Prenatal maternal stress effects in wild Assamese macaques (*Macaca assamensis*)

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Summary

Across mammals, adverse conditions experienced during specific ontogenetic sensitive periods can have persisting effects on neurodevelopment, acquisition of cognitive and motoric skills, immune function, and systemic diseases. Non-genetic maternal effects are among the most pronounced and lasting environmental effects on individual health and fitness and from conception onwards, maternal adversity can affect offspring developmental trajectories depending on the degree of the adversity, the timing of challenges, and sex of the offspring. Thus, sometimes the adult's health states are better explained by early adversity rather than by their interactions with the current environment.

The effects induced by adversity during sensitive phases of ontogeny could be interpreted as the product of constraints during development or as adaptive responses to conditions of future life-phases. According to the internal predictive adaptive response (iPAR), early adversities induce developmental constraints which translate into disadvantaged somatic states and consequent reduction in life expectancy. However, the offspring is thought to recalibrate its life-history pace in an effort to counter the reduced life expectancy. The recalibration would accelerate growth and reproduction at the cost of quality-related attributes and lower investment in maintenance functions that normally enhance offspring's health and thus longevity.

Using data on a wild long-lived mammal species, I tested the internal consistency of the iPAR hypothesis, and competing hypotheses on developmental plasticity induced by epigenetic prenatal maternal stress. The lack of available long-term data on life-time reproductive success did not allow to test for the presence of fitness-crossovers, and thus I focused on the adaptive trade-offs. Crucially, the timing of the exposure confirmed the central factor within the debate on the persistence of maternal effects and the adaptive nature of developmental plasticity.

Early-preGC (prenatal maternal GC levels during the first half of gestation) was a better predictor when compared with late-preGC (second half of gestation) and postGC (postanal phase during lactation). Across different statistical models and in all the studies, the results indicated that if experienced during early gestation, even moderate variation in maternal GC levels can lead to variation in offspring physiological phenotype without the need for catastrophic events.

Elevated early-preGC were associated with: (i) an hyperactivation of the HPA axis activity, (ii) a reduced diversity of the gut microbiome community with increased relative abundance of potentially pro-inflammatory organisms and reduced SCFA-producers; (iii) lower *Firmicutes* to *Bacteroidota* ratio; (iii) accelerated body growth and increased body-size index. Importantly, the variation in all these traits

persisted into adulthood with strong indications against short-term effects, and in favor of persisting phenotypic variations in response to maternal GCs during early gestation.

These findings suggest a higher allostatic load and “wear and tear” and gut-microbial dysbiotic states combined with accelerated growth. Overall, they indicate a trade-off in favor of an acceleration of the pace of life paid at the cost of reduced investment in maintenance functions and health in prenatally challenged individuals. Finally, the results confirmed previous findings on the trade-off between growth and immune response in immature wild Assamese macaques. They also indicate that such trade-offs are not limited to the first phases of life but are part of a long-term programmed life-history recalibration under the assumptions of the iPAR hypothesis.

Chapter 1 - General introduction

1 A small step into life-history theory

At the heart of evolutionary biology lies life-history theory, investigating how organisms allocate time and energy to different activities of their life cycle and explaining some of the most marked phenotypic variations (Del Giudice et al., 2011; Hill, 1993; Kaplan and Gangestad, 2005; Kappeler et al., 2003).

Ultimately, the costs and benefits of energy and time allocations to several activities promoting growth, survival, reproduction, and parental care shape an organism's evolutionary fitness (Del Giudice et al., 2011; Oosthuizen et al., 2021; Touitou et al., 2021). Life-history theory models optimization of such costs-benefits problems by providing theoretical explanations to processes that maximize reproductive success through different energy allocations strategies: adaptive solutions to concurrent fitness trade-offs (Del Giudice et al., 2011; Gluckman et al., 2005; Hill, 1993; Nettle et al., 2013; Oosthuizen et al., 2021; Spencer et al., 2022). Classic examples of life-history adaptive strategy are the trade-off between growth, maintenance and cognitive functions, and reproductive effort; and the trade-off between energy and time allocation in mating and conceiving versus parenting investment in conceived offspring (Del Giudice et al., 2011; Kaplan and Gangestad, 2005; Lu et al., 2019; Nettle et al., 2013). Such crucial decisions are condensed into trade-offs involving short-term and long-term costs-benefits and related fitness advantages mediated by the investments in current or future survival and reproduction, and in the quantity or quality of the offspring produced (Del Giudice et al., 2011; Ellis et al., 2009; Hill, 1993; Kappeler et al., 2003; Oosthuizen et al., 2021; Reichert et al., 2020; Wells et al., 2016). One fundamental postulate of life-history theory is that there is not a perfect solution that maximizes fitness for every ecological scenario, and the best strategy for a species, a population or an individual depends on a multitude of ecological aspects (e.g., environmental predictability, resources abundance, mortality risks).

Mammals present a striking variation in life-history traits such as life span, development, and maturation: some rodents can live one or few years and be sexually mature in weeks, while humans, elephants and whales mature after 10 years and live up to one hundred years (Hollister-Smith et al., 2007; Kappeler et al., 2003; Raia et al., 2003; Robeck and Monfort, 2006; Sibly and Brown, 2007). These traits dispose life-history strategies along a “fast-slow” continuum with organisms characterized by a shorter life span, accelerated development and maturation, and higher reproduction rates at the “fast” end of the continuum; while organisms with longer life span, slower development and maturation, and slower reproduction rates at the “slow” end of the continuum (Dobson and Oli, 2008; Healy et al., 2014;

Kappeler et al., 2003; Sibly and Brown, 2007; Stearns, 1992). The disposition of adaptive strategies along this continuum seems the product of limited options generated by energetic constraints lying on the essential trade-offs between growth, survival, and reproduction (Dobson and Oli, 2008; Spencer et al., 2022; Stearns, 1992).

Substantial contributions to life-history theory have been imported by studies of phenotypic adaptive plasticity which integrated ecological pressures on within-species variation. Such studies established that the differences in life-history traits are not circumscribed to between-species comparisons and exist (to a smaller degree) also between individuals of the same population (Berghänel et al., 2017, 2016; Dantzer et al., 2013; Dmitriew, 2011; Gluckman et al., 2005; Lu et al., 2019; Monaghan, 2008; Nettle et al., 2013; Oosthuizen et al., 2021; Spencer et al., 2022; Stearns, 1992). Adaptive plastic responses affecting individuals' life-history traits and generating phenotypic variations have been reconducted to epigenetic mechanisms which would operate by altering the transcription of genetic information (e.g., DNA-methylation) (McGowan and Matthews, 2018; V.G. Moisiadis and Matthews, 2014a). Probably the most investigated epigenetic pathway generating between-individual variation and offspring phenotype starts at conception and is mediated by the maternal stress response to environmental adversity during gestation and other sensitive periods of offspring ontogeny (Del Giudice, 2015; Del Giudice et al., 2011; McGowan and Matthews, 2018).

2 Adversities during development and adaptive plasticity in mammals

2.1 Maternal glucocorticoids and offspring programming

Glucocorticoids (GCs) are metabolic hormones secreted by the adrenal gland and regulated by the hypothalamic-pituitary-adrenal (HPA) axis in response to homeostasis-threatening situations (MacDougall-Shackleton et al., 2019; Selye, 1946). According to the allostatic model, GCs dynamically function to maintain homeostasis through changes and stressful conditions (McEwen and Wingfield, 2003; Romero et al., 2009): increased production of GCs temporarily inhibits body functions not urgently needed for survival like growth, reproduction, and digestion, and redirect energy allocation to re-establish the perturbed homeostasis (MacDougall-Shackleton et al., 2019; Sapolsky et al., 2000). Enduring adversities (e.g., low food abundance) likely reduce the energy balance leading to changes in energy-allocation strategies and variation of the HPA axis activity. By regulating GCs secretion and energy-allocation strategies, the HPA axis has a pivotal role in re-establishing the physiological homeostasis in response to stress (McEwen and Wingfield, 2003; Romero et al., 2009; Sapolsky, 2021; Sapolsky et al., 2000).

The HPA axis is very sensitive to environmental perturbations and increased GCs secretion has been largely associated with several types of adversities such as food scarcity, predation, social stress, and increased population density in several animal species (Capitanio and Cole, 2015; Dantzer et al., 2013; Foerster and Monfort, 2010; McGowan and Matthews, 2018; Patterson et al., 2021; Sheriff et al., 2011; Snyder-Mackler et al., 2016). Females experiencing adverse conditions during gestation can transfer GCs, and therefore the stress information, to the forming embryo/fetus via placenta in mammals, or yolk in other vertebrate species (McGowan and Matthews, 2018; Reynolds, 2013). Additionally, after birth, maternal stress can be conveyed to the offspring via maternal behavior or via maternal milk GCs causing postnatal mediating effects on offspring phenotypes (Bernardo, 1996; Dettmer et al., 2018; Maestripieri and Mateo, 2009; Novak et al., 2013; Petrullo et al., 2016; Stead et al., 2022). For example: in rhesus macaques, individuals receiving maternal maltreatment during the first 3 months of life exhibited higher minimum cortisol levels and a blunted response to acute stressors, indicating dysregulation of the HPA axis activity characterized by attenuated stress response (Petrullo et al., 2016).

Health and survival differences begin in utero and can be amplified by differential exposure to adversity during infancy and childhood as well (Campos et al., 2021; Lewis et al., 2000; Maestripieri and Mateo, 2009; McGowan and Matthews, 2018; Novak et al., 2013; Reynolds, 2013). Fetal exposure to GCs leads to long-term programming of neurological, cardio-vascular, metabolic, and reproductive functions in humans and other animal species (Braun et al., 2013; Chapman et al., 2013; McGowan and Matthews, 2018). GCs are a cardinal developmental trigger: they promote cellular differentiation and are involved in the organization of neural circuitry during development (Cottrell, 2009; De Kloet et al., 1988). In most mammalian species, increased GCs in fetal circulation during late gestation are essential for normal maturation of the lung, GCs are fundamental for developmental switches in the brain, thyroid, pituitary, kidney (De Kloet et al., 1988; Fowden et al., 1998), and prepare the fetus for the extrauterine life.

2.1.1 Programming of the holobiont

The HPA axis is both mediator and a sensitive target of phenotypic programming (Cottrell, 2009; McGowan and Matthews, 2018; Reynolds, 2013). Depending on the nature and the magnitude of the exposure, prenatal exposure to GCs affects the development and successive functionality of the HPA axis with potential higher or lower baselines, and hyper or reduced responsiveness to stressors in the offspring (Braun et al., 2013; De Kloet et al., 1988; Fowden et al., 1998; McGowan and Matthews, 2018; V.G. Moisiadis and Matthews, 2014b). Given the pivotal role in functionality regulation of metabolic, cardiovascular, and neurological systems, impaired functionality of offspring HPA axis is associated with programmed pathogenesis of psychiatric diseases and chronic diseases such as diabetes, obesity, and cardiovascular diseases (McGowan and Matthews, 2018; V.G. Moisiadis and Matthews, 2014b). In

rodent models, prenatally stressed offspring exhibited a significant decrease in social behavior, higher corticosterone release after social interactions, higher neuroinflammation and reduced oxytocin receptors, reduced serotonin metabolism in their cortex, and dysbiotic gut microbial states (Gur et al., 2019).

Crucially, epigenetic programming of the HPA axis functionality induced by adversities during ontogeny, the consequently impaired functionality translating into detrimental physiological conditions and poor health states of the offspring indicate a general higher somatic "wear and tear" which theoretically can reduce fitness (Bonier et al., 2009; Nunn et al., 2015; Reynolds, 2013; Romero et al., 2009; Thayer et al., 2018; Zipple et al., 2021a). Among primates, the hyperactivation of the HPA axis in developmentally challenged adults is associated with reduced survival: in wild baboons, higher cumulative GCs exposure is associated with a higher hazard of death and about 5.4years of differences in the predicted life expectancy of the normal versus developmentally challenged individuals (Campos et al., 2021; Tung et al., 2016); in muriquis, baboons and blue monkeys, early maternal death leads to decreasing offspring survival in the next generation (Zipple et al., 2021a); in wild olive baboons, maternal early-life adversity predicts increased maternal GC levels, reduced sociality, shortened life-span and increased offspring mortality risk (Patterson et al., 2021). However, whether in primates the higher "wear and tear" and consequently reduced fitness is resilient to social buffering or it can ameliorate with time it is still under debate and likely depends on the species' ecology and sociality, and the magnitude of the adversity (Del Giudice et al., 2011; Kappeler et al., 2003; Nunn et al., 2015). In wild baboons, cumulative adversity during development led to an increase of 9-14% of GCs and the effect was 11 times stronger than the mediating effect of weak social bonds (Rosenbaum et al., 2020), while immature wild chimpanzees showed altered diurnal cortisol slopes only if the mother died recently (before than two years) and the effect disappeared after few years suggesting a mediation effect of alloparental care or adoption (Girard-Buttoz et al., 2021).

Firstly conceptualized by Adolf Meyer-Abich, the term "holobiont" indicates the strong association between individuals or individuals of different organisms forming together anatomical, physiological, and immunological units which evolved synergically (Baedke et al., 2020). This concept introduces an explicit evolutionary and eco-evo-devo perspective on older symbiosis-related notions (e.g., "consortium" or "homobium" concepts) and explains evolutionary changes starting from associations processes of independent symbiotic organisms, and then holobionts evolving synergically into organ systems contributing to the "whole", or unit (Baedke et al., 2020). The holobiont includes all the organisms contributing in some way to the functioning of the unit and the most studied cases are the reef-building corals or host-microbiota units such as humans and other animals. Investigating developmental plasticity and programming of maternal GCs "with the lens" provided by Adolf Meyer-Abich offers the opportunity to dive into the complexity of evolutionary forces faced by "units", and not

individuals, and to understand the synergic strategies and energetic trade-offs operated by more complex unitary concepts.

In the last decades, the characterization of typical microbial communities inhabiting the gut of healthy subjects of different species during different periods of their life cycle allowed scientists to define hosts' health states by investigating variations in the diversity and composition of organisms living inside them (Amato et al., 2016; Clayton et al., 2018; Fujimura et al., 2010; McKenna et al., 2008; Nunn et al., 2015; Zmora et al., 2019). With the use of high-throughput methods, we started to grasp the intricacy of connections between the brain, gut, and immune system mediated by hundreds of microbial organisms, and by adopting the concept of the holobiont we integrated the study of individuals' health and related notions, to the study of the holobionts' health with a more holistic approach which now incorporates the microbiota-gut-brain synergic system (Carabotti et al., 2015; Clarke et al., 2013; Cryan et al., 2019; Cryan and Dinan, 2012). Since the health states of the hosts are directly influenced by hosted microbial species and vice versa, it is not trivial that evolutionary forces select the best-adapting holobiont and not just the host.

Imbalanced gut microbial community associated with disease (dysbiosis) in the infant's gut microbiome has been linked with the same long-term diseases as impaired functionality of the HPA axis (e.g., increased risk for metabolic and neuro-behavioral disorders) (Cryan and Dinan, 2012; Goulet, 2015), indicating the gut microbiome as a potential mediator of programmed stress-induced pathogenesis (Clarke et al., 2013; Foster and McVey Neufeld, 2013; Jahnke et al., 2021). Although very few studies investigated prenatal maternal effects on offspring gut microbiome, elevated maternal stress and GCs during gestation have been associated with altered gut microbial community and dysbiotic states in humans (Jahnke et al., 2021; Zijlmans et al., 2015), other primates (Bailey et al., 2004; Bailey and Coe, 1999) and rodent models (Gur et al., 2019, 2017).

The mechanisms through which higher maternal stress and GCs during pregnancy can impact the offspring's gut microbiome are not clear yet. Conventional pathways of maternal translocation of GCs and pro-inflammatory cytokines via the placenta, and their direct organizational effect on fetal enteric and brain development have been proposed in many studies, especially in primate and rodent models (Clarke et al., 2013; Coe et al., 2003; Gur et al., 2019, 2017; Patterson, 2009; Weinstock, 2008; Zhang et al., 2021). In support of this hypothesis, a study on rodents revealed that prenatal maternal stress affected gastrointestinal neurodevelopment and functionality, HPA axis activity, and gut microbiome of the offspring (Golubeva et al., 2015).

Alternative stimulating hypotheses have been formulated. Maternal stress during gestation has been associated with altered maternal vaginal and gut microbial communities in humans and other mammals (Golubeva et al., 2015; Gur et al., 2019; Jašarević et al., 2017; Zijlmans et al., 2015). At birth, the infant's

gastrointestinal tract is firstly colonized through ingestion of the maternal vaginal microbiome and in early postnatal phases it is consolidated via milk consumption (Jašarević et al., 2017; Petrullo et al., 2022). Disruption of the maternal vaginal microbiota and early postnatal phase with increased hormones deposition in milk can lead to alteration of the typical maternal colonization and increase the risks of early dysbiotic states and neurodevelopment disease in the offspring (Dettmer et al., 2018; Hinde et al., 2015; Jahnke et al., 2021; Jašarević et al., 2017; Thomson et al., 2018). Furthermore, because maternal GCs can be transferred to the fetus via the placenta and increase fetal GC concentrations, they can reprogram the fetal HPA axis and increase basal GC levels which can affect the fetus and infant's immune response (Gollwitzer and Marsland, 2015; McGowan and Matthews, 2018; Moisiadis and Matthews, 2014a). The altered immune response may increase gut permeability, disrupt mucosal and barrier functionality, and affect the gut microbiome composition of the offspring (Bailey et al., 2006; Clarke et al., 2013; Cryan and Dinan, 2012; Tong et al., 2013; Zijlmans et al., 2015). Finally, a more speculative although intriguing hypothesis suggests the possibility of microbial transfer through the placenta (Walker et al., 2017). Recent studies indicate that although the uterine environment has been thought to be sterile for a long time, the infant's gut microbiome can be actually seeded earlier than birth (Hu et al., 2013; Jiménez et al., 2005; Mackie et al., 1999). The placenta harbors a particular microbial population, very low in abundance (Aagaard et al., 2014), and stress exposure is a well-known driver of microbial translocation from the gastrointestinal tract to other organs (Bailey et al., 2011, 2006; Bailey and Coe, 1999). One possibility to be tested is that maternal stress during pregnancy may trigger microbial translocation via the placenta and alter *in utero* microbial community population increasing the risk of fetal dysbiotic states (Hartman et al., 2019; Walker et al., 2017).

