
<td>0.07</td>
<td>-0.28</td>
<td>0.00</td>
</tr>
<tr>
<td>group (oth)</td>
<td>0.10</td>
<td>0.08</td>
<td>-0.09</td>
<td>0.27</td>
</tr>
<tr>
<td>group (sst)</td>
<td>0.01</td>
<td>0.09</td>
<td>-0.16</td>
<td>0.17</td>
</tr>
<tr>
<td>group (stu)</td>
<td>-0.16</td>
<td>0.17</td>
<td>-0.46</td>
<td>0.24</td>
</tr>
<tr>
<td>daily fruit availability</td>
<td>0.16</td>
<td>0.11</td>
<td>-0.08</td>
<td>0.36</td>
</tr>
<tr>
<td>daily min temperature</td>
<td>0.00</td>
<td>0.04</td>
<td>-0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>daily min temperature&lt;sup&gt;2&lt;/sup&gt;</td>
<td>-0.02</td>
<td>0.01</td>
<td>-0.04</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**Figure 1.** Individual centered age did not correlate with fecal glucocorticoid metabolites (fGCs) (estimated slope and confidence intervals extracted from model B in Table 1).
Discussion

We found no evidence that within-individual age was associated with fGCs, suggesting no differences owing to aging. The between-individual age effect was not significant, suggesting that females on average older did not exhibit increased or decreased fGC concentrations compared to younger females. Therefore, cohort effects or selective disappearance could not explain the absence of within-individual age effects.

Despite great interest in understanding the contribution of HPA axis dysfunction to age-related pathologies in an aging human population, very few comparative studies have investigated the late-life course of GCs in other primates. Results are inconclusive, GCs increasing with age in female baboons (Altmann et al., 2010), and in both male and female chimpanzees (Emery Thompson et al., 2020a) irrespective of climatic and sociosexual dynamics, whereas no association was found in female rhesus (Maestripieri and Georgiev, 2016), golden lion tamarins (Bales et al., 2006), Barbary macaques (Shutt et al., 2007), and long-tailed macaques (van Schaik et al., 1991). Although the conclusion reached by some of these studies may be limited by small sample size and their cross-sectional design, results from our longitudinal analyses suggest that neither may be sufficient to explain a lack of age-related changes in GCs.

The relationship between social status, social integration (either measured by network size or bond strength) and fGCs is highly variable across the literature, even sometimes within species (Beehner and Bergman, 2017). An alternative interpretation of the results from humans, yellow baboons, chimpanzees, and captive non-human primates is that intense and frequent HPA triggering is required for age-related dysregulation of HPA axis to manifest. Evidence of increased baseline GCs with age in laboratory rodents (Sapolsky, 1992; Sapolsky et al., 2002) further supports that this dysregulation will arise under any (abnormally) chronically stressful environments. The results suggest that such stressful level of adversity is not encountered by female macaques living in their natural social and environmental conditions.

The ‘coping hypothesis of aging’ (Hämäläinen et al., 2015b) suggest that age-related dysfunction will only manifest under circumstances driving the greatest HPA activity. Consistent with this interpretation, GCs increased only during the dry season in female mouse lemurs (Hämäläinen et al., 2015b), and in mandrills the increase with age was magnified by lower rainfall and temperatures (Charpentier et al., 2018) (although note that age was not continuous but treated as dichotomic). On the contrary, age did not modify the association between cortisol and dominance status in chimpanzees (Emery Thompson et al., 2020a), although as social integration was not considered in the study, it remains possible that parallel age-related changes in affiliation strategies obscured the
relationship (Rosati et al., 2020). It would therefore seem that age-related changes in GCs are associated with the influence of environmental rather than social factors. The response of the HPA axis to variation in temperature and rainfall may therefore reflect the greater sensitivity to variation in thermoregulatory or energy needs in aging subjects, rather than enhanced responsiveness to social interactions. Late-life dynamics of the HPA axis are only beginning to be investigated in natural animal populations, and though studies have been mostly motivated by the search for features of a ‘normal’ primate aging, the present data suggest integrating context-dependence in GCs-age relationships is needed (Sapolsky, 2021; Schoenle et al., 2018; Vitousek et al., 2019a).

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Chapter 5

Aging gut microbiota of wild macaques are equally diverse, less stable but progressively personalized

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Abstract

Pronounced heterogeneity of age trajectories has been identified as a hallmark of the gut microbiota in humans and has been explained by marked changes in lifestyle and health condition. Comparatively, age-related personalization of microbiota is understudied in natural systems limiting our comprehension of patterns observed in humans from ecological and evolutionary perspectives. Here, we tested age-related changes in the diversity, stability, and composition of the gut bacterial community using 16S rRNA gene sequencing with dense repeated sampling over three seasons in a cross-sectional age sample of adult female Assamese macaques (*Macaca assamensis*) living in their natural forest habitat. Gut bacterial composition exhibited a personal signature which became less stable as individuals aged. This lack of stability was not explained by differences in microbiota diversity but rather linked to an increase in the relative abundance of rare bacterial taxa. The lack of age-related changes in core-taxa or convergence with age to a common state of the community hampered predicting gut bacterial composition of aged individuals. On the contrary, we found increasing personalization of the gut bacterial composition with age, indicating that composition in older individuals was increasingly divergent from the rest of the population. Reduced direct transmission of bacteria resulting from decreasing social activity may contribute to, but not be sufficient to explain, increasing personalization with age. Together, our results challenge the assumption of a constant microbiota through adult life in a wild primate. Within the limits of this study, the fact that increasing personalization of the aging microbiota is not restricted to humans, suggests the underlying process to be evolved instead of provoked only by modern lifestyle of and health care for the elderly.

Keywords

aging, senescence, stability, gut bacteria, commensals, social transmission, personalized, primate, microbiome, dysbiosis
Chapter 6

Non-invasive assessment of metabolic responses to food restriction using urinary triiodothyronine and cortisol measurement in macaques

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Abstract

Regulation of energy allocation and metabolic rate plays an important role in determining behavior and fitness in wild animals, calling for the validation of non-invasive markers of energetic condition. Recently, the thyroid hormone triiodothyronine (T3) has emerged as a promising marker as concentrations decrease to lower the metabolic rate during energetically challenging periods. However, it remains largely unclear whether T3 merely represents an alternative or provides additional information compared to other compounds involved in the regulation of energy acquisition and allocation, like cortisol and C-peptide, as few joint measurements have been conducted to date in non-invasively collected samples. We aimed to validate the non-invasive measurement of immunoreactive urinary total T3 (uTT3), in comparison to urinary cortisol (uCort) and urinary C-peptide (uCP), as a marker of metabolic response to variation in food intake in macaques, and to address a number of issues regarding the collection, storage and processing of samples which are important for application of uTT3 measurements under field conditions. We used daily samples and body mass measures from a prior food restriction-refeeding experiment over 4 weeks with six captive macaques and analyzed concentrations of uTT3 and uCort in samples collected prior to (fasting) and after morning feeding (non-fasting). Concentrations of uTT3 decreased in response to restriction in food supply and were also lower during weeks of food restriction compared to weeks of refeeding. Variation in uTT3 also correlated positively with variation in body mass and concentrations of uCP. As expected, uCort showed the reverse pattern, increasing during food restriction and decreasing following refeeding, but was not associated with variation in body mass. Generally, compared to fasting samples, concentrations were higher in post-morning feeding, i.e., non-fasting, samples for uTT3 but not uCort. Contamination of urine samples with fecal matter, but not soil, and exposure to UV light led to a decrease in uTT3. uTT3 was largely unaffected by repeated freeze-thaw cycles and by refrigeration for medium-term storage (2 days) but degraded substantially when stored at ambient temperature for the same period. In conclusion, uTT3 measurements inform on the effect of food intake and its associated metabolic response to variation in energetic status. Since uTT3 is reasonably robust to many issues associated with collection and storage of urine samples under field conditions, it is a promising biomarker for studies of energetic condition and basal metabolic rate in wild macaques.