2.1.2 Timing-effects on programming

Variation in maternal HPA axis activity and GCs secretion during gestation provides the developing offspring with information on the maternal physiological states and anticipated maternal investment in offspring development (Berghänel et al., 2016; Hanson and Gluckman, 2014; Lu et al., 2019; McGowan and Matthews, 2018; Monaghan, 2008). Starting from conception onwards, maternal adversity and consequent elevation of maternal GC levels can affect offspring developmental trajectories depending on the timing and magnitude of the adversity, and the sex of the offspring — whether fetal exposure increases during the early or late gestation can be critical to the formation of specific developmental trajectories and subsequent phenotypes (Berghänel et al., 2017; McGowan and Matthews 2018). Experimental manipulation of adverse conditions and maternal GCs established that the timing of early adversity can impact differently the existence, the strength, and even the direction of the effects on the offspring's HPA axis activity (Moisiadis and Matthews, 2014a, 2014b; Schneider et al., 1999). Crucially, the GC-induced switches in developmental trajectories need to be triggered at precise ontogenetic phases otherwise they may induce altered tissues and organs maturation leading to inappropriate organ

developmental trajectories (De Kloet et al., 1988; McGowan and Matthews, 2018; Moisiadis and Matthews, 2014a; Schneider et al., 1999). Altered developmental trajectories can detrimentally translate into impaired physiological functions making the GCs-programmed physiological alterations a well-known gateway to altered health states (Class et al., 2011; Davis and Sandman, 2010; McGowan and Matthews, 2018; Mueller and Bale, 2006; Reynolds, 2013). In guinea pigs, prenatal stress had a timing effect on the programming of cognitive function development, growth, HPA axis function, and stress-related response (Kapoor et al., 2009, 2009; Kapoor and Matthews, 2008, 2005). In humans, maternal anxiety during the last half of gestation and associated cortisol levels were positively correlated with higher cortisol stress response in infants and children (Davis and Sandman, 2010; Davis et al., 2011; Gutteling et al., 2005).

Despite the large number of studies investigating the programming of the HPA axis functionality in captive and wild species, it is not clear yet whether an altered functionality persists into adulthood of slow-developing and long-lived species, and which mechanisms make the potential programmed alteration irreversible. Most of the studies available are on rodent models (Dantzer et al., 2013; Gur et al., 2019; Kapoor et al., 2009; Kapoor and Matthews, 2005), lying on the fast end of the "fast-slow" continuum, and thus being characterized by quick development and advantaged by more accurate environmental predictability. Although highly informative on the mechanistic aspects driving epigenetic transgenerational effects, those studies inform only a little on the potential adaptive nature of such effects on the long run, when environmental predictability strongly decreases. Moving from the fast to the slow end of the continuum, the longevity of the species increases, and with it increases the difficulty of investigating long-term programming of adversity during sensitive periods and potential adaptive strategies: the number of studies investigating persisting effects in adults of slow-developing and long-lived species vigorously drops. While theoretically more informative on the adaptive nature of such adversities (Del Giudice, 2015, 2014), the large number of investigations on humans do not test direct correlations between maternal GC levels during pregnancy and GC levels of adult daughters and sons (Bevan and Kumari, 2021; Monteleone et al., 2020), for obvious technical difficulties. Furthermore, they start from the reasonable assumption that the stronger the stressor, the easier the possibility to see the transgenerational effect. Thus, these studies tend to focus on very traumatic experiences during pregnancy (Enlow et al., 2017) and assess maternal effects driven by severe stress spanning from very rare situations such as the Rwandan genocide against the Tutsi (Perroud et al., 2014), to the death of a family member or a car accident (Bevan and Kumari, 2021; Entringer et al., 2009).

Studies on captive primates are very informative considering the nice experiment-testing frameworks used with controlled conditions but may lack ecological perspectives and tend to focus only on postnatal adversities (Novak et al., 2013; Petrullo et al., 2016). Results from the few studies available that investigated the long-term effects of early-life adversity in wild primates are conflicting. Two studies

showed only temporary alterations of the HPA axis activity, and the adversity (i.e., maternal loss) occurred rather late during development (Girard-Buttoz et al., 2021; Rosenbaum et al., 2020). Another late adversity, the birth of a sibling, did not program adults' HPA axis activity (Rosenbaum et al., 2020). Instead, adversities experienced earlier during development such as a drought in the first year of life and low maternal rank were associated with persisting alterations of the HPA axis activity which lasted into adulthood (Rosenbaum et al., 2020). Studies investigating the effects of cumulative adversity in wild baboons revealed instead persisting elevated GC levels in adult females suggesting that the strength of the adversity may be a key factor inducing developmental plasticity (Rosenbaum et al., 2020; Tung et al., 2016). Such contrasting results point at several open questions on the nature of the effects induced by adversity during sensitive period of the offspring's ontogeny and on their persistence into adulthood in long-lived species.

Another largely studied phenotypic trait affected by maternal stress and GCs exposure is also body growth. Across animals, studies on prenatal effects on growth are inconsistent with the effect direction being reported as positive or negative even within the same species (Berghänel et al., 2016; Dantzer et al., 2013; de Vries et al., 2007; Emack et al., 2008; Hauser et al., 2007, 2006; Patin et al., 2002; Schöpper et al., 2012). What is even more puzzling is whether the decreased or increased body growth is merely the product of an altered and constrained ontogenetic environment (e.g., intrauterine growth restriction) or whether they can be the results of adaptive processes to such conditions. Timing of exposure and sensitivity to organizational effects seems pivotal in regulating such differences: across mammals, increasing prenatal maternal stress and GCs during early gestation tend to lead to higher fetal size and accelerated offspring growth with earlier maturation when compared with controls, or when compared with the effect of increasing maternal GCs during late gestation (Berghänel et al., 2017).

Such results indicate that the timing of GC exposures and fetal and offspring sensitivity to organizational effects are central factors shaping epigenetic-induced phenotypic variation by regulating the potential adaptive or detrimental responses to prenatal and early-life adversities.

2.2 The developmental origin of health and diseases

The rising prosperity brought by industrialization has been associated with a steep increase in circulatory diseases, and today one leading cause of death is coronary heart disease in most countries. Historically, genetic predisposition matching the high-fat westernized diet and the constant use of drugs has been investigated to understand the origin of coronary heart diseases (Barker and Osmond, 1986) and several programs have been introduced by governments to reduce its occurrence. Although preventive measures such as diet changes and blood cholesterol reduction, and elimination of smoking habits reduced its risk,

epidemiologists realized that such interventions did not provide a secure basis to prevent such adult disorders (Barker, 2007).

In the 80', while investigating the relationship between diet and coronary heart disease in England and Wales during the second world war, Barker and Osmond discovered the unexpected correlation between the spatial distribution of adult disease and the spatial distribution of infants mortality (Barker, 2007; Barker and Osmond, 1986). Based on these and other studies on childhood nutrition and ischemic disease (Barker and Osmond, 1986), Barker understood that lifestyle and genetic inheritance were not enough to explain the health states in adult subjects and formulated the first model on the developmental origin of health and disease (Barker, 2007; Barker et al., 1993; Barker and Osmond, 1986). He proposed that adult health states can be explained better by adversities experienced early during development and leading to organizational effects on physiology, body structure, and metabolism rather than by the interactions of the subjects with their current environment (Barker, 2007; Barker and Osmond, 1986).

Barker developed further his theory by reviewing the effects of fetal malnutrition at different gestational phases and underlined the link between hormone variation in the fetus and placental environment with the development of specific and divergent metabolic phenotypes (Barker et al., 1993). He added more emphasis on fetal nutrition and established that permanent alteration of body structure and metabolic conditions increasing the risk for coronary heart diseases can be caused by phenotypic reprogramming derived from nutrient lack during gestation (Barker et al., 1993; Wadhwa et al., 2009). A classic example supporting Barker's intuitions is the Dutch-famine study which showed that adults conceived during the war, under adverse conditions and lack of nutrients had higher increased heart disease, stress responsiveness, and obesity, contrarily to adults conceived under better nutritional conditions (Roseboom et al., 2006).

Broadly, theoretical models on the evolution of maternal effects can be grouped in two main groups: developmental constraints models and predictive adaptive response models (Hanson and Gluckman, 2014; Lea et al., 2015; Lu et al., 2019; Malani et al., 2022; Nettle and Bateson, 2015; Spencer et al., 2022). In the next sections, I briefly introduce some of the most discussed hypotheses and provide examples of empirical tests informing on the debate on the evolutionary origin of developmental plasticity.

2.3 Developmental constraints

Since Barker formulated the developmental origins of health and disease (Barker, 2007), the interest of epidemiologists, biologists, and ecologists in the fetal origin of adult disorders has been largely growing (Wadhwa et al., 2009). Today, there is an extensive body of literature on phenotypic variations driven by environmental conditions in humans and other animals and it is well established that conditions experienced during specific ontogenetic sensitive periods can have persisting effects on

neurodevelopment, acquisition of cognitive and motoric skills, immune function and systemic diseases (Berghänel et al., 2015; Conti et al., 2012; Elford et al., 1991; Gollwitzer and Marsland, 2015; Hanson and Gluckman, 2014; McGowan and Matthews, 2018; Schneider et al., 1999). Yet, the evolutionary origin of developmental plasticity is still under debate.

According to the developmental constraints hypothesis, early-life adverse conditions can negatively impact later-life performance by constraining the development of individuals living in poor conditions (Monaghan, 2008). Thus, individuals born in an advantaged environmental context will outperform those born under suboptimal conditions ("silver spoon" effect; Grafen 1988). Broadly, adversity prompts organisms to optimize energy allocation to processes that ensure immediate survival and early recalibrations lead to reduced fitness without the possibility of later life improvements (Berghänel et al., 2017; Lea et al., 2015; Lu et al., 2019; Monaghan, 2008). One example of the "silver spoon" effect came from a study on fitness in yellow ground squirrels where population density, as an adverse environmental condition, negatively affected the life span of adult subjects and predicted lifetime reproductive success (Vasilieva and Tchabovsky, 2020). Supporting the developmental constraints hypothesis, female baboons born in a higher-quality environment had higher fitness during a drought period than females born in a lower-quality environment and reproducing the same period, but such fitness advantage became null when both the high-quality and low-quality females reproduced under normal environmental conditions (Lea et al., 2015). One example of long-term developmental constraints model is the Biological embedding model (BEM). Biological embedding occurs when cumulative adversity "gets under the skin" altering human development and biology with stable and long-term effects influencing health and behavior (Hertzman, 1999). BEM posits that cumulative early adversity becomes embedded in permanently disrupted physiological systems (Grafen, 1988; Hertzman, 1999; Miller et al., 2009; Monaghan, 2008).

Body growth is a phenotypic trait particularly sensitive to constraints during development. According to the developmental constraints hypothesis, mothers living under adverse conditions face a reduction in the energy available for offspring development (or increased costs associated with such adversities). Offspring developing under suboptimal conditions show reduced prenatal and postnatal body growth as a direct consequence of a reduced maternal energetic allocation, which ultimately translates into a reduced life-expectancy (Merlot et al., 2013; Sheriff and Love, 2013; Hanson and Gluckman, 2014; Wells et al., 2016; Hinde et al., 2015; Berghänel et al., 2017). In support of developmental constraints' effects on growth, prenatal maternal stress alters placenta size and weight of the fetus (Barker et al., 1993; Hanson and Gluckman, 2014; McGowan and Matthews, 2018; Moisiadis and Matthews, 2014b).

2.4 External predictive adaptive responses (ePAR)

An alternative model argues that mothers transmit the environmental adversity information to the offspring and trigger predictive adaptive response (PAR) aimed at benefiting the offspring during later life stages (Gluckman et al., 2005; Hanson and Gluckman, 2014). The first formulated PAR hypothesis assumes that the environmental clue transmitted from the mother during ontogenetic periods of the offspring, basically the information on environmental adversity, prompts developmental responses calibrated to increase fitness only in future environments matching the ones predicted by the mother (Gluckman et al., 2005; Hanson and Gluckman, 2014; Lu et al., 2019). Differently from the developmental constraints hypothesis, the PAR hypothesis predicts an adaptive response triggered by prenatal stressful conditions. Elevated prenatal maternal glucocorticoid levels would inform the offspring about prenatal environmental adversities and thus would trigger phenotypical recalibration allowing the offspring to optimize its fitness in the future environment, only when the match occurs (Gluckman et al., 2005; Lu et al., 2019). In essence, the PAR predicts that lower-quality phenotype produced from poor environments can have increased fitness if also the later-life environment is equally poor. Because the PAR formulated by Gluckman and Hanson strongly relies on environmental forecast, it has also been named external PAR (ePAR) to differentiate it from another theoretical model (Gluckman et al., 2005; Lu et al., 2019).

Support for the ePAR hypothesis comes from studies on fast-living species such as birds and small mammals (Sheriff and Love, 2013). For example, zebra finches exhibited growth changes induced by temperature variations which translated into greater reproductive success only when the temperature experienced during early stages matched that one of the later-life phases (Mariette and Buchanan, 2016).

2.5 Internal predictive adaptive responses (iPAR)

Although the assumed environmental predictability between early conditions and later stages can be observed in fast life-history animals, it is unlikely for long-lived species with slow development and slow life-history pace (Nettle and Bateson, 2015; Spencer et al., 2022). In fact, contrary to what was observed in fast-living species, studies on slow-developing and long-lived mammals contradicted ePAR predictions. Investigations on fitness in wild baboons (Lea et al., 2015), bighorn ewes (Pigeon et al., 2017) and roe deer (Douhard et al., 2014) revealed that adversities or poor rearing environments brought fitness costs that persisted across time or increased when the later environment was also poor.

The whole ePAR hypothesis is grounded on the assumption that early environmental conditions will reliably predict later life environmental conditions. For this reason, its applicability to long-lived species has been largely questioned: longer gaps between early environmental clues and future environmental conditions decrease the probability of correct environmental prediction, and the chances of observing a

mismatch raise steeply (Nettle et al., 2013; Nettle and Bateson, 2015; Spencer et al., 2022). The longer the development — meant as the time between the moment the cue informs on the environmental state and the recalibration initiates, and the time of response completion — the higher the recalibration costs (Spencer et al., 2022). Theoretically speaking, long-lived organisms under ePAR face higher recalibration costs than fast-living ones, and at the same time will have lower environmental predictability with consequent higher chances of mismatch and a higher probability of lower fitness benefits (Spencer et al., 2022).

According to the internal PAR (iPAR) hypothesis, offspring experiencing developmental constraints may reprogram their life-history by accelerating their pace of life to ensure the best possible offspring production under a reduced life expectancy (Del Giudice, 2014; Nettle et al., 2013; Nettle and Bateson, 2015). That is, offspring should live faster under the expectation of dying younger. One important prediction of the iPAR hypothesis is that subjects who face early adversity and accelerate reproduction should gain higher fitness benefits than those facing the same adversity and maintaining delayed reproduction. In contrast, slower reproduction is optimal for individuals who do not face such adversity since it allows them to reach full body size without accelerating the pace of life. Such predictions generate a fitness crossover, an essential element to test PARs since it shows that the optimal phenotype is state-dependent (Lu et al., 2019; Nettle et al., 2013; Weibel et al., 2020). However, if resources are limited during ontogenetic sensitive periods, the proposed acceleration of life-history pace will necessarily rely on energetic trade-offs grounding the development and maintenance of other physiological functions like acquired immune defense (Dantzer et al., 2013; McGowan and Matthews, 2018; Patterson et al., 2021; Snyder-Mackler et al., 2016; Del Giudice, 2014; Nettle et al., 2013; Nettle and Bateson, 2015). Crucially, the ePAR and the iPAR models differ in the conditions required to be functional and therefore adaptive (Figure 1).

Although it does not test the fitness crossover prediction, partial support for adaptive developmental plasticity according to the iPAR hypothesis comes from a meta-analysis that included more than 700 studies across several mammal species and investigated the effects of prenatal maternal stress on growth: the study suggested that while maternal investment leads to reduced growth due to developmental constraints, adaptive recalibrations of the offspring's pace of life are possible if the exposure to prenatal maternal GCs intensifies during early gestation. Thus, adaptive growth plasticity would be possible in long-lived species not because of matched environmental forecasting induced by maternal GCs, but because maternal GC levels would inform the fetus of lower maternal investment and would induce an internal somatic-state-based PAR. Supporting the iPAR hypothesis, in wild Assamese macaques prenatal maternal GC levels increase infants' growth and body-size at 18 months and it is associated with a conjunctivitis outbreak indicating that infants traded off immune function with growth to maximize fitness (Berghänel et al., 2016). Quite puzzling, in contrast to what was observed in wild Assamese

macaques, a recent and unique test of the iPAR hypothesis and related fitness crossover in wild baboons found no evidence of adaptive responses (e.g., accelerated reproduction) to early-life adversities, but a generally increased lifetime reproductive success associated with accelerated reproduction, despite the adversity faced (Weibel et al., 2020). Such strong difference in the observed effects raise doubts that accelerated reproduction is indeed an adaptive response to early adversity in long-lived species and leave stimulating questions on developmental plasticity open.