Keywords

thyroid hormone, macaques, non-invasive, metabolism, energy allocation, food restriction
Chapter 7

General Discussion

The aim of this thesis was to investigate the manifestations of aging in natural animal populations by studying adult females from a population of wild Assamese macaques. The present work contributes to the emerging integration of social behavior into aging research (Chapter 2) and, by building on the established links between sociality and health in primates (Chapter 3), to investigations anchoring human-specific physiological aging within a broader comparative framework (Chapter 4 and 5). In the first study (Chapter 2), I combined a mixed-longitudinal and a within-individual centered statistical analytic approach to investigate age-related changes in social behavior and found that females experienced active social disengagement with advancing age (table 1). This social disengagement was concomitant with, rather than explained by, the expression of social selectivity towards preferred partners, and findings pointed towards increasing constraints limiting social relationships with age. The comparison of mixed-longitudinal and within-individual centered results further highlighted demographic processes, consistent with the selective disappearance of poorly socially connected individuals, contributing to dynamics of social aging at the population level. In the second study (Chapter 4), I focused on one hormonal system, the HPA axis, and found no evidence of age-related changes in activity of the HPA axis assessed from the measurement of fecal glucocorticoid concentrations (fGCs). In Chapter 5, I found that gut bacterial communities are equally diversified, less stable, and increasingly individualized with increasing age. Having established the patterns of age-related changes in those different traits independently, I attempted to draw connections between them by assessing the interdependence between social behavior and physiological aging (Chapter 4 and 5). However, I found no evidence suggesting that social behavior mediates age-related changes in physiological traits. In this final chapter, I will integrate these findings into a broader frame and discuss their implications.
Table 1. Summary table recapitulating age-related changes in female Assamese macaques and across chapters in female-female social behavior, fecal glucocorticoids concentrations (fGCs), and gut bacterial communities, measured mixed-longitudinally, within-individuals or between-individuals. Patterns can indicate an increase (↑), a decrease (↓), or no evidence for a change (→) with age. Mixed-longitudinal patterns result from a combination of within- and between-individual effects. Between-individual age effects solely driven by within-individual age effects were denoted 0, while between-individual effects differing from within-individual effects (in either direction or magnitude) were denoted with the corresponding arrow.

<table>
<thead>
<tr>
<th>Chapter 2 – female-female social interactions</th>
<th>Age</th>
<th>Mixed-longitudinal</th>
<th>Within-individual</th>
<th>Between-individual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grooming engagement</td>
<td></td>
<td>↓ Active (\rightarrow) Passive &amp; ↓ Active (\rightarrow) Passive &amp; ↓ Active (\rightarrow) Passive &amp; 0 Passive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grooming network size</td>
<td></td>
<td>↓ Active (\rightarrow) Passive &amp; ↓ Active (\rightarrow) Passive &amp; ↓ Active (\rightarrow) Passive &amp; ↑ Passive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spatial segregation</td>
<td></td>
<td>↑ &amp; ← &amp; ↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initiation of interactions</td>
<td></td>
<td>↓ Active (\rightarrow) Passive &amp; ↓ Active (\rightarrow) Passive &amp; ↓ Active (\rightarrow) Passive &amp; ↓ Passive</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Chapter 4 – fGCs                             |     | ← |
| Chapter 5 – Gut bacterial communities        |     | ↓ |
| Diversity                                    |     | ← |
| Stability                                    |     | ↓ |
| Similarity                                   |     | ← |
| Personalization                              |     | ↑ |

7.1 Social behavior in old age: compensation or decline?

Female Assamese macaques decreased their active engagement in social interactions with advancing age, associated with a reduction in the number of partners they groomed. The reduction of grooming network size on a few selected partners has been associated with benefits such as reduced HPA axis activity and faster return to baseline following an instable social period in female chacma baboons (*Papio hamadryas ursinus*) (Crockford et al., 2008; Wittig et al., 2008). However, in the chacma baboons, reduction in network size occurred without females reducing their rate of grooming interactions. On the contrary, the results presented in Chapter 2 suggest that for aging female Assamese macaques, reduction in network size is likely driven, at least partly, by the reduction in grooming activity.
The central question, then, is why do aging individuals decrease their engagement in social interactions? Evidence in non-human primates converges on the maintenance of interest in social interactions well into old age (Almeling et al., 2017; Rathke and Fischer, 2021; Schino and Pinzaglia, 2018; van Noordwijk and van Schaik, 1987). For instance, old Barbary macaques (*Macaca sylvanus*) maintain interest in social cues (Almeling et al., 2016; Rathke and Fischer, 2021; Rosati and Santos, 2017) and regularly vocalize when witnessing interactions (Almeling et al., 2016; Rathke and Fischer, 2021), but this does not lead to interactions (Rathke et al., 2022). Lack of motivation to interact is often interpreted as resulting from some form of cognitive impairment (Röhr et al., 2020; Siracusa et al., 2022a). However, some evidence in humans has shown that a healthy brain can also drive a reduction in motivation into old age (Soutschek et al., 2021). During an experiment conducted on one group of young and one group of old healthy subjects, researchers stimulated the participants’ frontopolar cortex, a brain region promoting reward obtainment and management of competing goals (Mansouri et al., 2017), and assessed the participants willingness to perform a task. Frontopolar cortex activity increased motivation to perform the task in young subjects but decreased motivation among elders. This suggested that a healthy aging brain may contribute to the shift from gain maximization to effort minimization with age, possibly as a response to a change in an individual psychological or physiological internal state (Cardini and Freund, 2020; Depping and Freund, 2011). The contribution of energetic deficit and frailty to social disengagement remains unclear given that both social decline and maintained social engagement with age are observed in provisioned and non-provisioned groups (Almeling et al., 2016; Archie et al., 2014; Corr, 2003; Nakamichi and Shizawa, 2003; Rathke and Fischer, 2021; Rosati et al., 2020; Schino and Pinzaglia, 2018; Siracusa et al., 2022b; Thompson González et al., 2021; van Noordwijk and van Schaik, 1987; Veenema et al., 1997). The non-invasive measurement of triiodothyronine, validated for application to field research in Chapter 6, could contribute to test whether decreasing triiodothyronine levels correlate with decreasing social activity, and thereby offer an opportunity to test directional causality from physiology to sociality.

It is important to keep an open mind, as age-related changes in behavior may not necessarily mean social aging, at least not in the sense of *deterioration* commonly associated with aging. Behavior offers great flexibility to individuals in managing external and internal pressures (Touitou et al., 2021a), so that modifications of behavior with age may represent the best strategy to compensate other age-related declines. In female Assamese macaques, I investigated age-related changes in fGCs and the gut microbiome to cover some physiological traits impacted by differences in social strategies (Chapter 3, 4 and 5). However, I found no evidence for age-related changes in fGCs, and no correlation between sociality (measured as bond strength to the top partners) and fGCs (controlled for age). Therefore, decreasing social engagement is unlikely to be an adaptation to HPA axis functional aging. Similarly,
female dyads that groomed longer did not have a more similar gut bacterial composition, suggesting no protective effect of reduced social contact with age. Overall, the joint examinations suggest decline as a likely cause of age-related social changes in female Assamese macaques.

7.2 Social and physiological aging: mediation or modulation?

I found no evidence for social interactions to act as a mediator (Chapter 4 and 5) of physiological aging. Concentrations of fGCs were neither correlated to the strength of the grooming bonds to the top partners nor age. When investigating the gut microbiome, group identity explained a small but non-negligible variance in similarity, consistent with previous findings (reviewed in Sarkar et al., 2020). Therefore, future investigations should also account for group membership when assessing the contribution of individual traits (e.g., age) to gut bacterial communities, otherwise unaccounted group effects will inflate the importance of individual traits (Risely et al., 2022). However, this influence of group membership only provides indirect evidence for social effects on the gut microbiome. Group members may exhibit higher composition similarity because they share a territory, interact frequently, or both. In one yellow baboon population (Papio cynocephalus) (Björk et al., 2022), similarity among group members arose in majority from roaming over a shared territory, which echoes the known infectious risk associated with shared-patch occupancy (Müller-Klein et al., 2019). On the contrary, neither in baboons nor in Assamese macaques did grooming interactions influence similarity (Björk et al., 2022; Chapter 5 of this thesis). In yellow baboons and Verreaux's sifakas (Propithecus verreauxi), genetic relatedness explained similarity better than matriline, suggesting stronger effects of genotype than social interactions (Björk et al., 2022; Rudolph et al., 2022).

Could this suggest that evidence for social transmission through grooming interactions arises from confounding effects of genetic relatedness (in a kin-structured network) and shared-patch occupancy? This is likely not the case. Previous research in both baboons and sifakas have found a correlation of grooming bond strength and similarity, while controlling for relatedness and spatial proximity (Perofsky et al., 2017; Tung et al., 2015). These overall conflicting results are better understood by considering time scales. Grooming bond strength influences similarity when samples are collected over short periods of time, such as a few weeks (Perofsky et al., 2017; Tung et al., 2015), but not over months or years in primates (Björk et al., 2022; Rudolph et al., 2022; Chapter 5 of this thesis). This also suggests that influence of social interactions on the aging gut microbiome are probably short-term, possibly attenuating age-related changes occurring in older individuals as long as close contact is maintained, without influencing patterns in the long run.

Another reason, which may explain why including social interactions did not alter the relationship between age and physiological traits, could be that sociality does not act as a mediator, but rather as
a modulator of physiological aging. There is repeated evidence that the effect of social bonds and integration on physiology and health is modulated by third variables, either individual traits, such as dominance status (Brent et al., 2011), reproductive state (Crockford et al., 2008; Emery Thompson et al., 2010), social partner' sex or dominance status (Fürtbauer et al., 2014; Sapolsky, 1992b), or context-dependences, such as timing to mating season or group-level social stability (Bergman et al., 2005; Sapolsky, 2005). For example, the benefits of female-female relationships in lowering injury risk and fGC concentrations are more pronounced in, or limited to, the non-mating season in female macaques (Fürtbauer et al., 2014; Pavez-Fox et al., 2022). Some of the modulation effects on HPA axis activity are thought to reflect differences in the degree of control over social relationships (Thompson, 1981). For example, in female chacma baboons (Papio hamadryas ursinus) having a more focused grooming network is associated with lower fGCs (Crockford et al., 2008), and buffers the rise in fGCs levels during a period of high instability (Wittig et al., 2008). In one population of rhesus macaques (Macaca mulatta), high-ranking females displayed lower fGC concentrations only when their social network narrowed, suggesting that a form of control over a more focused network modulates the relationship between dominance status and physiology (Brent et al., 2011). Consistent with this interpretation, social integration did not benefit survival in yellow-belied marmots (Marmota flaviventer), probably because increased social connectedness results from increasing population density rather than active social engagement in this facultatively social species (Blumstein et al., 2018). These findings demonstrate that the relationship between sociality and physiology is multidimensional, with a variety of optimal strategies.

This is important for our understanding of the benefits and consequences of sociality into old age. In Chapter 4, I found no evidence for correlations between fGCs and either age or bond strength to top partners. This could arise if the value of social bonds is different across ages, resulting in several age-specific social optima. The possibility that the added value of social bonds to survival and health changes over the lifespan has only rarely been directly addressed, and results are inconclusive (Brent et al., 2017; Holt-Lunstad et al., 2015; Yang et al., 2016). Describing how social bond strength, social status, and broader social level integration varies for specific age-classes will be a necessary first step requiring large enough cohort at different ages. It is further important to formulate hypotheses regarding which social system or environmental conditions should favor increasing, or conversely decreasing, benefits of sociality with age. In females from despotic macaque species, rank acquisition in adult individuals is constrained by inter-matrilne dominance hierarchy (Chapais, 1988; Chapais et al., 1991; Silk, 2009). Support from young and prime-aged mothers may be critical to the rank achievement of their first daughters, and the engagement of these prime-aged females may further contribute to stabilize inter-matrilne dynamics. With advancing age, females may be able to rely on
the engagement of their adult daughters, lowering the need for their own active involvement in social interactions, while retaining the value of social relationships. Consequently, we may observe decreasing active social engagement (Chapter 2) in the absence of negative consequences for physiology (e.g., GCs in Chapter 4) or health.

To test such hypotheses, it will be imperative to match measures of sociality to the underlying mechanism investigated. For example, when sociality was approximated by the number of available kin in the group, greater number of kin was associated with lower risk of injury (Pavez-Fox et al., 2022). At the same time, the positive influence of the number of kin on survival decreased from prime-age to old age (Brent et al., 2017). Although this may seem in agreement with the suggestion formulated above that sociality is less critical to aging females in despotic societies, the patterns are not actually consistent. Active disengagement from social interactions would not be costly if females can, anyway, rely on the direct and indirect support from their kin. Findings in rhesus macaques on the contrary suggest that kin support is not driving inter-individual differences in survival into old age. There is no definitive answer to this issue, and there is a possibility that decreasing added benefits of sociality into old age arises from a survivor bias in cross-sectional studies, a risk highlighted in Chapter 2.