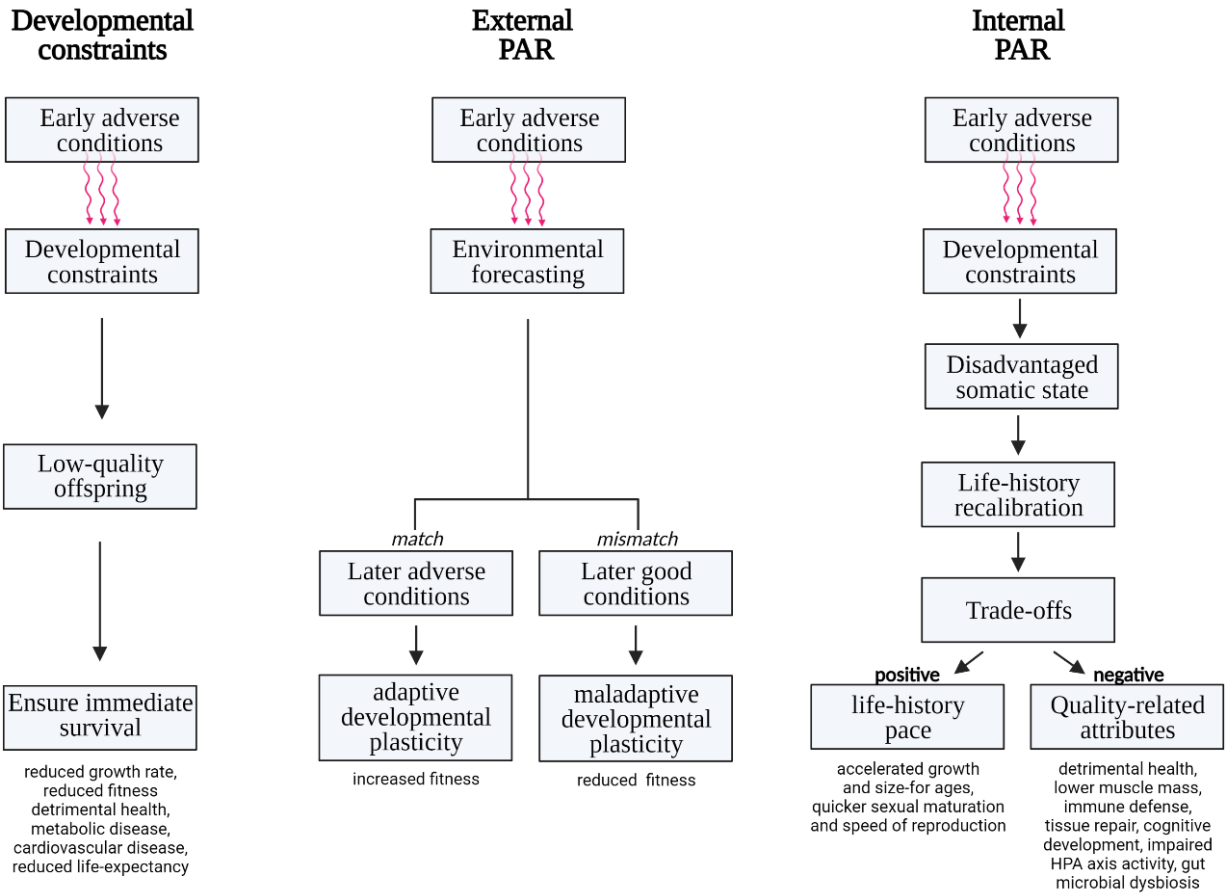

Figure 1 - Major models for developmental plasticity. The developmental constraints hypothesis predicts that organisms reproducing under poor environmental conditions will produce low-quality offspring which will ensure survival as short-term adaptation while both the external and the internal predictive adaptive response (PAR) hypotheses posit that organisms increase fitness through long-term adaptive recalibrations. The external PAR relies on environmental predictability, and it is truly adaptive only when the later life environment matches the one predicted during ontogenetic periods. The internal PAR hypothesis predicts that early adversity provides cues of environmental conditions and lower maternal investment leading to offspring with disadvantaged somatic states and reduced life expectancy which will maximize fitness through the accelerated pace of life (e.g., accelerated growth, sexual maturation, and reproduction) paid at the cost of quality-related attributes.

2.6 Adaptive Calibration model (ACM)

While developmental constraints models predict detrimental health and reduced fitness outcomes for challenged offspring, predictive adaptive response models predict that the phenotypic recalibration can

partially counter such negative fitness effects of the expected reduced life span with an increased effort in reproduction.

Mostly conceptualized to explain variation in the human-stress response, the Adaptive Calibration model (ACM) proposes physiological adaptive responses to stressors (i.e., HPA axis activity) translating into intra- and inter-individual variation. According to the ACM, such adaptations allow challenged individuals to adjust physiological responses during development in response to variations of socio-ecological adversities (Del Giudice et al., 2011). However, the ACM recognizes that offspring phenotype may be repeatedly recalibrated to current conditions (during consecutive sensitive phases), allowing for compensation or even reversal of earlier adversities effects on a physiological system like the HPA axis. Importantly, the ACM is the only model allowing the effects of the same adversity to change between offspring developmental phases and predicts adaptive plasticity of rather immediate physiological functions without necessarily yielding later-life effects, provided that socio-ecological conditions fluctuate (Del Giudice, 2015).

3 Assamese macaques as a model species

Assamese macaques are cercopithecine native to South Asia. They are mainly arboreal and spend 60% of their time away from the ground and low storey of the forest (Berghänel et al., 2016; Heesen et al., 2013; Schülke et al., 2011). Individuals live in multi-male multi-female groups, males migrate to other groups at approximately 4 years of age while females are philopatric and start to reproduce at age 6 (Fürtbauer et al., 2010). Assamese macaques are relaxed-income breeders with seasonal reproduction and about 80% of the births occur between April and June (Touitou et al., 2021), the average gestation length is 164 days (Fürtbauer et al., 2010; Ostner et al., 2013), and weaning age is approximately 12 months based on last observations of nipple contact (Ostner et al. 2013). However, because after the first 6 months infant suckling rates progressively drop, the lactation period is typically defined as the first 6 months of life (Heesen et al., 2013).

As the environmental conditions in south-east Asian forests are highly unpredictable, also the predictability of food and fruit abundances is very low (Berghänel et al., 2016) with repercussions on food consumed during different periods of the year and across the years (Heesen et al., 2013; Touitou et al., 2021). Despite variability across years, the population study lives in a habitat characterized by repeated patterns of fluctuating resources with a cold dry season (lean season: November-February) and a hot rainy season (rich season: March-October) (Touitou et al., 2021). The availability of fruits is crucial for energy intake and activity patterns (Heesen et al., 2013; Touitou et al., 2021), and in our study population food scarcity based on fruit abundance estimation increases GCs productions (Berghänel et al., 2016).

At the field site located in Phu Khieo Wildlife sanctuary (Thailand) the study population is frugivorous and the main part of the plant diet comprises fruit, pulp, and seed (Heesen et al., 2013; Schülke et al., 2011; Touitou et al., 2021). However, individuals spend a considerable part of their feeding time slowly foraging for animal matter and consume large amounts of aquatic mollusks, several species of insects and spiders, and also and less regularly small amphibians and reptiles like frogs, snakes, and lizards, but also birds and bird eggs, and small rodents (Schülke et al., 2011). They consume more than 150 known plant items belonging to about 120 plant species and only very few items are constantly consumed across the years suggesting adaptive capabilities to high diet variability. However, diversity in the diet is not rare among primate species (Milton, 1984; Lambert and Rothman, 2015). Although the main part of Assamese macaques' diet is based on plant parts, their gut microbial community endures unpredictable food variation and must maintain stability through dynamism with higher resilience and resistance to perturbations in order to provide energy from different sources. Studying Assamese macaques' gut microbial community offers the opportunity to investigate prenatal maternal effects on phenotypic recalibration of physiological systems providing adaptations to an unpredictable environment.

The population at Phu Khieo Wildlife Sanctuary provides a good model for testing the internal consistency of the iPAR hypothesis. External predictive adaptive responses rely on the assumption that early environmental conditions will reliably predict later life environmental conditions. Assamese macaques are slow-developing and long-lived mammals that inhabit a highly unpredictable environment and developmental plasticity in this species must rely on mechanisms other than good environmental prediction. Previous studies on the same study population have shown that prenatal maternal GC levels increase infants' body growth and correlate positively with an outbreak of conjunctivitis suggesting a potential trade-off of growth acceleration against immune function in infants (Berghänel et al., 2016, 2015). Crucially, these studies established that also a moderate and naturally driven prenatal stressor can trigger developmental plasticity effects according to what postulated by PARs and established that phenotypic recalibration occurred in infant Assamese macaques was based on somatic states (iPAR) given the excluded possibility of the environmental prediction (ePAR).

These studies paved the way for a direct test of predictions postulated by the iPAR hypothesis in a wild long-lived primate.

4 Study aims and approaches

The general aim of this thesis is to advance our understanding of the evolutionary origins of developmental plasticity by testing predictions of the iPAR hypothesis. The iPAR proposes that offspring enduring early adversities may use their own suboptimal and disadvantaged somatic states to recalibrate and optimize their future somatic states despite conditions of the future environment (Nettle

et al., 2013; Nettle and Bateson, 2015). Moreover, if resources are limited during sensitive ontogenetic periods, the proposed acceleration of the life-history pace will require a trade-off with functions of physiological maintenance.

I aimed to test the internal consistency of the iPAR hypothesis by investigating potential long-term phenotypic recalibrations associated with prenatal maternal GCs on a wild species. Wild Assamese macaques are long-lived mammal species with slow development and evolved to live in a highly unpredictable environment thus the possibility of developmental plasticity regulated by ePAR is excluded by definition (Berghänel et al., 2016).

This thesis is grounded on the long-term data on fecal maternal GCs provided by Julia Ostner and Oliver Schülke and collected by several field assistants and other Ph.D. students over more than one decade: without their work, none of this would have been possible.

To tackle the effects of prenatal maternal GCs on developmental plasticity I spent 13 months on the field after a period of 1 month of training. I collected fecal samples to derive information on offspring HPA axis activity and gut microbiome diversity and composition in a non-invasive way and I remotely collected a biometric measure via laser digital photogrammetry. I measured forearm length as a proxy for body size and once back from the field I performed DNA extractions and 16S rRNA amplification on more than 400 samples, I estimated forearm length and finally performed statistical analyses using different statistical methods.

With a cross-sectional approach, I tested in infants, juveniles, and adults whether increased maternal GCs during gestation and lactation were associated with the accelerated pace of life paid at the cost of quality-related attributes. The information on quality-related attributes tested was derived from analyses on the HPA axis activity and the gut microbial community of the offspring, whereas I used trajectories of body growth as a proxy for the pace of life. Since fetal GC sensitivity to organizational effects depends on the timing of exposure (Berghänel et al., 2017; De Kloet et al., 1988; McGowan and Matthews, 2018; Moisiadis and Matthews, 2014a), all the predictions tested were integrated with assessments of potential timing-effects of maternal GCs.

In the first study (Chapter 2) I tested whether prenatal maternal GCs were associated with long-term variation of HPA axis activity, with the prediction that their increase will impact offspring maintenance functions with potentially detrimental effects on health. The HPA axis is a physiological system very sensitive to environmental perturbations and a target of phenotypic plasticity— GCs reprogramming of its functionality critically links early adversities with the development of poor health states. Since elevated baseline levels, higher sensitivity, and alterations of HPA axis functionality have been largely associated with detrimental health states (Cacioppo et al., 2015; Capitanio and Cole, 2015; McGowan and Matthews, 2018), I tested whether increased maternal GCs during gestation and lactation led to

persisting hyperactivation of offspring's HPA axis and whether the altered activity was linked with timing of GCs exposure.

Testing the long-term programming of offspring's HPA axis activity informed on their current physiological states and allowed deeper interpretations of health states in the second investigation (Chapter 3): the tests on prenatal maternal GCs effects on gut microbial richness and composition. The tests were carried out under the assumptions that lower microbial diversity and richness are associated with community instability to perturbations and with several diseases (Amato et al., 2016; Clarke et al., 2013; Clayton et al., 2018; Dahl et al., 2017; Foster and McVey Neufeld, 2013; Le Chatelier et al., 2013). However, since microbial diversity and richness are not considered solid health indicators but a proxy for dysbiotic states and microbial community stability, I integrated the analyses with the challenging exploration of the effects of prenatal maternal GCs on gut microbial composition. I explored variations in the relative abundance of approximately 300 microbial species and characterized the microbial signature and dysbiotic states associated with maternal GCs measured during different developmental time windows. Additionally, the information derived in the first study (i.e., offspring HPA axis activity) was integrated into the second one and allowed me to speculate on potential plastic adaptations of the prenatally stressed holobiont.

In the third study (Chapter 4) I investigated Assamese macaques' developmental trajectories and milestones and validated the parallel-laser photogrammetry method used to remotely and non-invasively measure sizes of forearm length. I tested method accuracy through the estimation of several error measures and with a direct comparison with another method performed on the same study population (Berghänel et al., 2015, 2016). Finally, I explored the use of different statistical approaches to characterize Assamese macaques' growth trajectories and pseudo-velocity curves with the use of quadratic-plateau models and local polynomial regressions.

Method validation through different statistical approaches, error measures and methods comparison provided important clues on the best statistical solution to investigate the acceleration of life-history pace associated with increased prenatal maternal GCs (Chapter 5). With the optimal statistical method identified in Chapter 4, in the fourth study (Chapter 5) I applied both a linear and a non-linear approach with local polynomial regression to test one of the most important postulates of the iPAR hypothesis predicting phenotypic recalibration characterized by accelerated growth associated with prenatal maternal GCs (Berghänel et al., 2017). To do so, I predicted and compared different growth trajectories and pseudo-velocity curves at different values of prenatal maternal GCs during early and late gestation and controlled for the postnatal effect.

Chapter 2 - HPA axis activity

Prenatal glucocorticoids are associated with increased HPA axis activity from infancy to adulthood in a wild primate

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Abstract

The hypothalamic-pituitary-adrenal (HPA) axis has a dual role in the biology of developmental plasticity in mammals including humans — it provides offspring with cues to maternal physiological states, and it is the target of offspring developmental plasticity. To assess timing effects, we quantified maternal HPA axis activity as a source of early adversity during three developmental phases, and assessed its effect on offspring HPA axis activity in wild infant, juvenile, and adult macaques. Prenatal maternal glucocorticoid levels experienced early in gestation but not during lactation had similar enhancing effects on offspring HPA axis activity in all offspring age classes. Together with previous results, this small study suggests that offspring are sensitive to the fundamental programming of this and other physiological systems especially very early during development with effects on correlates of Darwinian fitness.

Manuscript in preparation for submission.

Introduction

Developmental plasticity has been demonstrated in mammals from humans via model organisms, to farm animals and a few species of wildlife (Nussey et al., 2007; Maestripieri & Mateo, 2009; Dantzer et al., 2013; Li et al., 2013; Douhard et al., 2014; Wells, 2019). Particularly the conditions experienced early in life can have pronounced effects on later life morphology, physiology, health, cognition, behavior, and ultimately fitness (Sandi & Haller, 2015; Berghänel et al., 2017; Manzari et al., 2019). For the embryo, fetus, and infant the mother provides the interaction with, but also a buffer to, external environmental conditions (Thayer et al., 2020). Therefore, offspring phenotype can be shaped by maternal effects that are independent of offspring and maternal genotypes (Bernardo, 1996).

The hypothalamic-pituitary-adrenal (HPA) axis and its end product, the glucocorticoids, play a dual role as a cue for the offspring to environmental conditions experienced by the mother and her physiological state on the one hand, and as a target of offspring phenotypic plasticity in reaction to such cues on the other (Meaney et al., 2007; Thayer et al., 2018). Offspring exposure to maternal glucocorticoids starts at conception and lasts throughout gestation with the placenta as a possible filter between the maternal and offspring bloodstream (Moisiadis & Matthews, 2014a). Yet, it continues after birth until weaning with milk glucocorticoid levels partly reflecting maternal plasma levels (Hinde et al., 2015; Dettmer et al., 2018). In addition, maternal effects will be mediated also by maternal behavior towards the offspring which may vary with maternal glucocorticoid levels as well (Maestripieri, 2009). Thus, a comprehensive study of HPA axis developmental plasticity will assess exposure to adversity and resulting offspring HPA axis phenotype each at different life stages.

Experimental manipulation of adverse conditions or maternal glucocorticoids established that the timing of early adversity can impact differentially the existence, strength, and even the direction of effects on offspring HPA axis activity (Schneider et al., 1999; Moisiadis & Matthews, 2014b). Maternal effects lasting into adulthood have been of particular interest because they may influence reproductive performance, health, longevity, and hence individual fitness (Nettle et al., 2011; Tung et al., 2016). However, assessing the half-life of maternal effects which may be short as suggested by a recent discussion of the adaptive calibration model of stress (Girard-Buttoz et al., 2021), or elucidating the mechanisms linking the initial adversity to a pathophysiological outcome (McGowan & Matthews, 2018) both requires that offspring phenotype is assessed at several points during development.

Theoretical models of the evolution of maternal effects can be grouped into developmental constraints models and predictive adaptive response models (Lea et al., 2018; Lu et al., 2019; Schülke et al., 2019). Developmental constraints models posit that (cumulative) early adversity becomes embedded in permanently disrupted physiological systems like the HPA axis (Grafen 1988; Monaghan 2008; Hertzman, 1999; Miller et al., 2009). According to predictive adaptive models, early adversity has

programming effects that alter offspring phenotype to better match later life external environmental conditions (Barker 2007; Gluckman & Hanson 2004) or internal conditions that can be predicted into adulthood from the early adversity (Nettle et al., 2013).

Developmental constraints models predict negative health and fitness outcomes for challenged offspring, whereas internal predictive adaptive response models predict that re-calibrated challenged offspring will partly counter the negative effects of the expected reduced life span by increased reproductive effort. A third model type recognizes that offspring phenotype may be adjusted to current conditions repeatedly in consecutive sensitive phases allowing for compensating or even reversing the effects of earlier adversity on physiological systems like the HPA axis (Adaptive Calibration model; Del Giudice et al., 2011); it is the only model type allowing the effects of the same early adversity to change between phases of offspring development.

In general, maternal effects on offspring HPA axis phenotype may either result from epigenetic reprogramming via altered DNA methylation (Palma-Gudiel et al., 2015; Kertes et al., 2016), small non-coding RNAs (Gapp et al., 2014; Rodgers et al., 2015), and expression of epigenetic regulators (McGowan & Mathews 2018) or from non-epigenetic changes in hormone activity, body composition, or post-translational modification of non-histone proteins (Lappalainen & Greally 2017; McGowan & Mathews 2018). Phenotyping offspring at different developmental stages will allow assessing whether early adversity immediately impairs HPA axis functioning causing rather stable effects across individual development or initial cell reprogramming causes pathological effects only later when cells exhibit changed responses to the same environmental cues (McGowan & Mathews 2018).