One practical statistical way to investigate age-specific optima would be to include interactions between age and sociality metrics. Throughout the present thesis, I mainly investigated the influence of age without interaction as I think it necessary to first assess which patterns are consistent at the population level throughout adulthood. For those traits that exhibit no significant change with age, it will be interesting to assess interaction effects. Taking the relationship between age, sociality, and fGCs as an example (Chapter 4), one practical application would be to introduce an interaction between social bond strength, within-individual, and between-individual age, investigating the possibility that social bond strength has varying influence on fGCs as individuals age (within-individual effect), and that this variation may not be linear across adulthood (between-individual effect). The inclusion of non-linear effects can constitute a statistical challenge, but it will be an important next step towards a more complete understanding of differences in the pace of aging between individuals, and within individuals across traits, in natural animal populations.

7.3 The aging gut microbiome

The aging gut microbiome has become a central topic of research in human and veterinary medicine as the involvement of the gut bacterial composition has been demonstrated for a rapidly growing list of diseases (Agirman et al., 2021; Amato et al., 2021; Levy et al., 2017; Sencio et al., 2022). Given the diversity of taxa making up the gut microbial ecosystem and the multitude of external and internal factors influencing its dynamics, a central question is whether disease- and age-related changes are
most individualized or shared by many. This question had only been addressed in natural animal populations with cross-sectional or mixed-longitudinal short-term studies investigating the influence of host identity and age on the gut bacterial communities with a limited number of metrics. Recently, four studies (Table 2) have contributed new perspectives to these issues, which I will now discuss. Although each study covers several topics, I will focus on individualization and age, and measures of bacterial inter-sample similarity (i.e., beta-diversity), intra-individual compositional stability, and composition dynamics, and briefly evoke at the end age-related changes in intra-sample diversity (i.e., alpha-diversity) and diurnal cyclicity in the gut bacterial composition.

Table 2. Summary table of studies investigating individualized gut bacterial composition over time and the correlates of host aging on gut bacterial communities, in spotted hyenas (Rojas et al., 2022), Assamese macaques (Chapter 5 of this thesis), yellow baboons (Björk et al., 2022; Grieneisen et al., 2021), and meerkats (Risely et al., 2022, 2021). Studies are arranged by alphabetical order of species’ Latin name.

<table>
<thead>
<tr>
<th>Species</th>
<th>Study years</th>
<th>N° subjects</th>
<th>N° samples</th>
<th>Samples per subject</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spotted hyenas (<em>Crocuta crocuta</em>)</td>
<td>23</td>
<td>12</td>
<td>301</td>
<td>Mean = 25 (range = 13–48)</td>
</tr>
<tr>
<td>Assamese macaques (<em>Macaca assamensis</em>)</td>
<td>1.5</td>
<td>51</td>
<td>543</td>
<td>Mean = 11 (range = 3–13)</td>
</tr>
<tr>
<td>Yellow baboons (<em>Papio cynocephalus</em>)</td>
<td>14</td>
<td>600</td>
<td>17,265</td>
<td>Median = 19</td>
</tr>
<tr>
<td>Meerkats (<em>Suricata suricatta</em>)</td>
<td>22</td>
<td>157</td>
<td>965</td>
<td>Mean = 7.5 (range = 4–14)</td>
</tr>
</tbody>
</table>

A first key finding is that the gut bacterial composition is strongly individualized over short time scales. In macaques, baboons, and meerkats, intra-individual similarity was highest for samples collected close in time (about 10 days apart in primates, less than two months in meerkats). Over longer periods of time, all studies found individual identity (ID) to explain the largest portion of variance in similarity (19.6, 8.6, 11.3, and 19 percent in macaques, baboons, hyenas, and meerkats, respectively). This means that, when considering the entire population of samples, ID is important to understand how samples are organized in the compositional space. However, how much individuals differ from each other is not as clear. In meerkats, intra-individual repeatability (another way to express individual signature) decreased to become undistinguishable from inter-individual repeatability for samples collected a year apart. In macaques, I found a small average difference between intra- and inter-individual similarity when analyzing all samples collected over one and a half years. In baboons, the
difference between intra-individual and inter-individual similarity appeared to be larger and for longer, although the use of a different similarity metric precludes direct comparison between studies.

Across studies, the high intra-individual similarity over very short time scales appears to result from the additive effect of environmental effects and host specificities. The individualized gut bacterial composition in baboons arises mostly from subtle interindividual differences in the relative abundance of taxa present, on average, at low relative abundance. In macaques, personalization was also the result of small interindividual differences in relative abundance, rather than in the presence/absence of taxa. In baboons, taxa contributing the most to interindividual differences also exhibit the highest heritability, which suggests that interindividual differences in host’s genetics drive, at least partly, this phenomenon. As opposed to these differences in the relative abundance of low abundance taxa, environmental factors are responsible for high turn-over in the bacteria present in the gut and for inducing major variations in relative abundances. In meerkats, rapid changes in the presence/absence of taxa explained the lack of intra-individual consistency over time, and in my study, the seasons had a major role in explaining overall composition.

Following this argument, what could explain the persistence, over a year or longer, of individualized composition in some cases (here baboons) and not others (here meerkats)? Seasonality exerted a strong influence on composition and interannual composition dynamics in the primates, but not in meerkats. In the latter, the year of sample collection had the largest influence on composition, yet year did not account for similarity in baboons or hyenas. Long-term individualized composition could rely, partly, on the long-term stabilizing effect of seasonality on gut bacterial composition. Seasonality may contribute to cyclic colonization, or preferential growth, of the same taxa across years, which in interaction with host ‘baseline bacterial signature’, drives peaks of increasing intra-individual similarity over time. Although seasonality was not assessed in hyenas, the population ranges in south Kenya in a similar environment to the baboons and experience seasonality due to variation in abundance of migratory prey species. Interestingly, all hyenas experienced a major shift in bacterial communities over two years, without composition ever returning to the anterior state. Despite this shift, ID was still the strongest predictor of variance explained over the entire study, whereas the year of collection was not important. If as suggested by Mallott (2022), this pattern arises from combined individualized composition and individualized dynamics, hyenas would exhibit individualization patterns very similar to those seen in the sympatric baboons. I could not test the interannual effect of seasonality in macaques because each season was included only once. Despite marked seasonality, the environment at PKWS is rather unpredictable (Heesen et al., 2015, 2013), with some of the tree species constituting a significant part of the macaques’ diet displaying irregular fruiting across years. This would be an
interesting opportunity to test whether intra-individual similarity increases across years for which seasons are comparable, and not when seasons are less consistent.

Are all individuals equally individualized? In macaques and meerkats, gut bacterial composition was increasingly individualized with advancing age. In macaques, I found that sample minimum dissimilarity (expressing the distance to the closest bacterial neighbor) linearly increased with age. In meerkats, intra-individual repeatability increased from young age to prime adulthood, and again from adulthood to old age. The consistency between studies is especially interesting as only females were sampled for my thesis, leaving a possibility that, with age, females would exhibit compositions closer to males, rather than being increasingly individualized. Both sexes were sampled in meerkats, yet age-related personalization remained. Together, these studies provide the first evidence for increasing personalization of gut bacterial composition with host age in non-humans (Figure 1).

Although the mechanism driving this personalization is not demonstrated in either study (see also Chapter 5 for a discussion focused on macaques), both investigated age-related changes in compositional stability as a potential driver. Surprisingly, older meerkats exhibited higher stability, whereas older female macaques displayed lower stability, and in both cases, age-related changes in stability were interpreted as promoting personalization. This could reveal important differences between the phenomenon observed. In meerkats, old individuals exhibited a more stable repartition in the relative abundance across taxa, possibly enhanced by a decrease in the acquisition of taxa from the environment. In macaques, older females exhibited faster changes in relative abundance across taxa than younger females, which, combined with seasonal effects which tend to homogenize compositions across individuals, could have resulted in a decreasing similarity to other females (i.e., personalization). To summarize, results from other study species bring a new perspective on my results and tend to strengthen the interpretation that increasing personalization with age results from dysbiosis rather than greater individual consistency with advancing age.
Figure 1. Age-related increase in non-core and highly heritable taxa and decrease in environmentally acquired taxa contribute to individualized age-related changes in the gut bacterial composition, which result in unstable personalized gut bacterial composition, or stable individualized gut bacterial composition. Suggested links are depicted with black arrows. Demonstrated associations are depicted with dashed arrows in green for positive, and red for negative effects. Circled numbers refer to (1) Chapter 5 of this thesis, (2) (Björk et al., 2022; Grieneisen et al., 2021) and (3) (Risely et al., 2022, 2021). Definitions for personalized and individualized are as introduced in Chapter 5 of this thesis and (Risely et al., 2022), respectively. Silhouette images are by Oscar Sanisidro (Crocuta crocuta) and T. Michael Keesey (Suricata suricatta) on PhyloPic.com. Image created with Biorender.

Aside from changes in similarity and stability, age correlated with slightly decreasing diversity in hyenas and baboons, although the significance of the very small effect in the latter probably results from the large sample size. In hyenas, age also explained a small portion of variance in similarity, about ten times the variance explained in macaques, and 1,000 times the effect in meerkats. It is unclear how the very low number of individuals, and possible changes in reproductive state which were not accounted for, may contribute to this result. In the meerkats, most bacterial taxa exhibited cyclic variation in relative abundance throughout the day, which were not influenced by age.

Overall, age does not consistently influence the gut bacterial diversity, the compositional similarity across individuals, the relative abundance of most taxa, or the diurnal cyclicity in their relative
abundance. However, evidence suggests that age could be associated with changes in stability. Whether these changes are the cause for increasingly individualized composition with advancing age needs further testing. As none of the studies discussed here differentiated changes arising within- from between-individuals, some results may also be due to selective disappearance effects. For example, six out of 12 hyenas died during the study conducted by Rojas and colleagues (2022). In meerkats, intra-individual consistency may increase with age if chances to succumb to other pathogens (e.g., tuberculosis (Müller-Klein et al., 2022)) are increased for those with unstable gut bacterial composition, creating an apparent increase in gut bacterial stability among the older individuals. Differentiating between within- and between-individual aging is not yet in practice in longitudinal analysis of microbiome data but could allow testing, in addition to aging, the survival benefits of individualized gut compositions into old age.

7.4 Conclusion and future research perspectives

“ [...] though I feel a professional obligation to say something about the natural history of senescence, there is no time to do so, and even if there were, there would not be much to say. [...] Whether animals can, or cannot, reveal an innate deterioration with age is almost literally a domestic problem; the fact is that under the exactions of natural life they do not do so. They simply do not live that long.” Medawar, 1952, p.11-13.

At the time of Medawar inaugural lecture, evidence for aging in natural animal populations was indeed scant. Since then, our knowledge on the natural history of senescence has grown, and the emerging picture reveals a complexity and diversity across the tree of life, on which much is still to be said. In this thesis, I set out to investigate the manifestations of aging in the social behavior and physiology of adult female Assamese macaques living in their natural habitat, while using the sociality-health nexus as a framework to address the complex interdependences between aging traits. I found that, with age, females reduce their active engagement in social interactions with others, even though they do not experience decreasing opportunities to do so. I went on to test different scenarios compatible with the socio-emotional selectivity theory of aging, to find that the one supported was compatible with maintained selectivity into old age. As maintained selectivity cannot explain decreasing engagement, my findings emphasize that selectivity and engagement are independent social dimensions in the study population, which follow different trajectories into old age. To link social and physiological aging, I reviewed established benefits and risks for health associated with sociality in primates, and highlighted gaps and promising avenues of research. Starting my investigation of physiological aging with one of the best documented traits, I investigated aging in the activity of the HPA axis assessed from the concentrations of glucocorticoid metabolites in fecal samples and found no evidence of age-related
changes. Moving to less explored biomarkers of aging in natural populations, I found age to correlate with changes in the gut microbiome. Although age has seldom been found to influence the gut microbiome in free-living animals, combining several metrics offered insights on some rarely explored components of this multidimensional ecosystem. Overall gut bacterial composition was not associated with host age but became increasingly personalized, less stable, and enriched in pro-inflammatory bacteria with advancing age. This reveals some degree of host specificity in aging trajectories of the gut microbiome, which I suggested could arise from age-associated dysbiosis in this population.

Contrary to my predictions, I found no evidence that social aging contributes to age-related changes in physiological traits. This does not undermine the value of social relationships as some evidence could indicate the selective disappearance of poorly socially connected individuals in the population. However, it suggests that social aging is unlikely to contribute to age-related mortality via its influence on physiological functions. Although I found no evidence for the effects of sociality on physiology, I contributed to open new opportunities to test effects from physiology to sociality by validating the measurement of urinary triiodothyronine hormones as a biomarker of the metabolic response to energy shortage. As predicted, urinary triiodothyronine decreased when energy intake decreased, and variation in urinary hormone concentrations reflected fluctuations in body mass, offering an interesting opportunity to assess metabolic responses and body condition non-invasively. With this work, I have contributed to our understanding of age-specific changes in traits much less investigated than the better-known actuarial senescence patterns, and I would now suggest some further research directions.

The pattern of social aging revealed in this thesis is intriguing for several reasons. With age females decreased their active engagement in grooming, and lost several partners grooming them, despite receiving grooming at a constant frequency. This suggests that remaining old females’ partners compensate for network’s size reduction. This leaves a first important question unanswered: How closely related to the aging females are these partners? As female Assamese macaques bias their affiliative relationships to both maternal and paternal kin (De Moor et al., 2020), complete relatedness pedigree are necessary to answer. If, as I suspect, relationships with older females are increasingly one-sided due to decreasing engagement from aging females, we should ask: Is the investment of those partners dependent on their own age and the presence of immature offspring? How stable are the relationships with aging females? And are there any costs (e.g., reproductive, energetic) to maintaining relationships with aging females? This would allow investigating the consequences of aging, not only from the side of the aging individuals, but also for the collective. As more behavioral and physiological data are collected for the long-term project at the PKWS, estimating social bonds reciprocity, stability, and possible costs of bonds will be possible for more female dyads.
Social aging is intriguing for a second reason, namely the inter-individual variability in the slope of social engagement with age. Although inter-individual differences in the slope of disengagement with age was not addressed here because of the limited sample size, some individual trajectories suggest such differences. The question is what underlies these differences: Is the slope of disengagement with age associated with the number of close kin in the group? Are more distant kin also important? For those females with little available female support, could a solution be to capitalize on long-lasting male-female relationships (Ostner et al., 2013)?