With this work, we aimed at informing evolutionary hypotheses and adding ecological validity to the assessment of early life maternal effects on offspring HPA function. Hence, we investigated in a wild long-lived mammal, correlations between prenatal or postnatal maternal glucocorticoid levels and those of offspring (both measured as concentrations of fecal glucocorticoid metabolites) at different life stages in a prospective cross-sectional design. Mean values of fecal glucocorticoid metabolites are best interpreted as a measure of average HPA axis activity, but they offer hints to HPA axis reactivity as well, if combined with minimum and range of levels across repeated samples. In the study population of Assamese macaques (*Macaca assamensis*) living in their natural habitat — northern Thailand — food abundance fluctuates within and between years and food scarcity is associated with increased maternal glucocorticoid output (GCs) (Berghänel et al., 2016; Touitou et al., 2021). This variation in prenatal maternal GCs is associated with the differences in growth velocity of infant offspring and its motoric skill acquisition (Berghänel et al., 2016). This study extends offspring phenotyping to HPA axis activity and beyond infancy into the juvenile and adult life phase, and links prenatal maternal condition to offspring reproductive performance.

Results and Discussion

Across several statistical models, prenatal maternal GCs early in gestation (first half) had a consistent enhancing effect on offspring HPA axis activity quantified as the mean, range, or maximum fecal GC metabolite levels in 9.5±3.5 (mean±SD) samples per offspring (N=32 offspring, all from different mothers; Figure 1). In the same models, the effects of prenatal maternal GCs late in gestation were in the same direction but weaker. In contrast, postnatal maternal GCs during lactation were not associated with mean, range, or maximum GC levels in the offspring, but positively correlated with minimum levels (Figure 1, Appendix 1 – Table 1). Together with earlier results from the same population, this suggests that it is particularly the adversity experienced during early prenatal development, during organogenesis, that may cause programming effects on growth, motor development, immune function (Berghänel et al., 2016), and now also HPA axis activity. The lack of evidence for postnatal maternal GCs affecting offspring GCs may result from this timing effect, from variation in maternal postnatal GCs not being a severe enough stressor to trigger a reaction, or from a weak correlation of maternal fecal and milk GC levels (Dettmer et al., 2018).

The increased offspring HPA activity in response to prenatal maternal GCs is consistent with results from controlled experiments with infant (de Vries et al., 2007) and adolescent monkey offspring (Clarke et al., 1994). Adult offspring also show increased average fecal GC metabolite levels in response to cumulative early life adversity experienced before or after birth in two populations of wild baboons (Onyango et al., 2008; Rosenbaum et al., 2020; Patterson et al., 2021). Likewise, different prenatal stressors including increased maternal GCs were consistently associated with increased offspring HPA activity or reactivity in a meta-analysis of 39 studies on 14 mostly captive vertebrate species (Thayer et al., 2018).

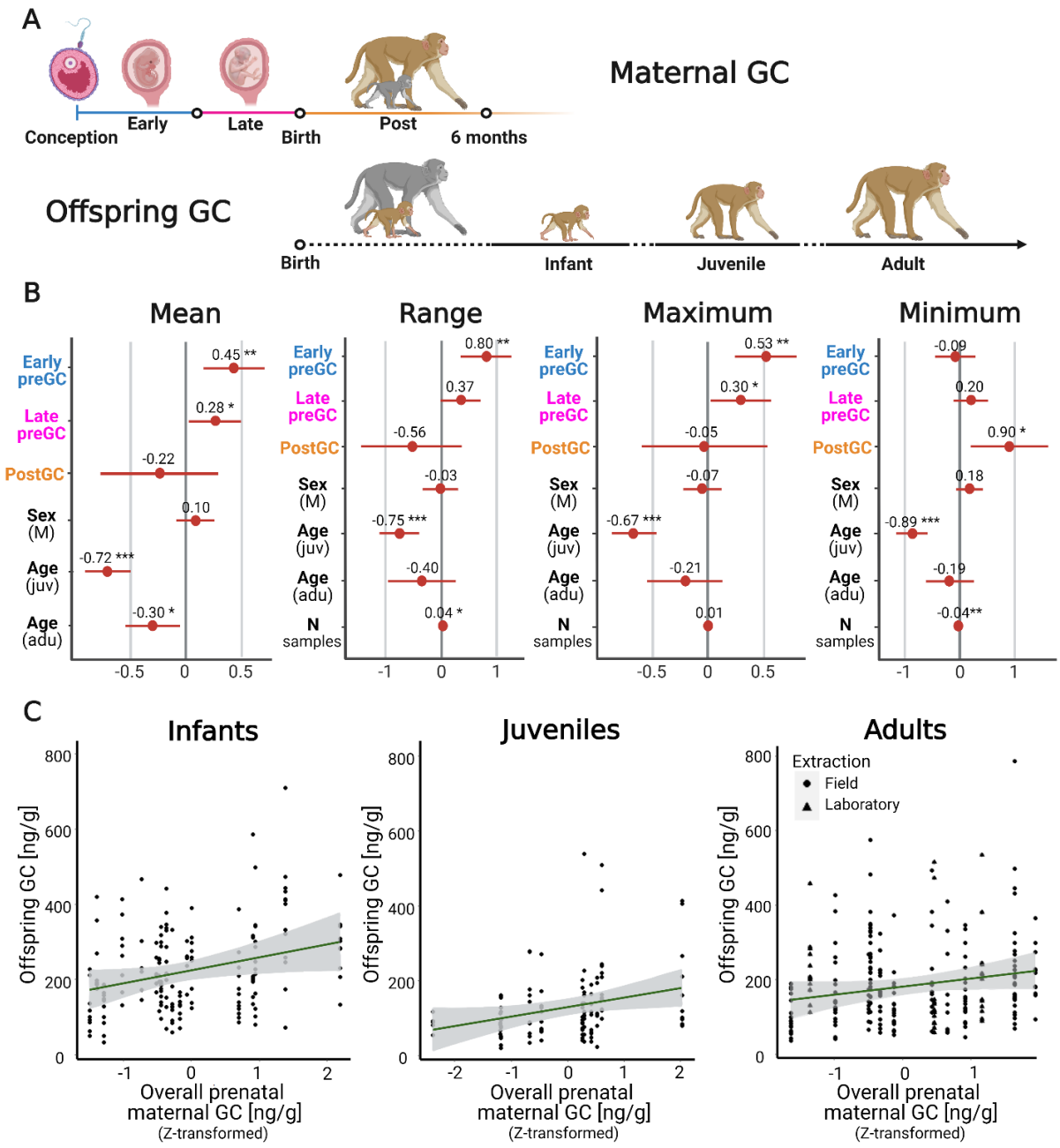

Figure 1 Maternal glucocorticoid metabolite (GC) levels measured in feces in three different time windows, and their effect on infant, juvenile, and adult offspring levels. Increased mean, range, and maximum offspring GCs in all age classes are associated with increased early-prenatal, but not postnatal exposure to maternal GCs. Increased minimum offspring GCs is associated with increased postnatal maternal GCs in all age classes. (A) the blue line indicates sampling of early-prenatal maternal GCs (early), the magenta line indicates sampling of late-prenatal maternal GCs (late), and the orange line spanning the first six months of lactation indicates the sampling of postnatal maternal GCs (post). The black line indicates cross-sectional sampling of offspring GCs at ages from six months on for infants, 3-4yrs. for juveniles, and 9-10yrs. for adults. (B) Forest plots show estimated effects sizes and confidence intervals on $\log_n$-transformed responses from reduced models 1-4 after removal of non-significant interaction terms for age class with prenatal and postnatal maternal GCs (n=32, significance codes: <0.05 *, <0.01 **, <0.001 ***, exact p-values and model estimates in Appendix 1 – table 1). (C) Effect of overall mean of prenatal maternal GC (z-transformed with mean = 0 and standard deviation = 1) on offspring GC for infants (n=18), juveniles (n=11) and adults (n=14) estimated from GLS models ran on single GC values as data points (model estimates in Appendix 1 – Table 2). The z-transformation of prenatal maternal GCs metabolites in adults' samples have been performed separately according to extraction methods, and then pulled together into the model. Circles denote field-extracted samples, triangles lab-extracted samples. The data to generate this figure are uploaded as Figure 1 - Source data 1. Figure created with BioRender.com

We argue that our results may not only reflect variation in HPA axis activity but also in reactivity. Fecal GC metabolite levels cannot quantify directly the very short-term HPA axis response to an acute stressor (Romero & Beattie 2021). Instead, GC metabolites accumulate in feces over several hours when the fecal material travels through the gut (Heistermann 2010). Yet, if different individuals experienced similar stressors at a similar rate, higher mean, range, and maximum fecal GC metabolite levels as observed here in association with increasing early prenatal maternal GCs, are consistent with a stronger HPA axis reactivity or a slower return to baseline after an acute stress response, but not with a blunted HPA axis response. Blunting would result in a narrowing range of fecal GC metabolite values coupled either with increasing or with decreasing baselines in challenged offspring; no matter the stress exposure during the preceding hours, GC metabolite levels remain rather stable, either constantly elevated or low. In this study, all offspring were sampled during the same field season to ensure all were exposed to the same ecological stressors. Offspring GC range increased with early and non-significantly with late prenatal maternal GCs, it was not related to postnatal maternal GCs, and was neither negatively related with minimum nor maximum GC levels (Figure 2) providing no evidence for a blunted response.

Blunted responses of the HPA axis have been described for offspring that experienced natural or experimental adversity after birth in non-human primates (Petrullo et al., 2016; Novak et al., 2013) and humans (Young et al., 2021) and are consistent with the flattening of diurnal urinary cortisol slopes in wild chimpanzees that experienced early maternal loss before the age of 5 years (Girard-Buttoz et al. 2021). These seemingly contradicting results may be a consequence of pronounced timing effects prohibiting comparison across studies (Moisiadis & Matthews, 2014b).

Particularly when experienced very early, prenatal adversity may cause epigenetic changes to components of the HPA axis yielding elevated baseline GC levels and increased reactivity (McGowan & Mathews, 2018). Within the longer postnatal immature period, challenges to the offspring may have less direct effects on HPA axis function, perhaps depending again on the timing relative to sensitive periods (McGowan & Matthews, 2018). For example, non-lethal adversity caused by maternal loss may occur too late in development to cause epigenetic changes lasting into adulthood which may explain why maternal loss is associated with a labile change in offspring HPA axis functioning in wild chimpanzees that is not detectable in adult offspring (Girard-Buttoz et al., 2021). Likewise, maternal loss did not have an isolated effect on offspring HPA axis activity in adult female baboons (Rosenbaum et al., 2020), but was only effective in combination with other stressors that occurred earlier in development like a low maternal dominance rank at birth or a severe drought experienced in the first year of life (Rosenbaum et al., 2020).

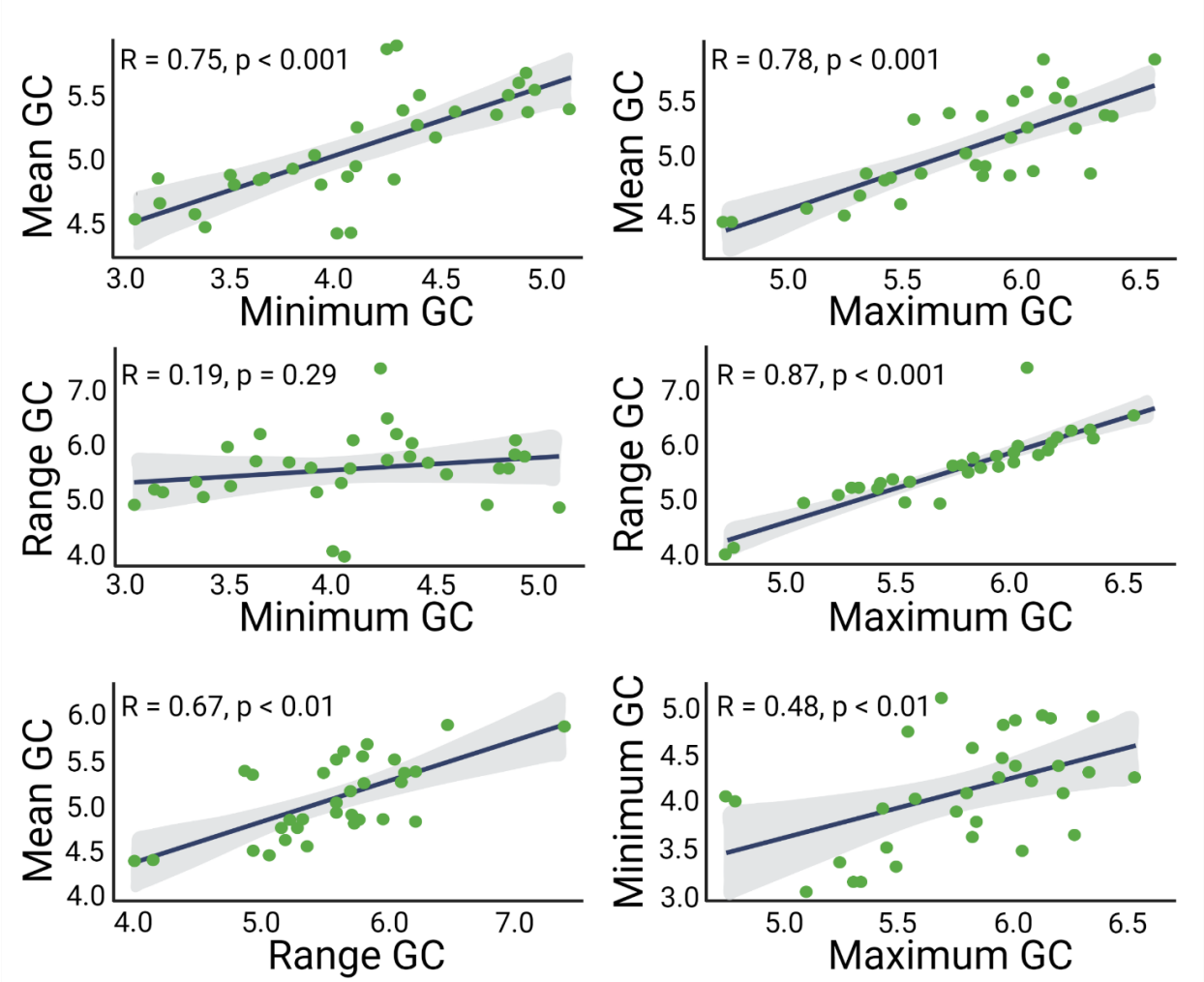

Figure 2 Correlations between $\log_n$-transformed GC responses (concentration of fecal glucocorticoid metabolites [ng/g]; n = 32). If HPA response was blunted and baseline levels were either increasing or decreasing, the more early adversity an individual had faced, the range of offspring GC values should be negatively correlated to the maximum or the minimum. Instead, range and maximum are positively correlated ($p < 0.001$) and range and minimum are not associated ($p = 0.29$). The data to generate this figure are uploaded as Figure 2 - Source data 1.

Beyond those timing effects, the direction and strength of early adversity effects may also depend on offspring sex and the target element of offspring phenotype (Malalaharivony et al., 2021). Carefully designed field experiments on red squirrels (Dantzer et al., 2020), and a metanalysis across mammals (Berghänel et al., 2017), demonstrated that timing of early stress exposure can even reverse the effects on offspring growth phenotype. Yet, in the same red squirrel study system, the manipulation of maternal GC levels during gestation or lactation had only minimal effects on offspring HPA axis reactivity after challenge (Westrick et al., 2021). Even more, the timing effects and differences between targets may vary between species (Sheriff et al., 2017) and possibly between the sexes (Westrick et al., 2021; Dantzer et al., 2020).

Across models of mean, range, maximum, and minimum offspring GC, the statistical interactions of age class with maternal predictor variables were all non-significant, providing no evidence for changes of maternal effects with increasing age of offspring (Figure 1; Appendix 1 – Table 1). Post-hoc tests

revealed no differences between slopes for different age classes (all p-values $0.275 < p < 0.965$ for Tukey-adjusted contrasts between age classes, Appendix 1 – Table 3). Since the sample size was limited and therefore power to detect the interaction effects low, we ran additional analyses. We first pooled early and late prenatal GC measures into one average prenatal estimate which allowed the inclusion of individuals lacking data in one of the phases. A poolability test suggested no difference in the two regressions (Chow test: $F = 0.026$, $df_1 = 2$, $df_2 = 68$, p-value = 0.974). Then we built three separate models, one for each offspring age class which corroborated the result that prenatal, but not postnatal GC levels are positively associated with mean offspring GC from infancy into adulthood (Figure 1, Appendix 1 – Table 2). Examining possible programming effects on HPA axis activity very early in offspring development has important implications for the interpretation of adult physiological phenotypes (McGowan & Matthews, 2018). Without providing direct evidence, the fact that prenatal maternal effects on adult HPA activity can be detected already in infants suggests that the HPA axis is programmed as early as the time of exposure to elevated maternal GCs and that the same effect lasts into adulthood (McGowan & Matthews, 2018). An alternative developmental pathway is not supported; early adversity could cause effects expressed in different tissues of adult offspring which then secondarily cause changes in HPA axis activity only in adults but not in infants or juveniles (McGowan & Mathews, 2018). Much more and ideally longitudinal data on long-lived mammals are needed to conclusively resolve this issue.

By assessing outcomes at different offspring life stages, this study informs on evolutionary models of developmental plasticity, e.g. the discussion of the biological embedding (BEM: Power and Hertzman, 1997) versus the adaptive calibration model of stress (ACM: Del Giudice et al., 2011) as recently described (Girard-Buttoz et al., 2021). While the BEM hypothesizes that early life adversity will have detrimental repercussions on physiological states across life, the ACM predicts that adaptive plasticity concerns rather immediate functions without necessarily yielding later life effects provided that socio-ecological conditions fluctuate. Our small sample did not produce evidence for different effects of prenatal maternal stress in infants (closer to the adversity experienced), juveniles, or adults. Instead, our results concur with models of more permanent effects.

To differentiate between developmental constraints models like the BEM and internal predictive adaptive response models (iPAR: Nettle et al., 2015), we tested the iPAR prediction of pace-of-life acceleration in response to early adversity with data on female reproductive rate quantified as the annual production of offspring surviving to one year of age. In support of the iPAR prediction, prenatal GCs of 13 females were positively related to surviving birth rate in a partial correlation controlling for adult offspring GC levels (partial Pearson's $r=0.59$, $p=0.043$; Figure 4). In this seasonal species, there was too little variation in age at first reproduction to test how it related to early adversity. A similar increase in fertility in response to early adversity has been described for free-ranging rhesus macaques only during

their middle ages (*Macaca mulatta*; Luevano et al., 2022) but contrasts with findings in wild baboons, African bush elephant, and roe deer where early adversity is negatively associated with offspring survival (baboons: Zipple et al., 2019; Patterson et al., 2021), surviving birth rate (baboon: Weibel et al., 2020), first-year survival of calves (elephant: Lee et al., 2021) and probability of weaning two fawns in one year (Douhard et al., 2014). Longevity data are not yet available for the study population and thus, the internal PAR model cannot be tested conclusively yet by examining the fitness cross-over for offspring with or without accelerated reproduction that did or did not experience early adversity (Weibel et al., 2020).

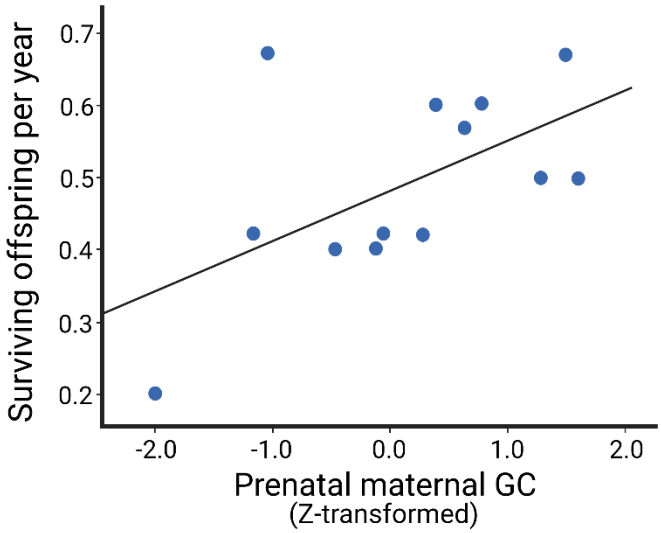

Figure 3 Overall prenatal maternal GC levels predict surviving birth rate (n = 13, partial Pearson's r=0.59, p=0.043). To increase variable interpretability, the variable *Prenatal maternal GC* (ng/g) was $\log_n$-transformed, and then z-transformed with mean = 0 and standard deviation = 1. The data to generate this figure are uploaded as Figure 3 - Source data 1.

As an important lesson also for human developmental medicine (Hawkley & Capitanio, 2020), these results suggest even moderate variation in maternal GC can lead to variation in offspring physiological phenotype, without the need for catastrophic events like maternal loss, serious drought, starvation, or extreme experimental procedures. It is not only the broad interannual variation in food scarcity, but also the relative timing of conception, and consequently gestation, within the four-months breeding season and resulting among-female variation in exposure to food scarcity as a stressor during this critical time that affected maternal GCs in ways relevant to offspring development (Berghänel et al., 2016). Thus, furthering our understanding of early maternal effects on various outcomes will not only contribute to more comprehensive models in evolutionary ecology, but also to preventive and intervention medicine (Lea et al., 2018).

Material and Methods

Key Resources Table				
Reagent type (species) or resource	**Designation**	**Source or reference**	**Identifiers**	**Additional information**
biological sample (Macaca assamensis)	Fecal sample	This study		Freshly collected from wild study animals
antibody	Anti-11ß-hydroxyetio-cholanolone (sheep, polyclonal)	In-house made (IZW Berlin)	Ganswindt et al., 2003	(1:480000)

Ethics statements

Our study was based on non-invasive collection of fecal samples, did not involve experimental work, and adhered to the ASAB/ABS Guidelines for the Use of Animals in Research (https://www.asab.org/ethics). It has been approved by the Thai National Research Council and the Department of National Parks, Wildlife and Plant Conservation Thailand (permits 0002.3/2647 April 2nd 2009, 0002/17 January 2nd 2013, 0002/2424 April 23th 2014, 0002/470 January 26th 2016, 0002/4139 June 9th 2017, 0002/2747 May 4th 2018, 0402/2798 October 4th 2019).

Data collection

Data were collected from a wild population of Assamese macaques from 2008 to 2019. The individuals lived in their natural habitat at the Huai Mai Sot Yai study site (16°27′ N, 101°38′ E) located in a large, protected forest (Phu Khieo Wildlife Sanctuary, Thailand). Assamese macaques reproduce seasonally with 78% of births occurring from April through June (O. Schülke and J. Ostner personal communication June 2021) and the average gestation length is 164 days (Fürtbauer et al., 2010). In this population, female age at first reproduction is 5.9 ± 0.5 years (mean ± SD, range 4.99 - 7.0 years, N = 40; O. Schülke and J. Ostner personal communication June 2021). Age at weaning is 12 months estimated from the last observations of nipple contact but observed suckling rates indicative of intense nursing during the day already decreased to zero by six months of age (Berghänel et al., 2016). Therefore, we measured postnatal maternal GCs only for the first six months of life. All fecal samples from infants were collected later than that (between 7 and 12 months of an infant's life), thus postnatal maternal GCs and infant offspring GCs were assessed at different times. Fecal samples for assessment of prenatal and postnatal

maternal stress levels had been collected during other studies as part of the long-term project. For this study, we phenotyped in one field season the offspring of mothers that were sufficiently sampled during early and late gestation as well as early lactation, together with an unplanned number of offspring born that year. The sample size was not planned according to statistical power analyses, because sample size was due to change in response to emigration, death, and unpredictable birth events. For the main analysis, we included fecal samples belonging to 32 different mothers and collected from 2010 to 2018 during early gestation (n = 193, mean ± SD per female 6.03 ± 3.41), late gestation (n = 186, mean ± SD per female 5.81 ± 3.05), and lactation (n = 564, mean ± SD per female 17.6 ± 6.2; postGC). The samples from the corresponding 32 offspring (n = 303, mean ± SD per individual 9.5 ± 3.5) were collected between June 2018 and July 2019. Infant offspring were from the 2018 birth cohort (10 males and 6 females), juveniles were born during the 2014-2016 (5 males and 4 females) and adults were born before 2012 from mothers that had been sampled at the time (2 males, 5 females). These data were used to test whether the timing of adversity relative to birth (early and late prenatal or postnatal) affected offspring HPA axis activity (mean, range, maximum and minimum offspring GC).

To substantiate the finding that prenatal adversity effects did not differ between age classes, we increased the sample size by pooling all prenatal data (early and late) into one average value and ran age-specific statistical analyses. The procedure permitted to include individuals for which no samples were available for one of the two prenatal phases. We ran models on specific age classes including samples from 18 infants, 11 juveniles, and 14 adults (see Fecal sample preparation and GC metabolite analyses) conceived as far back as 2008 and included 110 additional fecal samples and obtained a total of 1356 fecal samples analyzed for this study.

A detailed treatise of the STRANGE framework (Webster & Rutz 2020) for assessing biases in the selection of study subjects is provided below. We cannot rule out that mortality selection and differential social dispersal have biased the sample which would result in a lack of subjects with the highest levels of early adversity (mortality selection) with detrimental effects for statistical power or in case of differential dispersal in currently unpredictable effects.

STRANGE framework

Concerning the Social background, we selected as offspring subjects all individuals born into three fully habituated study groups in 2018 plus those juveniles and adults for whose early developmental phases we had maternal fecal samples stored from previous studies and who were still present in these groups in 2018. Thus, mortality selection may have affected our analyses. We followed up on the 2018 birth cohort at 3.5 years of age in November 2021 and found four offspring had disappeared from our study groups. Three of these offspring most likely were dead, because they disappeared before one year of age

either alone or with their mother in this female philopatric species. One individual dispersed into a neighboring group at two years of age and disappeared after seven months with an unknown fate. Maternal GC values were variable for these offspring: early preGCs were low relative to the cohort mean in three but very high in another individual, late preGCs were low in one, average in two, and high in the fourth individual, postGCs were average in one and high in three individuals. Based on these few cases, we cannot say whether mortality and disappearance introduced a clear bias in our sample.

In response to variation in Trappability and self-selection, the sample could be biased by variation in male dispersal strategies, if predictor variables were associated with age at first/secondary dispersal or the tendency to disperse into an unhabituated group versus one of the study groups so that they are lost from our sample. The Rearing history of subjects is the explicit topic of this study in the sense that all subjects were born to habituated mothers without experimental interference, but with mothers varying in fecal glucocorticoid metabolite levels and perhaps postnatally also in maternal behavior. Acclimation and Habituation are unlikely to have affected results because all subjects were long-term residents of the study groups. Because of known Natural changes in responsiveness, we excluded adult offspring samples from gravid females in consideration of GC levels increment through gestation.

We expect no systematic biases from variation in Genetic make-up because all subjects came from the same well-mixed open population and both sexes were represented and highlight that inter-individual differences in environmental conditions experienced during development make Experience the explicit topic of this study.

Fecal sample preparation and GC metabolite analyses

We collected fecal samples immediately after defecation from the ground or vegetation. After manual homogenizing, roughly 1g of fresh material was transferred into a 15ml vial with 5ml 80% watery ethanol. Upon return to the field camp, we extracted samples as described by Berghänel et al. (2016) using a validated field extraction method (Shutt et al., 2012). We pipetted 2ml of supernatant into a polypropylene cup and extracts were subsequently stored frozen at -20°C until export to the endocrinology lab at the German Primate Center for the analyses of fecal glucocorticoid metabolites. We analyzed fecal extracts for immunoreactive 11ß-hydroxyetiocholanolone (GC), a major metabolite of cortisol in primate feces (e.g. Heistermann et al., 2006), using an enzyme immunoassay. The assay has been validated for assessing adrenocortical activity in numerous primate species (e.g. Heistermann et al., 2006; Shutt et al., 2012), including the study species (Ostner et al., 2008; Fürtbauer et al., 2014). Extracts were diluted 1: 200 – 1: 4000 with assay buffer before assays, which were then carried out according to the method described in detail by Heistermann and colleagues (2004). The sensitivity of the assay was 12pg/ml. Intra- and inter-assay coefficients of variation of high- and low-value quality

controls were <10% and <15%, respectively. We removed one offspring fecal sample before the estimation of the offspring GC mean, range, maximum and minimum values due to unrealistic concentration of fecal glucocorticoid metabolites detected (1654.94 ng/g). For the extended analysis of maternal effects by age class (Figure 1, Appendix 1 – Table 2), we included 3 adult offspring with maternal GC samples that had been stored differently than all other samples. Instead of extracting the fresh sample into ethanol the same day and storing the extract, fresh samples were frozen at -20°C until export and successive extraction in the hormone lab (Fürtbauer et al., 2014). GC concentrations generated with the two methods were mean-centered and standardized (z-transformation) separately. Before entering the extended analysis for the adult age class, we validated data poolability by comparing the two method in a regression against the offspring GC value ($F = 1.79$, $df_1 = 2$, $df_2 = 241$, p-value = 0.169).

Statistical analyses

We ran all statistical analyses using the software RStudio 1.3.1093 (RStudio Team, 2020) and the packages *glmmTMB* (version 1.0.2.1; Brooks et al., 2017) for general linear models, *nlme* (version 3.1-151; Pinheiro et al., 2022) for generalized least square models, and *gap* (version 1.2.3-1; Zhao 2021; function *chow.test*) for data poolability. We performed all GLM models and GLS models with no customization of the optimizers by using the default option provided within the respective command function. No additional arguments were passed to the default optimizer function. We ensured all test assumptions were fulfilled using the package *performance* (version 0.7.0: Lüdecke et al., 2021). The package for model diagnostics computes variance inflation factors (VIFs) and allows for visual inspection of scatterplots, residual plots, Q-Q plots to check for normality, linearity, homogeneity of variance, model singularity and overdispersion.

To assess the independent effects of early versus late prenatal as well as postnatal maternal GCs we built four GLMs with (i) mean, (ii) range, (iii) maximum, and (iv) minimum offspring GCs as the response calculated from 9.5±3.5 (mean±SD) samples per offspring. We included as model predictors the mean concentration of maternal fecal GCs during the early gestation, during the late gestation, and in the postnatal phase. Each of these predictors was included in interaction with offspring *age* class (infant, juvenile, adult). Finally, we included offspring *sex* as a categorical predictor.

We included models of the maximum and minimum GC value detected in each offspring to decompose the effect of maternal stress on the offspring range value. The range response variable was estimated by subtracting the minimum from the maximum value, and the number of offspring fecal samples was included as a control variable in the range, maximum, and minimum models. All variables expressing GC concentration (i.e., *early-preGC, late-preGC, postGC, mean, range, maximum,* and *minimum* of

offspringGC) were $\log_n$-transformed to achieve a more symmetrical distribution and meet test assumptions. We performed full-null model comparisons to test whether the inclusion of predictors improved model fit. Null model 1 included only the intercept, while null models 2-4 included as a control variable the number of samples collected and used to estimate range, maximum and minimum value of offspring GCs. The detection of non-significant two-way interactions led to the reduction of models to include only main effect predictors. Note that after excluding two non-significant interaction terms from the maximum offspring GC model, a third interaction term was no longer significant and excluded in a second step. We excluded all samples of gestating adult offspring because GCs are known to increase towards the end of gestation (Fürtbauer et al., 2014; Touitou et al., 2021).

Predictors *early-* and *late-preGC* were not correlated (N = 32, Pearson's r = 0.29, p = 0.11), whereas *early-preGC* and *postGC* were moderately (N = 32, r = 0.61, p = 0.001) and *late-preGC* and *postGC* were weakly correlated (N = 32, r = 0.39, p = 0.015). This collinearity did not affect the interpretation of model results as independent effects though, as indicated by all VIFs being no larger than 2.05 (Queen et al., 2002). Variation in maternal GC levels during different reproductive phases is the result of stable inter-individual differences (e.g., resulting from own early adversity) and differential exposure to stressors. Regardless of age class, we sampled all offspring in this study under the same ecological conditions emphasizing stable inter-individual differences in response to the same stressors. Instead, mothers were exposed to different conditions and stressors varying between and within years so that adversity experienced during different reproductive phases also varied between mothers; some mothers may have experienced little change in food abundance from early through late gestation into lactation, whereas other mothers may have suffered from food scarcity early in gestation with conditions increasing during late gestation and being favorable during lactation so that their GC changed accordingly.

To corroborate the finding that the prenatal adversity effects did not differ between the age classes we ran 3 models and analyzed each age class separately: infants, juveniles, and adults. We fit the age-specific linear models using generalized least squares (GLS) which allow the errors to be correlated. We included as predictors (i) the mean value of overall prenatal maternal GC, (ii) the postnatal maternal GC, (iii) sex of the offspring, and (iv) the time of sample collection as a continuous variable to control for possible diurnal effects in offspring fecal GC metabolite levels. We analyzed the residual autocorrelation and partial autocorrelation function from the GLS models and identified an autoregressive process of order 1 as the correlation structure class for all the GLS models. Thus, we fit the GLS models specifying the date of collection as time covariate within the grouping factor of subject identity, and therefore applied the correlation structure only to observations within the same subject. Finally, we ran each age-specific model with age-specific autocorrelation structure (ρ_{inf} = 0.31; ρ_{juv} = 0.24; ρ_{juv} = 0.35) and with the offspring GC level in a single sample as a single data point in the response variable (Figure 1,

Appendix 1 – Table 2). To test whether early adversity affected reproductive performance, we ran partial Pearson's correlations of overall mean prenatal GCs of 13 adult females and their annual rate of giving birth to surviving offspring corrected for the GC levels these mothers exhibited in our field season in Statistica 13.5.0.17 (TIBCO Inc. 2018). Simple bi-variate correlations between mean prenatal GCs and surviving birth rate ($N = 13$, $r = 0.57$, $p = 0.042$) or more specifically only early preGC and surviving birth rate ($N = 10$, $r = 0.82$, $p = 0.004$) yielded very similar results.

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Data availability

The data have been deposited on GRO.data: https://doi.org/10.25625/Y03YD4

Competing Interests

The authors declare they do not have any financial or non-financial competing interests.