Despite a growing understanding of the environmental and individual factors shaping the composition of the gut microbiome in natural populations, evidence is still lacking showing how these variations influence host health and fitness, especially in mammals. Because the gut microbiome is functionally redundant (Tian et al., 2020), some of the taxonomic changes may bear little consequences to the host. Functional annotations of metagenomes will help determine how fluctuations in taxonomic composition translate into differential functional capacities. Our understanding of the gut microbiome would benefit greatly for synergies between moderately invasive, and non-invasive wildlife studies. When deemed feasible, weighting and sampling blood in free-ranging populations will provide a more complete picture of the consequences of gut microbiome composition on host’s physiological state via the measurement of serum fatty acid levels and inflammation markers. While this will strengthen the link between gut microbiome and host physiological state, long-term studies with detailed behavioral data will be in a privileged position to investigate how much changes in the gut microbiome can be compensated by the host at minimal cost (Touitou et al., 2021a).

The completion of more life-history trajectories in the Assamese macaque population will offer new perspective on the study of aging. Complete life-histories including age at death are the missing piece to explore how age-dependent and age-independent declines interact to shape age-distributions at the population-level. Information on age at death combined with advanced statistical analyses will allow investigating further the value of social bonds for survival and provide more decisive answers. If as many times stated, the hallmark of human aging is its heterogeneity (Badal et al., 2020; Müller and Ellwardt, 2022), then future investigations should take up the challenge of assessing inter-individual differences in aging trajectories in the wild. Although the small sample sizes of natural populations are less suited to model individualized aging, the inclusion of individual life-history parameters could be an easily applicable first step. Specifically, investigating how accumulated reproductive effort in combination with age at death may influence the onset of social, physiological, and actuarial senescence would allow integrating trade-offs to the sociality-health-aging nexus. Long-term animal studies collecting detailed behavioral data are also in a privileged position to investigate the late-life consequences of early life. While recent evidence in this macaque population suggests that the
influence of early-life on physiological functions persists into adulthood (Anzà, 2022), it is unclear whether such modifications benefit or harm individual fitness, and whether the pace of aging may be altered.

Because interdependencies between social and physiological systems are bidirectional, future research should aim to explore feedback loops. These loops are critical because they may accelerate decline in non-linear fashions, creating transitions between multiple discrete health states, some of which may significantly increase the probability of death. Thus, age may be associated with a gradual linear decline in some traits, and in other traits, with an increasing probability to enter a pathological transitory state (e.g., sickness). The combined used of linear models and multistate analyses could reveal how multifaceted aging processes leads to death.

At a broader level, these data will contribute to ongoing investigations on the link between sociality, aging, and lifespan across species. It remains unknown whether differences in age-distribution and lifespan arise from differences in baseline mortality or in the rate of aging, even across populations of closely phylogenetically related species. Finally, the recent emphasis on the loss of diversity in the gut microbiome with age in the new Copenhagen synthesis of human aging (Schmauck-Medina et al., 2022), shows the value of comparative research in reminding us to distinguish species-specific age-related changes from truly universal patterns.


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Thank you Prof. Susann Boretius for being on my thesis committee, for the support renewed at each stage of the project, and for agreeing to review my thesis. I want to thank Prof. Rolf Daniel for welcoming me to the Institute of Microbiology and Genetics in the Department of Genomic and Applied Microbiology, and for being on my examination board. Special thanks to Dr. Dominik Schneider for his irreplaceable contribution to the microbiome work presented in this thesis, and for answering my questions on the best way to process microbiome data over and over again without ever losing his cool.

I also thank Prof. Julia Fischer for agreeing to be on my examination board, for the encouragement and the lively scientific discussions during the joint journal club, and for her insights especially on social aging. Thank you to Prof. Peter Kappeler for your investment in the Sociality and Health in Primates (SoHaPi) Research Unit, for encouraging me and other PhDs to collaborate on the redaction of a book chapter, and for being on my examination board. I also thank Dr. Verena Behringer for agreeing to be on my examination board and for always being so reachable and thrilled to exchange about how fascinating physiology can be, even when it becomes (frustratingly!) complicated.

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The time I spent at the PKWS was fascinating, challenging, stimulating, and full of joy, which I must thank several people for. Thank you to Miranda Swagemakers for training me in the many aspects of fieldwork, camp life, data handling, map use (I might need some extra training there), and for the friendly atmosphere you create for the team. Thank you, Thawat Wisate and Jureerat Wanart, for your kindness, the incredible details in your observations, the many stories you tell so well of the monkeys, and for pointing out how often the English language is so tricky (I agree!!). Sunantha Nurat, thank you for your sense of humor, for turning any gathering into a party with songs, music, and dancing. Apisit Boonsopin thank you for your calm spirit, for the great pictures, and for being my running buddy in the park. Thank you, Daam Thanatkit, for your enthusiasm, your energy, and welcoming everyone with a smile. Finally, Kanchanok Phumkhonsan thank you for always being in a good mood and with a joke in mind, for being a precious guide through markets, restaurant menus, and celebrations.

My time is Thailand is also precious for I shared it with James Stranks and Niels Kil. Thank you James for our discussions on movies and politics, for your insane musical knowledge, your humor, and your collection of whiskey. Your ability to sleep through a Thai military parade still amazes me. Could it be the key to your extreme endurance in the field? Or is it rather the loads of snacks packed in your bag? Further research is needed. Niels thank you for being my second brain in the field, for finding my lost belongings dispersed in the forest, and for your positive attitude and combativity in front of all hurdles, big or small. The three of us made a fun and complementary field trio, and I’m sure that we are welcome back anytime on the stage of Loy Krathong festival!

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Thanks to the PhDs of the Behavioural Ecology Department Nadine, Luz, Sonia, Shivani, Simone, Joanna, Anna Lucia, Ben, Sofia for the friendly atmosphere, the dancing nights, and dinners. Especially, thank you Luz for your good mood, the pictures of the monkeys and the postcards from the field, and Nadine for your valuable feedback on a draft of this thesis. Thank you Senja, Pearl, Carina, and Durré for your contribution to the long-term dataset and to the lab work. Thank you, Ben, for the passionate stats discussions and your friendship in the last months of this PhD. And finally, thank you Delphine, for your communicative passion for what we do, for your support when the moral was low, for intellectually challenging me all the time, and for being such a generous person in work and friendship.
J’ai une pensée particulière pour la Société Francophone de Primatologie, qui accompagne mon aventure scientifique depuis le début, et pour ses membres Victor, Julie, Hélène, Cécile, Charlotte, François, Sébastien, Helen que je suis toujours heureux de retrouver lors des colloques. Au-delà des primates, nous avons cette envie commune d’une science et d’une société plus juste, plus cool, plus fun, plus à vélo, et c’est toujours une bouffée d’air frais d’en rigoler autour de verres bien remplis.