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Source data file list

Figure 1 – Source Data 1 contains the data used to generate Figure 1B and 1C

Figure 2 – Source Data 1 contains the data used to generate Figure 2

Figure 3 – Source Data 1 contains the data used to generate Figure 3

Source Code 1 contains the R script used to estimate the effect of prenatal maternal glucocorticoids on offspring's HPA axis activity reported in tables showed in Appendix 1 from the data uploaded on the online repository https://doi.org/10.25625/Y03YD4

Appendix 1

Predictor	β	SE	CI 2.5-97.5%	Z	LRT	P value	β	SE	CI 2.5-97.5%	Z	LRT	P value
Mean offspring GC (pseudo R^2 = 0.68)							**Reduced model Mean offspring GC (pseudo R^2 = 0.65)**					
(Intercept)	1.19	1.90	-2.53 – 4.91	-	-	-	2.76	1.03	0.73 – 4.78	2.67	-	-
[1]Early-preGC	0.33	0.21	-0.08 – 0.73	1.59	-	-	0.45	0.15	0.16 – 0.73	3.05	8.157	**0.004**
[1]Late-preGC	0.35	0.22	-0.07 – 0.78	1.62	-	-	0.28	0.13	0.03 – 0.53	2.22	4.565	**0.033**
[1]postGC	0.14	0.47	-0.78 – 1.06	0.29	-	-	-0.22	0.27	-0.76 – 0.31	-0.82	0.660	0.416
[2]Age [adu]	-0.14	3.38	-6.76 – 6.48	-0.04	-	-	-0.30	0.13	-0.56 – -0.05	-2.37	28.239	<**0.001**
[2]Age [juv]	0.44	2.77	-4.99 – 5.88	0.16	-	-	-0.72	0.11	-0.92 – -0.51	-6.72		
[3]Sex [M]	0.08	0.09	-0.10 – 0.26	0.88	0.764	0.382	0.10	0.09	-0.08 – 0.28	1.14	1.279	0.258
[1]Early-preGC * age.cat [adu] [2]	1.03	0.81	-0.56 – 2.62	1.27	1.586	0.452						
[1]Early-preGC * age.cat [juv] [2]	0.09	0.35	-0.59 – 0.77	0.26								
[1]Late-preGC * age.cat [adu] [2]	0.22	0.42	-0.60 – 1.05	0.54	0.829	0.661						
[1]Late-preGC * age.cat [juv] [2]	-0.14	0.30	-0.74 – 0.45	-0.47								
[1]*postGC* * age.cat [adu] [2]	-1.24	0.78	-2.77 – 0.29	-1.59	2.605	0.272						
[1]*postGC* * age.cat [juv] [2]	-0.17	0.68	-1.51 – 1.16	-0.26								
Range offspring GC (pseudo R^2 = 0.57)							**Reduced model Range offspring GC (pseudo R^2 = 0.48)**					
(Intercept)	2.79	3.14	-3.36 – 8.94	0.89	-	-	2.23	1.86	-1.41 – 5.87	1.20	-	-
[1]Early-preGC	0.56	0.34	-0.11 – 1.24	1.63	-	-	0.80	0.26	0.30 – 1.30	3.14	8.607	**0.003**
[1]Late-preGC	0.20	0.36	-0.51 – 0.91	0.54	-	-	0.37	0.23	-0.08 – 0.81	1.62	2.527	0.112
[1]postGC	-0.29	0.78	-1.82 – 1.24	-0.37	-	-	-0.56	0.49	-1.51 – 0.39	-1.15	1.295	0.255
[2]Age [adu]	-5.18	5.64	-16.24 – 5.88	-0.92	-	-	-0.40	0.31	-1.00 – 0.21	-1.28	13.347	<**0.001**
[2]Age [juv]	-5.70	4.71	-14.93 – 3.53	-1.21	-	-	-0.75	0.19	-1.11 – -0.39	-4.05		
[3]Sex [M]	-0.04	0.16	-0.35 – 0.27	-0.28	0.076	0.781	-0.03	0.17	-0.36 – 0.30	-0.20	0.039	0.843
[1]Early-preGC * age.cat [adu] [2]	2.91	1.53	-0.08 – 5.91	1.91	3.448	0.178						
[1]Early-preGC * age.cat [juv] [2]	0.21	0.57	-0.91 – 1.33	0.36								
[1]Late-preGC * age.cat [adu] [2]	0.42	0.71	-0.97 – 1.81	0.59	0.355	0.837						
[1]Late-preGC * age.cat [juv] [2]	0.07	0.50	-0.91 – 1.05	0.15								
[1]*postGC* * age.cat [adu] [2]	-2.30	1.48	-5.20 – 0.61	-1.55	4.349	0.114						
[1]*postGC* * age.cat [juv] [2]	0.69	1.16	-1.58 – 2.97	0.60								
Number of samples	0.06	0.02	0.02 – 0.10	2.85	7.243	**0.007**	0.04	0.02	0.00 – 0.08	2.18	4.425	**0.035**
Maximum offspring GC (pseudo R^2 = 0.73) (*)							**Reduced Model Max. Offspring GC (pseudo R^2 = 0.62) (*)**					
(Intercept)	1.60	1.82	-1.96 – 5.17	0.88	-	-	1.89	1.16	-0.38 – 4.16	1.63	-	-
[1]Early-preGC	0.39	0.20	-0.00 – 0.78	1.95	-	-	0.53	0.16	0.22 – 0.85	3.36	9.686	**0.002**
[1]Late-preGC	0.24	0.21	-0.17 – 0.65	1.16	-	-	0.30	0.14	0.03 – 0.58	2.14	4.284	**0.038**
[1]postGC	0.19	0.45	-0.70 – 1.08	0.41	-	-	-0.05	0.30	-0.64 – 0.55	-0.16	0.025	0.875
[2]Age [adu]	-2.11	3.27	-8.52 – 4.30	-0.65	-	-	-0.21	0.19	-0.59 – 0.16	-1.11	23.224	<**0.001**
[2]Age [juv]	-3.78	2.73	-9.12 – 1.57	-1.38	-	-	-0.67	0.12	-0.90 – -0.45	-5.84		
[3]Sex [M]	-0.09	0.09	-0.26 – 0.09	-0.94	0.864	0.353	-0.07	0.10	-0.27 – 0.14	-0.64	0.412	0.521
[1]Early-preGC * age.cat [adu] [2]	2.09	0.89	0.36 – 3.83	2.36	5.309	0.070						
[1]Early-preGC * age.cat [juv] [2]	-0.03	0.33	-0.68 – 0.62	-0.09								
[1]Late-preGC * age.cat [adu] [2]	0.28	0.41	-0.52 – 1.09	0.69	0.744	0.591						
[1]Late-preGC * age.cat [juv] [2]	-0.01	0.29	-0.58 – 0.56	-0.03								
[1]*postGC* * age.cat [adu] [2]	-1.94	0.86	-3.62 – -0.25	-2.26	8.976	**0.011**						
[1]*postGC* * age.cat [juv] [2]	0.65	0.67	-0.67 – 1.97	0.96								
Number of samples	0.03	0.01	0.00 – 0.05	2.20	4.514	**0.034**	0.01	0.01	-0.01 – 0.03	1.06	1.101	0.294
Minimum offspring GC (pseudo R^2 = 0.70)							**Reduced model Min. Offspring GC (pseudo R^2 = 0.63)**					
(Intercept)	-3.27	2.28	-7.73 – 1.19	-1.44	-	-	-0.18	1.35	-2.83 – 2.46	-0.14	-	-
[1]Early-preGC	0.12	0.25	-0.61 – 0.37	-0.47	-	-	-0.09	0.19	-0.45 – 0.28	-0.48	0.225	0.635
[1]Late-preGC	0.43	0.26	-0.09 – 0.94	1.62	-	-	0.20	0.16	-0.12 – 0.52	1.21	1.428	0.232
[1]postGC	1.29	0.57	0.18 – 2.40	2.27	-	-	0.90	0.35	0.20 – 1.59	2.53	5.851	**0.016**
[2]Age [adu]	4.19	4.10	-3.84 – 12.22	1.02	-	-	-0.19	0.22	-0.63 – 0.25	-0.85	27.596	<**0.001**
[2]Age [juv]	1.74	3.42	-4.96 – 8.44	0.51	-	-	-0.89	0.13	-1.15 – -0.62	-6.60		
[3]Sex [M]	0.16	0.11	-0.07 – 0.38	1.36	1.811	0.178	0.18	0.12	-0.06 – 0.42	1.44	2.011	0.156
[1]Early-preGC * age.cat [adu] [2]	0.61	1.11	-1.57 – 2.78	0.55	0.762	0.683						
[1]Early-preGC * age.cat [juv] [2]	-0.25	0.41	-1.06 – 0.56	-0.60								
[1]Late-preGC * age.cat [adu] [2]	0.23	0.51	-0.78 – 1.24	0.45	2.019	0.364						
[1]Late-preGC * age.cat [juv] [2]	-0.39	0.36	-1.10 – 0.32	-1.07								
[1]*postGC* * age.cat [adu] [2]	-1.69	1.08	-3.79 – 0.42	-1.57	3.300	0.192						
[1]*postGC* * age.cat [juv] [2]	0.13	0.84	-1.53 – 1.78	0.15								
Number of samples	-0.03	0.01	-0.06 – -0.01	-2.40	5.285	**0.022**	-0.04	0.01	-0.07 – -0.02	-3.26	9.187	**0.002**

Table 1 Models and Reduced models explaining: (1) the mean concentration of offspring GC values, (2) the range of offspring GC, (3) the maximum and (4) the minimum offspring GC concentration. Control variables are in italics and significant *P* values of explanatory variables are in bold. Because of non-significance of the two-way interaction terms, analyses were repeated with a reduced model including only the main effects. All models and reduced models were tested via full-null model comparison: *Model 1* $\chi2$ = 36.36, df = 12, P < 0.001); *Reduced model 1* $\chi2$ = 33.42, df = 6, P < 0.001; *Model 2* $\chi2$ = 27.24, df = 12, P = 0.007; *Reduced model 2* $\chi2$ = 20.725, df = 6, P = 0.002; *Model 3* $\chi2$ = 41.99, df = 12, P < 0.001; *Reduced model 3* $\chi2$ = 30.851, df = 6, P < 0.001; *Model 4* $\chi2$ = 38.38, df = 12, P < 0.001; *Reduced model 4* $\chi2$ = 31.86, df = 6, P < 0.001. [(1)] Log_n-transformed; [(2)] Infant coded as reference category; [(3)] Female coded as reference category; [(*)] After excluding the non-significant interactions of *age category* with *early-preGC* and *late-preGC*, the interaction term *postGC * age category* was not significant, so it was also excluded from the reduced model.

Predictor	Estimate	SE	CI 2.5-97.5%	Z	LRT	P value
Infants (pseudo R^2 = 0.13)						
(Intercept)	150.38	38.26	74.84 – 225.92	3.93	-	-
[1]Overall preGC	35.62	12.17	11.58 – 59.66	2.93	8.25	**0.004**
[1]PostGC	3.44	12.58	-21.40 – 28.28	0.27	0.08	0.782
[2]Sex [M]	4.52	19.70	-34.38 – 43.42	0.23	0.05	0.816
[3]*Time of the day*	6.55	3.12	0.39 – 12.71	2.10	4.32	0.038
Juveniles (pseudo R^2 = 0.11)						
(Intercept)	166.43	36.58	93.96 – 238.91	4.55	-	-
[1]Overall preGC	24.81	10.77	3.47 – 46.16	2.30	5.36	**0.021**
[1]PostGC	7.66	10.77	-13.67 – 28.99	0.71	0.53	0.468
[2]Sex [M]	26.25	19.89	-13.16 – 65.66	1.32	1.79	0.181
[3]*Time of the day*	-3.92	2.86	-9.58 – 1.75	-1.37	1.86	0.173
Adults (pseudo R^2 = 0.10)						
(Intercept)	168.68	37.38	94.63 – 242.74	4.51	-	-
[1]Overall preGC	28.18	13.68	1.07 – 55.29	2.06	4.33	**0.037**
[1]PostGC	11.06	16.13	-20.90 – 43.02	0.69	0.49	0.484
[2]Sex [M]	24.26	20.68	-16.70 – 65.22	1.17	1.41	0.235
[3]*Time of the day*	-3.91	2.87	-9.60 – 1.77	-1.36	1.84	0.174

Table 2 GLS models explaining the GC value detected in a single sample belonging to infants (n = 18), juveniles (n = 11), and adults (n = 14). Control variables are in italics and significant *P* values of explanatory variables are in bold. (1) Z-transformed with mean = 0 and standard deviation = 1; (2) Female coded as reference category; (3) Time of the day estimated as number of hours after midnight (0-24).

Contrast	Estimate	SE	df	Statistics	P-value
Early-prenatal maternal GC					
infants-adults	-1.03	0.81	18	-1.27	0.429
infants-juveniles	-0.09	0.35	18	-0.26	0.964
adult-juveniles	0.94	0.84	18	1.12	0.514
Late-prenatal maternal GC					
infants-adults	-0.23	0.42	18	-0.54	0.843
infants-juveniles	0.14	0.30	18	0.47	0.888
adult-juveniles	0.37	0.40	18	0.90	0.648
Postnatal maternal GC					
infants-adults	1.24	0.78	18	1.59	0.275
infants-juveniles	0.18	0.68	18	0.26	0.965
adult-juveniles	-1.07	0.80	18	-1.33	0.396

Table 3 Post-hoc comparison of slopes for different age classes estimated for the effect of prenatal and postnatal maternal GC on the mean of offspring GC value.

Chapter 3 - Gut microbiome

The long-term gut bacterial signature of a wild primate is associated with a timing-effect of pre- and postnatal maternal glucocorticoid levels

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Abstract

Background: During development, elevated levels of maternal glucocorticoids (GCs) can cause detrimental effects on offspring morphology, cognition, and behavior as well as physiology and metabolism. Depending on the timing of exposure, such effects can vary in strength or even reverse in direction, they may alleviate with age or concern more stable and long-term programming of phenotypic traits. Maternal effects on gut bacterial diversity and composition, and the persistence of such effects into adulthood of long-lived model species in the natural habitats remain underexplored.

Results: In a cross-sectional sample of infant, juvenile, and adult Assamese macaques, the timing of exposure to elevated maternal GCs during ontogeny was associated with the gut bacterial community of the offspring. Specifically, naturally varying maternal GC levels during early but not late gestation or lactation were associated with reduced bacterial richness. The general effect of maternal GCs during early gestation on the gut bacterial composition exacerbated with offspring age and was 10 times stronger when compared with the effect associated with exposure during late prenatal or postnatal periods. Instead, variation in maternal GCs during the late prenatal or postnatal periods had less pronounced or less stable statistical effects and therefore a weaker effect on the entire bacterial community composition, particularly of adult individuals. Finally, higher early prenatal GCs increased the relative abundance of several potential pro-inflammatory bacteria and decreased the abundance of *Bifidobacterium* and other anti-inflammatory taxa, an effect that exacerbated with age.

Conclusions: In primates, the gut microbiota can be shaped by developmental effects with strong timing effects on plasticity and potentially detrimental consequences for adult health. Here, maternal GC levels were associated with dysbiotic states that may hamper energy extraction and storage particularly during the rich season, when adult female offspring prepare for upcoming reproductive events. Together with results on other macaque species, this study suggests potential detrimental developmental effects similar to rapid inflammaging which suggests prenatal stress is a common cause underlying both phenomena. Our results await corroboration in functional and causal analyses, and longitudinal studies of long-lived ecologically flexible primates in their natural habitat which ideally will consider also developmental effects originating before birth.

Keywords: Development, prenatal stress, dysbiosis, macaques, primates, programming, health, long-term, bacteria, 16S rRNA gene

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Background

Gestation is a developmental phase highly sensitive to environmental exposures; from conception onwards, adverse conditions and the consequent prenatal maternal stress response can affect offspring developmental trajectories depending on the type and degree of the adversity, the timing of such challenges, and offspring sex [1]. Among mammals, maternal stress and increased activity of the hypothalamic-pituitary-adrenal axis (HPA) are associated with increased glucocorticoid (GC) production which can permeate the placenta and reach the developing embryo/fetus during gestation. Prenatal maternal GCs are usually associated with long-lasting effects on morphological, cognitive, and physiological traits of the offspring [2,3]. Specifically, phenotypic functional alterations of physiological and behavioral traits associated with increased exposure to GCs during gestation involve the HPA axis of the offspring and its immune system, but also altered gene expression, behavior, and brain functionality [1,4,5]. The brain communicates constantly with the gastrointestinal tract and vice versa so that alterations in brain functionality can translate into changes in gut functionality, and vice versa [6–11]. An altered gut-brain system can be associated with detrimental variation of physiological traits such as impaired response to stressors and gut inflammation, and altered behavior [6–8,12–18]. In the last decade, the intricacy of the connections between brain, gut, and immune system mediated by hundreds of microbial species inhabiting the gut are beginning to be understood, and the study of health and the gut-brain axis is being broadened by adopting a more holistic approach incorporating the microbiota-gut-brain synergic system [19]. Yet, little is known about the programming effects of maternal stress on the gut microbiota, the possible mediating role of offspring GC levels, and the potential permanence of dysbiotic states into adulthood in long-lived mammals.

Starting from birth, the gut microbiota plays a fundamental role in regulating host physiology and behavior: it modulates metabolic and immune responses and affects brain development and adult behavior. Studies on the microbiota-gut-brain axis in germ-free animals show that bacteria-deprived individuals differ considerably in the development of physiological systems typical of individuals hosting microbial organisms such as impaired immune system, increased HPA axis activity, and altered metabolism [19], and emphasized the fundamental role of the gut microbiota in modulating the HPA axis activity and the stress response. One example is the modulation of the exaggerated HPA stress response in germ-free mice reduced by *Bifidobacterium infantis* colonization or increased by mono-association with enteropathogenic *Escherichia coli* [20]. Another example again from germ-free mice showed that *Enterococcus faecalis* reduced corticosterone levels, and thus the physiological stress response, following social stress [21]. However, studies on germ-free mice can overestimate stress-related

physiological outcomes associated with variation in abundance of single bacterial species since isolation and lack of host-environment interactions may conceal more complex microbial community dynamics. Thus, studies on animal models interacting with the environment may help unveil hidden microbial dynamics and provide a deeper understanding of the link between stress-related physiology and the gut microbiota.

Stress can alter the secretions and permeability of the gastrointestinal tract, impact regenerative processes of the mucosa, and affect gastrointestinal motility [22]. Bailey and Coe [23] investigated variation in rhesus macaques' gut bacteria associated with maternal separation and showed that the stress from separation at 6-9 months of age caused a decrease in several taxa, especially lactobacilli. The decrease was associated with stress-related behavior and with opportunistic bacterial infection and therefore vulnerability to disease [23]. Indeed, the release of catecholamines produced during the stress-response can stimulate the proliferation of gram-negative organisms [12] with direct or indirect consequent variation in the abundances of taxa like *Clostridium, Bacteroides, and Lactobacillus* [23]. A recent investigation on the effect of stress caused by habituation in gorillas revealed the association of fecal glucocorticoid metabolites with increased abundance of the genus *Oscillobacter*, *Clostridium* cluster XIV, and the family *Anaerolineaceae* [24]. However, despite changes in a few specific taxa, the authors found no significant association between fecal glucocorticoid metabolites and bacterial alpha diversity and concluded that stress from habituation had only minor effects on the overall gut microbial composition [24].

Higher diversity and richness in microbial community can provide higher resilience to perturbations and resistance to pathogens, can improve stability in the microbiota-gut-brain axis, and ultimately can promote health. In humans, gut microbial richness correlates with metabolic markers, and subjects with lower richness tend to show a pro-inflammatory phenotype, dyslipidemia, and insulin resistance[25]. An impoverishment in gut microbial alpha diversity can be associated with detrimental health states and altered behavior in human and non-human primates. For example, in mouse lemurs, rhesus macaques, chimpanzees, and humans, lower microbial richness is associated with infection by adenovirus, SIV, HIV, or enterocolitis [26–29]. A lower richness is also associated with depressive-like behaviors, stress, and anxiety-like behavior in humans and other primates [8,30,31], with Alzheimer's disease in humans [32], and with infections of helminths in yellow baboons and red colobus [33]. Interestingly, a study on the gut microbiota in humans and rats showed that fecal microbiota transplantation from depressed patients (and with lower alpha-diversity) to microbiota-depleted rats induced alterations in tryptophan metabolism, depression-like, and anxiety-like behaviors in the recipient animals [32]. Although there is a growing body of literature showing evidence of the negative relationship between health and microbial diversity and

richness there are also several studies showing no association and the debate on the use of diversity and richness as informative health markers is ongoing [24,34,35].

The microbiota-gut-brain system is very sensitive to stress, however, the relations between specific microbial taxa and host physiology are complex and usually vary across host species, groups, and individuals. Yet, some generalizations on the effect of specific taxa are possible. The phyla *Firmicutes* and *Bacteroidota* (formerly *Bacteroidetes*), represent two of the most abundant gut taxa in several mammalian species including humans, their prevalence is affected by environmental and genetic factors. Potentially, variation in the *Firmicutes*/*Bacteroidota* ratio can lead to dysbiotic states and metabolic dysfunctions in humans and other primates as well [36–38]. This hypothesis is supported by rodent models, and studies on the effect of early-life stress on adults' microbiota revealed a reduced ratio of *Firmicutes*/*Bacteroidota* in adult females and an increased abundance of organisms associated with inflammation like *Prevotella*, *Akkermansia*, and *Flexibacter* [39].

Among mammals, prenatal stress can strongly affect developmental trajectories of the synergic microbiota-gut-brain system and thus impact the adults' health state [1,3,40,41]. This was also observed for groups of wild Assamese macaques (*Macaca assamensis*) studied here, prenatal maternal stress during early but not late gestation or during lactation is associated with hyperactivation of the HPA axis activity in the offspring which persists into adulthood (see Chapter 2). Indeed, the effects of prenatal maternal adversity and fetal exposure to GC on offspring phenotypes can have different, sometimes opposing directions depending on the timing of the exposure — whether the exposure increases during the early or late gestation or after birth can be critical to the formation of specific developmental trajectories and subsequent phenotypes [1,42–44], with potential consequences on reproduction, health, and fitness [45,46]. Studies on the effect of maternal stress on offspring gut microbiota are still limited and target a few host species, generally rodent models, in a controlled environment or captivity [4,47–49]. To date, we know of only one study that tested the timing-effect of prenatal stress on offspring gut microbiota in a long-lived animal, this study on rhesus macaques was done in a controlled environment, using artificially induced stress and investigated effects in infants only [50]. Little is known about the ecological validity of such models, and there is even less information on the potential permanence of maternal effects on the gut microbiota of adults of long-lived animal species.

With this study, we aim to provide data to fill this knowledge gap by investigating the link between the variation in maternal GCs driven by naturally occurring stressors, measured during different sensitive periods of gestation

and lactation, and the gut microbial diversity and composition of the offspring in wild Assamese macaques. In a cross-sectional design during one field season (2018-2019), fecal samples were collected per individual and season from infants (< 1 year), juveniles (4-5 years), and adults (6-10 years) for the concurrent analysis of GC levels and gut bacterial community composition. Information on maternal GC levels during early and late gestation as well as during lactation were available from previous studies on the same population. We predicted increased prenatal and postnatal maternal GCs to be negatively associated with measures of microbial alpha diversity, the *Firmicutes*/*Bacteroidota* ratio, and with dysbiotic states evident from changes in the relative abundance of specific taxa. We further investigated whether these effects may be mediated by increased offspring GCs in developmentally challenged offspring by including offspring GC levels as a variable in all statistical models.

Methods

Study population and data collection

From 2010 to 2019 we collected fecal samples from a wild population of Assamese macaques living in their natural habitat. The study location is Phu Khieo Wildlife Sanctuary, a large, protected area that is part of a >6,500 km² system of connected protected forests in northeast Thailand. The study site (Huai Mai Sot Yai, 16°27′ N, 101°38′ E) is characterized by hilly terrain with a dry evergreen forest. The habitat exhibits two distinct seasons: a hot and rainy season (rich: March-October), and a colder dry season (lean: November-February) [51–53]. Although fruit and food availability are higher during the rich season, resource availability fluctuates between years, and forest productivity is considered rather unpredictable [51–54].

The study population is mainly frugivorous, and the main part of the plant diet comprises fruit, pulp, and seeds. Individuals spend an important part of their feeding time slowly foraging for animal matter like insects, spiders, gastropods, small amphibians, and reptiles [51–53]. Assamese macaques are relaxed-income breeders reproducing seasonally with 78% of births occurring between April and June (Schülke and Ostner unpublished data). Female reproduction depends on forest productivity and the probability of conception is positively associated with food availability and female condition [52]. The average length of gestation is estimated at 164 days [55]. Although infant suckling is observed through the first year of life, it is higher during the first 6 months and later it quickly decreases to zero [54]. Thus, we measured maternal postnatal GCs only for the first 6 months of life. All infant

fecal samples used for DNA extraction and GC analysis were collected later, thus postnatal maternal GCs and infant offspring GCs were assessed at different times. Between 2010 and 2019, fecal samples from 30 mothers were collected during their gestation (n = 379, mean ± SD: 11.9 ± 5.7 per individual; preGC) and lactation (n = 564, 17.6 ± 6.2 per individual; postGC). Between June 2018 and July 2019, we collected fecal samples from the corresponding 30 offspring of different ages that lived in three different social groups: 231 (mean ± SD: 7.7±2.3 per individual) samples were used to extract the information on GCs, and 217 (mean ± SD: 7.3±1.9 per individual) were used to extract the information on gut bacterial communities. We measured fecal glucocorticoid metabolites and extracted information on the gut bacteria from offspring of the 2018 birth cohort (11 males and 5 females), juveniles born between 2014 and 2016 (4 males and 3 females), and individuals born before 2012 (adults, 2 males 5 females). Since the average length of gestation is 164 days, we divided gestation at day 82 into early gestation (gestation days 1-82) and late gestation (days 83-day of birth) and calculated specific means of GC concentration separately. We used two aliquots of the fecal samples from offspring individuals to extract the information on GC metabolites and gut microbial community separately (see below).

Fecal sample collection and glucocorticoid metabolite (GC) analysis

Fecal samples of mothers and offspring were collected directly after defecation from the ground or vegetation, and without urine contamination. The fecal material was homogenized and about 1g of material was transferred into a 15 ml vial with 5 ml 80% ethanol. The samples were extracted at the field camp using a validated method [56] as described by Berghänel et al. [54]. We pipetted 2 ml of the resulting fecal extract containing the GCs into a polypropylene cup and successively stored all extracts at -20°C until transportation to the endocrinology lab of the German Primate Center for GC analysis. The extracts were analyzed using an enzyme immunoassay for the measurement of immunoreactive 11ß-hydroxyetiocholanolone, a major metabolite of cortisol in primate feces [57]. The assay has been validated for assessing adrenocortical activity in numerous primate species [57], including Assamese macaques [58,59]. Extracts were diluted 1:200 – 1:4,000 with assay buffer before assays which were then carried out according to the method described in detail in Heistermann et al. [60]. Assay sensitivity was 12 pg/ml, and intra- and inter-assay coefficients of variation of high- and low-value quality controls were <10% and <15%, respectively.

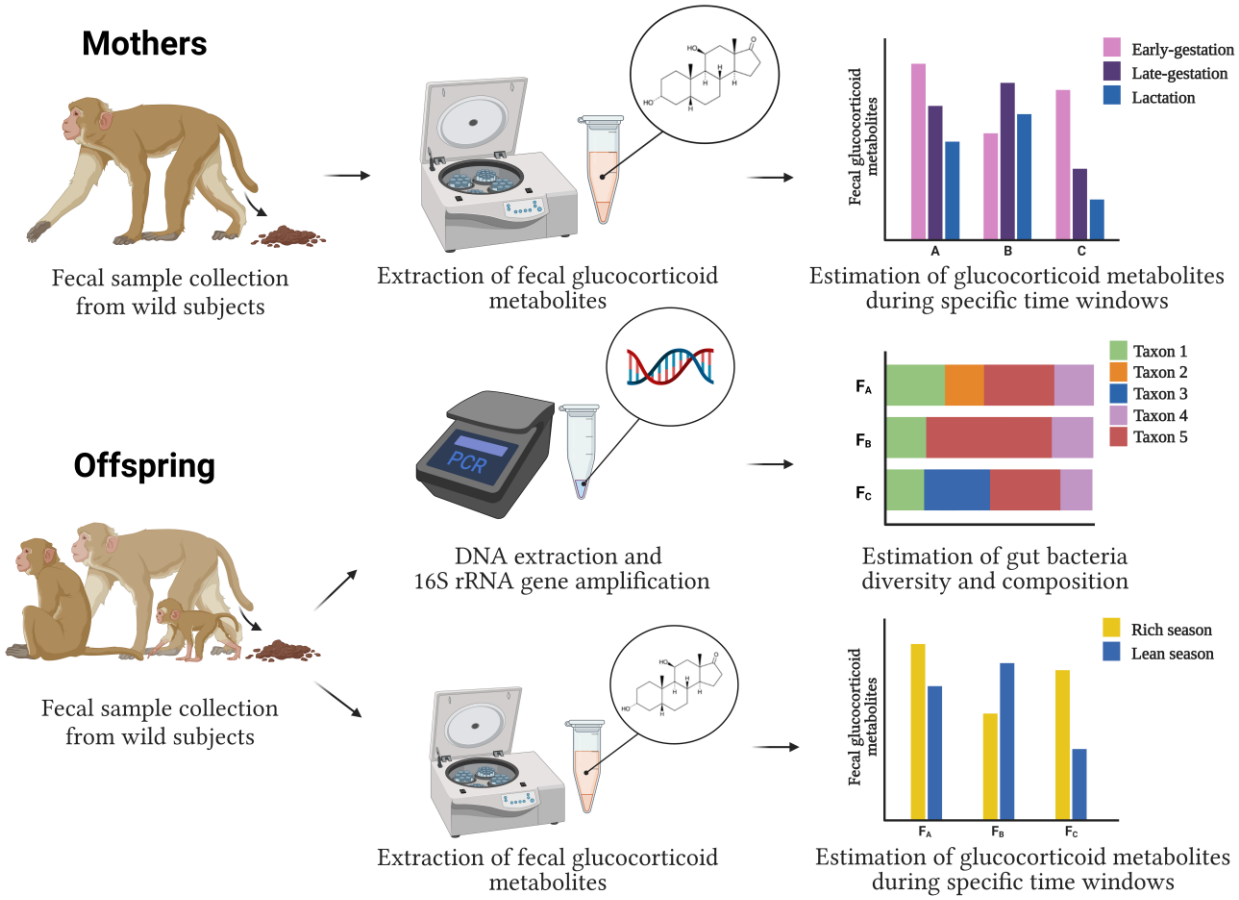


Figure 1 Methodological approach performed to investigate the effect of maternal GCs on offspring gut bacterial community. $F_{a\text{-}c}$ refers to offspring descendent from mothers A-C of wild Assamese macaques. Output graphs are icons only and do not represent actual data. Made with Biorender.com

Fecal sample collection, DNA extraction, amplification of 16S rRNA genes, and sequencing

We collected fecal samples of offspring directly after defecation (Figure 1). We homogenized the samples by kneading them with gloved hands and put them in sterile 2 ml screw-top polypropylene cups filled with 1 ml RNAlater buffer solution, shook the samples, and then stored them for 24 hours in the dark at room temperature. Subsequently, the samples were frozen at -20°C until export to the Laboratory at the University of Göttingen. Once in the lab, the samples were stored at -80°C.

Samples stored at -80°C were thawed on ice for 30 minutes. RNAlater was removed by centrifuging samples for 10 min at 13,000 rpm on a Thermo Electron Corp Heraeus Pico 21 (ThermoFisher Scientific). DNA was extracted from 150 mg of fecal matter with DNeasy PowerSoil Pro Kit (QIAGEN, Cat. No. / ID: 47016) following manufacturer instructions. DNA quantity and quality were assessed by spectrophotometry on a NanoDrop ND-

1000 Spectrophotometer (Thermo Fisher Scientific). Samples yielding a DNA concentration < 6 ng/µl were discarded and DNA extraction was repeated. We standardized DNA concentration per sample by dilution to 10 ng/µl. Using PCR primers as described by Klindworth and colleagues [61] we amplified the V3-V4 region of the 16S rRNA gene. Primers included adapters for MiSeq sequencing (underlined, forward primer: S-D-Bact-0341-b-S-17 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-CCTACGGGNGGCWGCAG-3′, reverse primer: S-D-Bact-0785-a-A-21 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-GACTACHVGGGTATCTAATCC-3′). PCRs were performed in triplicates with thermocycling protocols listed in Supplementary material (SI Amplification of 16S rRNA genes and sequencing: full procedure). Sequencing was conducted by the Göttingen Genomics Laboratory using Illumina MiSeq platform using dual indexing and MiSeq reagent kit v3 (600 cycles) as recommended by the manufacturer (SI Amplification of 16S rRNA genes and sequencing: full procedure).

16S rRNA gene sequence data deposition

The raw sequence data from the 16S rRNA gene amplicons were deposited at the National Center for Biotechnology Information and can be assessed under the BioProject accession number PRJNA795139.

Bioinformatic processing of amplicon data

Raw paired-end sequences were quality-filtered using fastp v0.20.0 [62] with a minimum phred score of 20, minimum sequence length of 50 bp and sliding window size of 4, read correction by overlap, and adapter removal of sequencing primers. Quality-filtered reads were merged with PEAR v0.9.11 [63], and 16S rRNA gene primers were trimmed with cutadapt v2.5 [64]. VSEARCH v2.15.0 [65] was used to sort and filter the sequences by size (allowed minimum length ≥ 300 bp), remove duplicates (--derep_fulllength), and denoise (--cluster_unoise, default settings). We further performed de novo chimera removal (--uchime3_denovo) followed by reference-based chimera removal (--uchime_ref) against the SILVA SSU 138.1 NR database [66] resulting the final set of Amplicon Sequence Variants (ASVs). Finally, merged and quality filtered sequences were mapped against ASVs with VSEARCH (--usearch_global) with default sequence identity threshold of 0.97. The bacterial lineage of each ASV were assigned by using BLASTn v2.9.0+ against SILVA SSU 138.1 NR [66]. Best hits were only accepted if $(\frac{\%identity + \%coverage}{2}) \geq 93$ following the recommendation of SILVA team [66]. A total of 25,283,812 reads

corresponding to 3,934 ASVs were obtained from the 411 samples, with all samples achieving high read counts (mean ± SD = 61,517 ± 31,959 reads per sample, median = 54,126 , range = 21,010-262,994).

Statistical analyses

To investigate the link between maternal GCs and potential detrimental dysbiosis in offspring's gut bacteria we began by exploring variation in microbial alpha diversity linked with prenatal maternal GCs during early and late gestation, postnatal maternal GCs, and associated with offspring GCs, sex, age, group and season of data collection. The study subjects experienced the same environmental conditions in terms of food availability and climate during data collection. All analyses were performed in R Studio (Version 3.6.1) using the packages *glmmTMB* [67], and *ancombc* [68]. We ran GLMMs with a full-null model comparison to investigate several alpha diversity measures: pure richness-based estimators (ObservedASVs, Chao1, ACE; Model 1a-1c), Faith's Phylogenetic Diversity (PD) as phylogenetic richness estimator (Model 1d), and richness-evenness estimators (i.e., Shannon-Weaver, Inverse Simpson: Model 1e-1f). We ran all the models regarding alpha diversity measures (Model 1a-1d) always including the same predictors: the average value of maternal GCs during early gestation (*Early-preGC*) and late gestation (*Late-preGC*), the average value of postnatal maternal GCs (*PostGC*), the average value of offspring GCs estimated separately for the rich and the lean season (*OffspringGC*), and the information on offspring sex, age category (infant, juvenile, adult), group (MST, MOT, SST) and season of data collection (rich, lean). We included the random intercept individual ID and the random slopes of *Early-preGC, Late-preGC, PostGC,* and *OffspringGC* within individual ID in all models. Because the inclusion of the random slopes of *Early-preGC, Late-preGC, PostGC,* and *OffspringGC* within individual ID caused no convergence of all full models 1a-1f, we excluded them and re-ran all full models 1a-1f without random slopes. We removed all not significant interactions and included main terms in reduced models. Because the inclusion of random slopes caused no convergence of Model 1a, we removed the random slopes of *PostGC* and *OffspringGC* within individual ID which had variance = 0 (Variance of *PostGC* within ID = 7.055e-32; *OffspringGC* within ID = 3.099e-66) and re-ran reduced model 1a. All the covariates were $\log_n$-transformed and then z-transformed to achieve model requirements and improve the interpretability of predictors. We checked model stability and collinearity of predictors using the package *performance* which identified no collinear predictors (all VIFs < 2). Each alpha diversity model dataset included 192 data points belonging to 30 subjects (mean = 6.4, SD=2.07).

Successively, we performed the analysis of compositions with bias correction ANCOM-BC [68], which estimates the unknown sampling fractions and corrects the bias induced by their differences among samples. The absolute abundance data were modeled by ANCOM-BC using a linear regression framework. ANCOM-BC provides a statistically valid test with appropriate p-values, confidence intervals for differential abundance of each taxon, and controls the false discovery rate while maintaining adequate power in a computationally simple way of implementing the models. In total we ran six separate ANCOM-BC analyses at phylum, family, and genus level for rich and lean season. We conducted separate analyses for the rich and the lean season since *ancombc* does not allow longitudinal analysis.

Before running ANCOM-BC we merged the information on differential abundance derived from seasonal biological replicates of each subject into a single mean value per individual and season (Rich season: 3.7±1.0; Lean season: 4.5±1.5 samples). The predictors included in each ANCOM-BC model were always coded in the same way as the alpha diversity models except *Age* that was included as a continuous variable and z-transformed with mean = 0 and sd = 1 to avoid convergence issues and improve interpretation of each interaction term. We excluded the collinear predictor *Group* (VIF > 5). P-values were adjusted to control for false discovery rate [69] (Benjamini-Hochberg corrected p-values: $p_{Bh} < 0.05$). The model datasets on ANCOM-BC during the rich and lean seasons included 16 and 30 data points, respectively, because some individuals could not be adequately sampled in the rich season. To understand whether the effect of maternal and offspring GCs on gut microbial composition was constant across different seasons we investigated the number of taxa significantly affected by the same model predictors during the rich and the lean season separately. Thus, we analyzed whether the effect direction of each predictor on different taxa was concordant or discordant between seasons.

Finally, we performed general linear models on the *Firmicutes* to *Bacteroidota* ratio (*Firmicutes*/*Bacteroidota*) using the same model predictors to investigate dysbiosis associated with maternal and offspring GCs moderated by age. We detected and excluded the collinear predictor *Group* (VIF > 5). We conducted a likelihood ratio test and obtained the p-values for each predictor variable using single-term elimination with the drop1 function in R [70]. The model datasets on *Firmicutes*/*Bacteroidota* during the rich and the lean seasons included 16 and 30 data points, respectively.