I must also thank you Roger for all the suggestions, advice, and contribution to the statistics throughout this thesis, but most importantly for being always so nice, open-minded, and happy to chat about any science or life topic, especially if it comes with a nice drink! I also thank Alan and Lauren for their friendship, Nora for the bike tours, the lockdown fitness challenges and the movie evenings at the flat, Marie for our Lüneburger Heide hike, Veronika, Derek, William, Tiffany, Judith, Federica, and Matthis for the game nights, Christof for our discussions on stats and research, and Lea for super fun dj parties. A fun group of psychologists has also shown me that one can chat about science without mentioning monkeys! So, for that, thank you Ricarda, Martina, Anika, Yasaman and Francesco, but mostly thanks for welcoming me to your GEMI crew. Thank you, Oli, for our L’Olivier-cinema combo, for always knowing what’s going on in the city and answering present for a time out, precious for my slight social hyperactivity. I especially want to thank Antoine, Lina, Clémence and Pierra for the barbecues at the lake, the crêpes, the support you have been through the lockdowns, and all the time we have spent at the climbing hall or in front of a (far too steep) cliff.

I end by thanking my parents, my brothers Jules and Sébastien, and the many friends by my side for years from Paris, and from the vet studies. You are the reason I come back home and wish I could do so more often. Merci le Tiekar, merci le Bronx for your invaluable friendships. Anna, your presence has meant everything to me in the last months of this thesis. Thank you for helping to find the motivation, the strength, and the peace of mind to complete it, while listening for hours at why do monkeys do all the strange things they do?! You have already achieved the impossible at keeping me quiet for a full ten-minute yoga session, and I have convinced you that running can be fun. Our next ride will take us by the sea. I look forward to the many future steps we take.
Curriculum Vitae

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Date of birth: 27 February 1992
Place of birth: Paris – France
Nationality: French

Research Experience

Graduate Researcher
Since May 2019
Georg-August-Universität Göttingen, Dept. Behavioral Ecology
German Primate Center, Research Group Social Evolution of Primates

- Managed research camp, observed wild animals, collected and processed biological samples under remote tropical field condition in Thailand. Work required long day of physical activity in the forest, managing a team with culturally varied background and representing the lab in exchanges with local officials.
- Led a project bringing together PhD students from 3 institutions resulting in the publication of a chapter for an academic book, co-organized 3 conferences both online and in presence gathering over 300 cumulated international participants.
- Developed learning agility, statistical skills, and scientific writing skills by publishing 7 peer-reviewed articles in the field of behavioral ecology, endocrinology, and automated data processing.
- Expanded the laboratory database by implementing a new procedure for data preparation and storage on servers. Handling data in Excel and R, co-coordinating the work of a team of seven people.
- Supervised undergraduate students in designing, conducting, analyses and writing of their pilot research projects.

Undergraduate research program
Jan – July 2018
Centre de Primatologie, Strasbourg, Unistra

- Capture, handling, anesthesia and sampling of blood and hair on captive macaques for a veterinary dissertation research project. Behavioral observation, selection, and analysis of hematological and biochemical blood tests. Performed all analytical steps from the sampling of hair to the measurement of cortisol and testosterone with immunoassays.

Eco-physiology and behavior
National Museum of Natural History, Naturelle, France, République Démocratique du Congo, Ouganda

- Sampling of urine and saliva to validate a cortisol immunoassay for urine samples of wild-living bonobos, assess salivary cortisol responses to a behavioral enrichment in captive orangutans,
and measure urinary triiodothyronine to investigate thyroid axis functions following exposure to pesticides in wild-living chimpanzees.

Education

Since May 2019  **Doctoral Studies**  
*Georg-August Universität Göttingen, Germany – Department of Behavioral Ecology*

- Thesis: “Effects of progressing age on energy, sociality and health in wild female Assamese macaques”
- Supervisors: Prof. Julia Ostner and Dr. Oliver Schülke

2017 – 2018  **Masters of Research**  
*Primate Biology Behavior and Conservation*  
*Roehampton University, London, UK – Department of Life Sciences*

- Master of Research, Grade: *Distinction*
- Supervisors: Dr. Julia Lehmann, Prof. Stuart Semple and Dr. Colette Berbesque
- Behavioral observations and biological sampling on captive non-human primates
- Performed all steps from the processing of hair samples and use of hormonal immune assay for the measurement of cortisol and testosterone
- Learnt coding in R and linear mixed modelling for the research project conducted

2013 – 2018  **Studies of Veterinary Medicine**  
*ONIRIS – Nantes-Atlantic National College of Veterinary Medicine, Food Science and Engineering, France*

- Doctor of Veterinary Medicine, Grade: *Distinction and Mention for Thesis Prize* (in French “Mention très honorable avec proposition pour Prix de Thèse”)
- Medicine and surgery of small and large animals
- Animal welfare, epidemiology, and concept OneHealth
- Production and Biosafety of food production

2009 – 2013  **Undergraduate Studies of Biology**  
*Sorbonne Université Campus Pierre & Marie Curie Paris, France  
University of Edinburgh, United-Kingdom. Erasmus exchange 3rd year*

- Bachelor of Science in Biology Grade
- Admission into the excellence training program to prepare the admission to veterinary studies

Research skills

**Technical skills**

**Data analysis**  
Data tidying and analysis with R (RStudio interface) and Excel. 
Generalized mixed models analyses, parametric and non-parametric statistics.

**Laboratory**  
Microbiome genetics DNA 16S extraction, amplification (PCR) and post-sequencing analysis. 
Hormone assay (ELISA) in-house design, validation and use for various biological samples.

**Animal Biology**  
Detailed systematic observation of animal behavior and interactions often under harsh climatic conditions and physical load.
**Soft skills**

**Management**
Planification, task attribution and processing of long-term data from the lab team. Involves R procedure, coordinating with a colleague PhD student on the supervision of the process and lab assistants involved in the process.

**Language**
English (professional proficiency) and French (native speaker). German (A2 level).

**Courses Attended**

**Jan 2023**
How to find your postdoctoral position by Dr. Dagmar Sigurdardottir. Hosted by the GAUSS Career Service Georg-August Universität Göttingen.

**Sep 2022**
Leadership Skills for Scientists by Dr. Alexander Britz. Advancing as a scientist, both out- as well as inside academia, comes with increased leadership responsibilities. In this interactive workshop, the participants learn the essential leadership skills which are necessary to be successful as a PhD student or Postdoc and to later facilitate a successful transition to a senior position at a research institution or the dream job outside academia. Hosted by the GAUSS Career Service Georg-August Universität Göttingen.

**Feb 2022**
Analyzing dyadic data with Bayesian methods by Dr. Aura Raulo, University of Oxford. Understanding the challenges associated to the modelization of dyadic data and the solutions to their analyzing provided by Bayesian statistics. Hosted by the Research Training Group 2070.

**Jun 2021**
Linear Models and their application in R by Dr. Roger Mundry. The course treated linear models from simple regressions to General and Generalized Linear Mixed Models (GLMM) modelling the impact of multiple predictors, categorical predictors and interactions, logistic, Poisson, zero-inflated, and negative binomial regression, the inclusion of grouping variables or ‘random effects’, formulating scientifically meaningful models. Host by the Leibniz Science Campus Primate Cognition.

**Nov 2020**
Time and career management by Peter Kronenberg from NaturalScience.careers. Hosted by SoHaPi Research Unit.

**Sep 2020**
An introduction to Bayesian multilevel modeling in R by Jordan Scott Martin, University of Zurich. Modeling of complex behavioral processes, specification of model priors, plotting and analysis of outcomes.

**Teaching and Supervision Experience**

**Feb 2022**
Course in Wildlife Monitoring Techniques Spring Semester 2022. “Macaques in the forest: Observing monkeys in the wild” invited talk by Dr. Sostra Chakrabarti, MacAlester College, United-States. Online lecture co-hosted with Dr. Delphine De Moor and James Stranks.

**Since 2020**
Georg-August Universität Göttingen, Dept. Behavioral Ecology
Co-supervised undergraduate students in designing, conducting, analyzing, and writing their pilot research projects and bachelor’s thesis.

- Master’s laboratory research rotation. Winter Semester 2022. Supervision of Pearl Amber Lee Váth.
- “Does allogrooming promote access to huddles in female Barbary macaques (Macaca sylvanus) at “Affenberg Salem?”. Winter Semester 2021.
- “How does age influence the physical and social life of semi-free living Barbary macaques (Macaca sylvanus) at “Affenberg Salem?”. Winter Semester 2021.
• “The link between social relationships, physiological stress and aging in primates and other mammals”. Summer Semester 2021.
• “Primate behaviour and the gut microbiome”. Summer Semester 2021.

**Research Funding, Honors and Awards**

2022  LiebnizScience Campus conference visit grant (600€)
2022  Acquired funding from the Research Training Group 2070 for the organization of a workshop for PhD students and post-docs on Bayesian statistics by Dr Aura Raulo (University of Oxford) (700€).
2018  Best Masters overall performance in the Life Sciences, University of Roehampton
2014  Laureate of Merial Student Award 2014 (1000€)
2014  Laureate of France Vétérinaire International Award 2014 (100€)

**Conference Attendance and Contribution**

Invited Talks

2022
Invited talk with Marie Hirel by the French Ambassy in Germany for the 6th Café des Sciences “At the origins of life in society: mechanisms and consequences of social ties in primates” (in French “Aux origines de la vie en société: des mécanismes aux conséquences des liens sociaux chez nos cousins primates”). Berlin, Germany. Online conference https://www.youtube.com/watch?v=GtbjFakBJ5s&t=1516s

2019
Radio interview Le labo des savoirs Radio Prun’, Nantes, France “Bonobo, chimpanzés: comprendre notre heritage primate”. Broadcasted on Prun’ on the 13/02/19, on France Culture on the 18/02/19 (cycle conférence), and on RCF le 20/02/2019. https://www.radiofrance.fr/franceculture/bonobos-chimpanzes-comprendre-notre-heritage-primate-3572122

Publications

2022

2022

2022

2022

2021
Sadoughi, B., Lacroix, L., Berbesque, C., Meunier, H. & Lehmann, J. Effects of social tolerance on stress: hair cortisol concentrations in the tolerant Tonkean macaques (Macaca tonkeana) and the despotic long-tailed macaques (Macaca fascicularis). Stress 0, 1–9 (2021).

2021

2021

2018

Further Professional Experience

Veterinarian Practitioner
Jun – Jul 2017 Clinique Vétérinaire des Pommiers, Countances – France
Jan – Apr 2018 Clinique Vétérinaire des Trois Valets, Mussidan – France
• Short-term contracts to support teams in times of high workload
• Performed companion and farm animal medicine
• Applied diagnostic and surgical skills, scientific and interpersonal communications abilities with other health professionals and clients.
• Conducted public health screening missions on cattle which allowed to get familiarized with the farming industry and welfare regulations. Required scheduling visits and constant communication.

**Veterinarian Apprentice**

Aug 2016

*Simian Laboratory Europe (Silabe)*

• Veterinary care, surgery and epidemiology of pathogens in primate
• Assisted the emergency surgery of an inguinal hernia in a macaque and published the case report in peer-reviewed scientific journal

**Service & Outreach**

Since 2019 **Member of the Ethics Committee for clinical and epidemiological veterinary research** at Oniris (Cervo in French) for the reviewing and approval of research involving animals (main reviewer for 8 research proposals, voting member on more than 30).

2018 – 2021 **Board member for the French Speaking Society of Primatology**

• Communication and social media coordinator for Twitter and Facebook. Launch a weekly post on primates directed to a general audience of followers. Interviewed scientists, video edited, and shared on Facebook and Youtube.
• Organized one online conference (Nov 2020) and one in presence conference (Nov 2021). Prior to conference, I contacted sponsors, planned the logistic and sanitary COVID-19 regulations, and relayed information between the board and the organization team on-site. During the conference, I co-chaired the Physiology & Health session.
• Piloted the estimation of conferences’ carbon footprint.

2020-2021 **Peer-reviewing** for international peer-reviewed journals (e.g., Scientific Reports, Hormones & Behavior) and selection of conference abstracts for the French Speaking Society of Primatology (conference editions 2019, 2020 and 2021)

Feb 2022 Invited Dr Aura Raulo (University of Oxford) to give a statistical workshop on Bayesian statistics for the Research Training Group 2070.

Nov 2021 Invited Prof. Alexandra Freund (University of Zurich) to give a talk entitled “Exhaustion and Recovery: What’s motivation got to do with it?” at the Leibniz Science Campus Primate Cognition (online).

2014-2017 Elected Student Representative in the Board for Teaching and Student affairs at Oniris (Conseil de l’Enseignement et de la Vie Etudiante in French).

**Membership in professional societies**

**Animal Behavioral Research Societies**

• Animal Behavior Society (ABS) – former
• French Speaking Society of Primatology (SFDP) – active
• International Primatological Society (IPS) – former
• Primate Society of Great Britain (PSGB) – former

**Animal Welfare and Veterinary Medicine**

• National France Order of Veterinarian (Ordre National des Vétérinaires)
• Compassion in World Farming (CIWF) France
Declaration

I hereby declare that all parts of my thesis titled ‘The manifestations and interdependence of social and physiological aging in wild female Assamese macaques (Macaca assamensis)’ were written by myself. Assistance of third parties was only accepted if scientifically justifiable and acceptable in regards to the examination regulations. Assistance or contributions to the individual chapters are indicated and all sources have been quoted.

Göttingen, 10th of April 2023

Baptiste Sadoughi