Results

General gut bacterial community composition of free-living Macaca assamensis

The general gut bacterial community composition was analyzed for the study population from 320 fecal samples taken from 51 subjects (6.3±2.6) of which not all had data on maternal GCs during development. About 97% of the microbial composition was represented by seven bacterial phyla with similar composition during the rich and the lean season (Figure 2A). Overall, the phylum *Firmicutes* was the most abundant one (59.3±7.1%), followed by *Bacteroidota* (17.9±5.2%), *Spirochaetota* (9.3±6.5%), *Proteobacteria* (3.5±3.2%), *Verrucomicrobiota* (2.9±1.9%), *Actinobacteriota* (2.9±5.3%), and *Cyanobacteria* (1.6±1.1%). All together the phyla *Fibrobacterota* (0.5±0.7%), *Desulfobacterota* (0.4±1.5%), *Campylobacterota* (0.3±0.3%), *Elusimicrobiota* (0.1±0.4%) represented the remaining 3% (including 1.4% of unclassified bacteria).

At the family level, almost 80% of the overall main composition was represented by 15 families (~85% by 20 families): *Lachnospiraceae* (17.19±6.2%) represented the most abundant family, followed by *Prevotellaceae* (10.1±5.7%), *Spirochaetaceae* (9.2±6.6%), *Ruminococcaceae* (8.6±3.1%), *Oscillospiraceae* (7.7±2.8%), UCG-010 of the order *Oscillospirales* (3.9±1.8%), *Rikenellaceae* (3.2±1.9%), uncultured bacteria from the Clostridia vadinBB60 group (2.9±1.6%), *Erysipelatoclostridiaceae* (2.8±2.3%), uncultured bacteria from the Clostridia UCG-014 (2.4±1.4%), *Christensenellaceae* (2.1±1.0%), uncultured bacteria from the order WCHB1-41 belonging to the class of *Kiritimatiellae* (2.1±1.6%), *Succinivibrionaceae* (1.8±3.1%), *Butyricicoccaceae* (1.7±1.5%), uncultured bacteria from the Coprostanoligenes group of *Oscillospirales* (1.7±0.8%), and finally *Muribaculaceae* (1.6±1.0%) (Figure 2B).

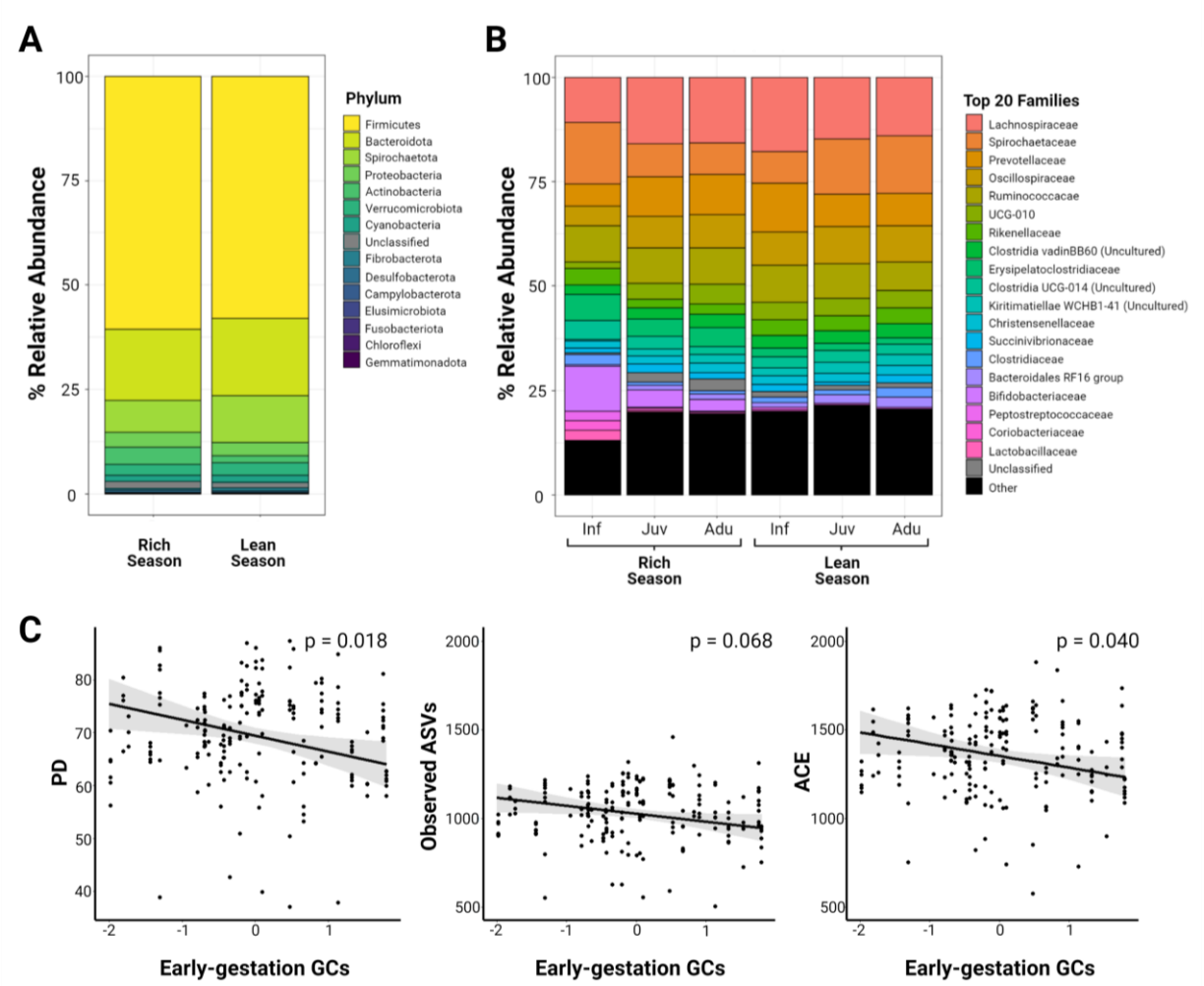


Figure 2 Gut bacterial community and effect of maternal GCs during the first half of gestation (*Early-preGC*) on alpha diversity measures of Assamese macaques during the study period from June 2018 to June 2019. Panel A shows the relative abundance of all bacterial phyla averaged according to *Season*. Panel B shows the relative abundance according to *Age* and *Season* of the 20 most abundant families which accounted for 84.2% of the total composition. Panel C shows the effect of *Early-preGC* (z-transformed with mean = 0 and SD = 1) on Faith's phylogenetic diversity (PD), Observed ASVs, and Abundance-based Coverage Estimator (ACE) indices. Each model line is plotted with all the other predictors at their average value.

Maternal GCs during early gestation alter offspring's bacterial diversity

Next, samples were filtered and only those belonging to individuals with complete information on maternal GCs during (early and late) gestation and lactation were retained. Thus, we estimated gut microbial alpha diversity from 192 fecal samples belonging to 30 subjects (6.4±2.07 samples per subject). Overall, all the full-models on alpha diversity consistently revealed no interaction effect of age with maternal GCs, and with offspring GCs (Supplementary Table 1-2). Therefore, we ran reduced models without non-significant interaction terms (Supplementary Table 3). Reduced model 1a-1c revealed consistent results and all the predictors together clearly influenced the offspring's gut bacteria alpha diversity in all the models (Model 1a: $\chi2 = 72.85$, df = 11, $p < 0.001$; Model 1b: $\chi2 = 91.98$, df = 11, $p < 0.001$; Model 1c: $\chi2 = 66.38$, df = 11, $p < 0.001$; Table 1, Supplementary Table

3). Increasing prenatal maternal GCs during early but not late gestation was significantly associated (or tended to be in one case) with a decrease in alpha diversity (*Early-preGC*: p_{1a}=0.068, p_{1b}=0.040, p_{1c}=0.018, Figure 2C; *Late-preGC*: p_{1a}=0.503, p_{1b}=0.670, p_{1c}=0.999). Postnatal maternal GCs had a positive effect on alpha diversity although only two models revealed a statistical trend (*PostGC*: p_{1a}=0.089, p_{1b}=0.054), and one revealed a non-significant effect on phylogenetic diversity (*PostGC*: p_{1c}=0.148). Surprisingly, the seasonal offspring GCs mean value had no effect on the model responses (*OffspringGC:* p_{1a}=0.395, p_{1b}=0.313, p_{1c}=0.987). Very consistently, *Age*, *Season,* and *Group* had the same significant effect in all the alpha diversity models, while *Sex* did not affect any response (Supplementary Table 3).

Table 1 - Effects of model predictors on Faith's Phylogenetic Diversity of offspring's gut bacteria.

Model 1c - Faith's Phylogenetic Diversity (R^2 = 0.29)					
Predictors	**Estimates**	**SE**	**CI 2.5-97.5%**	**Z**	**P-Value**
(Intercept)	68.21	2.01	64.26 – 72.15	33.88	-
Early-preGC	-2.09	0.88	-3.82 – -0.36	-2.37	**0.018**
Late-preGC	0.00	0.85	-1.67 – 1.67	0.00	0.999
PostGC	1.24	0.86	-0.44 – 2.93	1.45	0.148
OffspringGC	-0.01	0.77	-1.52 – 1.50	-0.02	0.987
[1]Sex [M]	0.20	1.30	-2.34 – 2.74	0.15	0.879
[2]Age [adu]	8.62	1.86	4.99 – 12.26	4.65	<**0.001**
[2]Age [juv]	11.66	2.33	7.08 – 16.23	4.99	<**0.001**
[3]Season [Rich]	-7.00	1.45	-9.85 – -4.15	-4.82	<**0.001**
[4]Group [2]	-3.11	1.64	-6.32 – 0.10	-1.90	0.058
[4]Group [3]	-4.51	1.89	-8.21 – -0.81	-2.39	**0.017**

Reduced model 1c explaining the Faith's Phylogenetic Diversity (PD) of offspring's gut bacteria. Significant p-values of explanatory variables are in bold. R^2 indicates the conditional coefficient of determination. All covariates are $\log_n$-transformed and then z-transformed (mean = 0, SD = 1) to meet model requirements and increase model interpretability. (1) coded with "female" as the reference category, (2) coded with "infant" as the reference category, (3) coded with "lean" as reference category, (4) coded with "MOT" as reference category.

The models analyzing the richness-evenness estimators (Model 1d-1e) showed different results (Supplementary Table 4). Although the reduced-null model comparison revealed that all predictors together influenced the offspring gut bacteria alpha diversity (reduced-Null model comparison of Model 1d: $\chi2$ = 29.26, df = 15, p = 0.015; Model 1e: $\chi2$ = 20.08, df = 11, p = 0.044), only *Season* and *Group* had a significant effect on the Shannon index (Model 1d), while only *Age[juv]* and *Group* significantly affected the Inverse Simpson index (Model 1e). In both Model 1d and 1e, prenatal maternal GCs during early and late gestation, and postnatal maternal GCs had no statistically significant effect on richness-evenness estimators (Supplementary Table 4).

Maternal GCs, offspring GCs, and overall variation in bacterial composition

The analysis of gut bacterial compositions (ANCOM-BC) identified differentially abundant taxa according to predictors of interest such as Early-preGC, Late-preGC, PostGC, OffspringGC, Age and Sex and their interactions. If a predictor variable was part of a significant interaction term, we do not report the summarized statistics used to estimate variation in overall composition because of the very limited interpretability. We provide a quantitative interpretation of predictor effects based on the percentage of taxa significantly affected, operating under the assumption that irrespective of the effect size, the greater the number of taxa significantly affected, the higher the potential relevance of the model predictor in shaping the overall gut bacterial community composition.

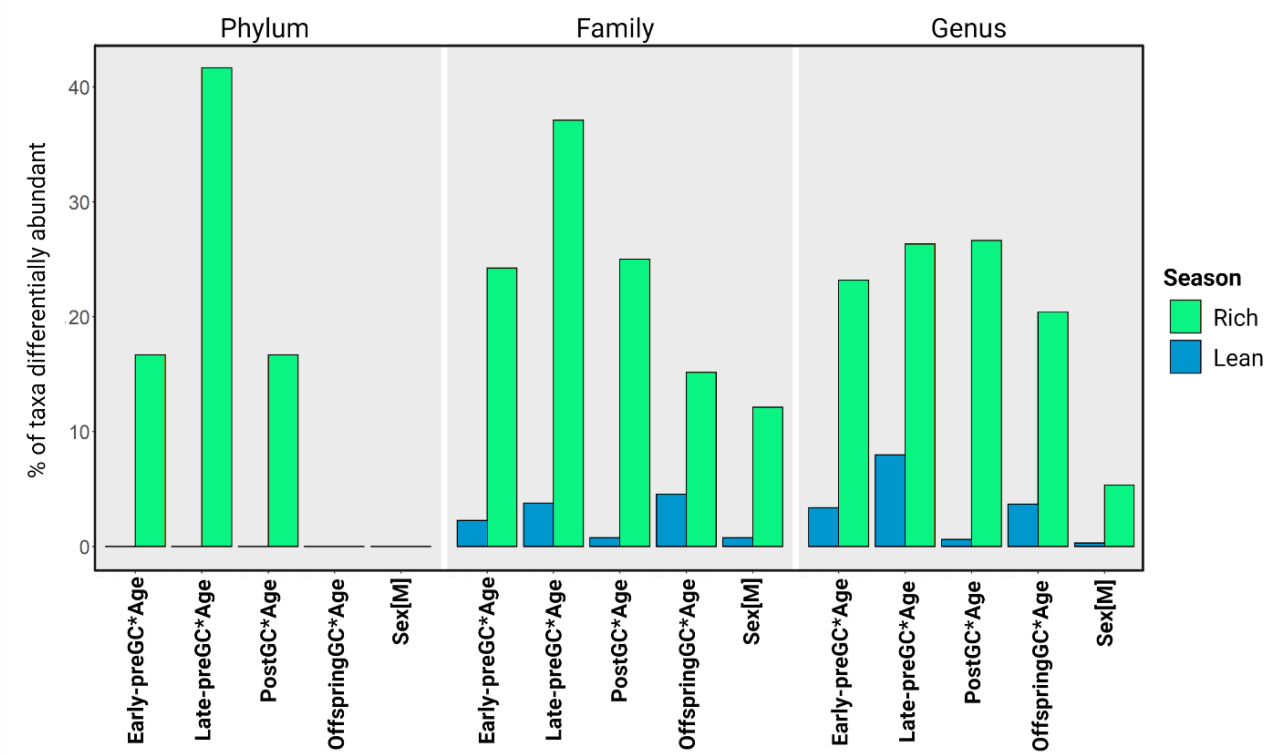
Table 2 – Quantitative characterization of model predictors' relevance

% of taxa significantly affected	Variation in the overall composition	Relevance
0-5	no variation/similar composition	not relevant
5-10	minor differences	slightly relevant
10-20	moderate differences	mildly relevant
20-40	considerable differences	considerably relevant
>40	high differences	highly relevant

Ranges of the percentage of taxa significantly affected by predictors used to quantitatively characterize the relevance of model predictors in shaping variation in overall microbial composition.

Rich season At the phylum level, early prenatal maternal GCs and postnatal maternal GCs in interaction with age were associated with moderate differences whereas late prenatal GCs in interaction with age had considerable effects on bacterial composition: 1SD of variation in maternal GCs with 1SD of variation in age was associated with significant changes in the abundance of 10-20% and 20-40% of phyla, respectively. Offspring GC or sex in interaction with age did not affect gut bacterial composition in relevant ways. At the family and genus level, variation in maternal GCs during all three time-windows in interaction with age had considerable effects on bacterial composition, and offspring GC effects increased to a moderate level (Figure 3, Table 2).

Figure 3 Percentage of bacterial taxa differentially abundant according to model predictors moderated by offspring's age. The composition is estimated for the rich and the lean season at the phylum, family, and genus level separately. Significance of the main terms of each interaction has limited interpretability and therefore they are not depicted. All the variables but sex have been z-transformed with mean = 0 and SD = 1 to increase model interpretability. Sex is coded with "female" as the reference category. Early-preGC = prenatal maternal GCs during early gestation, Late-preGC = prenatal maternal GCs during late gestation, PostGC = postnatal maternal GCs during lactation, OffspringGC = seasonal offspring GCs.



Lean season Overall, the gut bacterial composition during the lean season was more anchored and less influenced by predictors. Minor variation in composition was observed at the family and genus level in response to an interaction of age with late preGCs and offspring GC (Figure 3).

Bacterial composition and the effect of maternal GCs, offspring GCs, and age

Subsequent analyses focused on the direction of effects associated with prenatal and postnatal maternal GCs, and offspring GCs in interaction with age. We report summary statistics on the modeled effect of maternal and offspring GCs at the mean of the variable age (equivalent to juveniles age class), mean+1SD (equivalent to adult age class), and mean-1SD (roughly equivalent to infant age class). Exposure to maternal GCs before and after birth had significant effects on bacterial community composition depending on the age of the offspring and sampling season, but these effects during the lean season concerned less than 5% of taxa only (with one exception) and were typically less stable in the direction across age compared to effects during the rich season.

Rich season From a total of 319 genera analyzed, 23.2% (74) were significantly affected by maternal GCs during early gestation moderated by age (*Early-preGC*Age*). The effect of a 1SD increase in *Early-preGC* on differential abundance amplified with *Age* in 14.1% of the genera (positive: 4.7%; negative 9.4%), it diminished with *Age* in 0%, and reversed in 9.1%. Late prenatal maternal GCs moderated by age (*Late-preGC*Age*) had a similar effect on microbial composition in terms of number of genera significantly affected (84 out of 319, 26.3%). However, the effect of an increase of 1SD of *Late-preGC* amplified with *Age* in only 1.6% of genera (positive: 0.6%; negative 1%), reduced in 3.8% (positive: 1.0%; negative: 2.8%) and reversed in as many as 20.9% of genera. A total of 85 out of 319 genera (26.6%) were affected by postnatal maternal GC moderated by age (*PostGC*Age*). The effect of *PostGC* amplified with *Age* in 1.9% of genera (positive: 0.3%; negative: 1.6%), weakened with *Age* in 4.1% (positive: 2.5%; negative: 1.6%) and mainly reversed with age in 20.6% of genera (Figure 4). The ANCOM-BC analysis was repeated including only juveniles and adults and showed a similar effect of early-preGC on these genera (Supplementary Table 6).

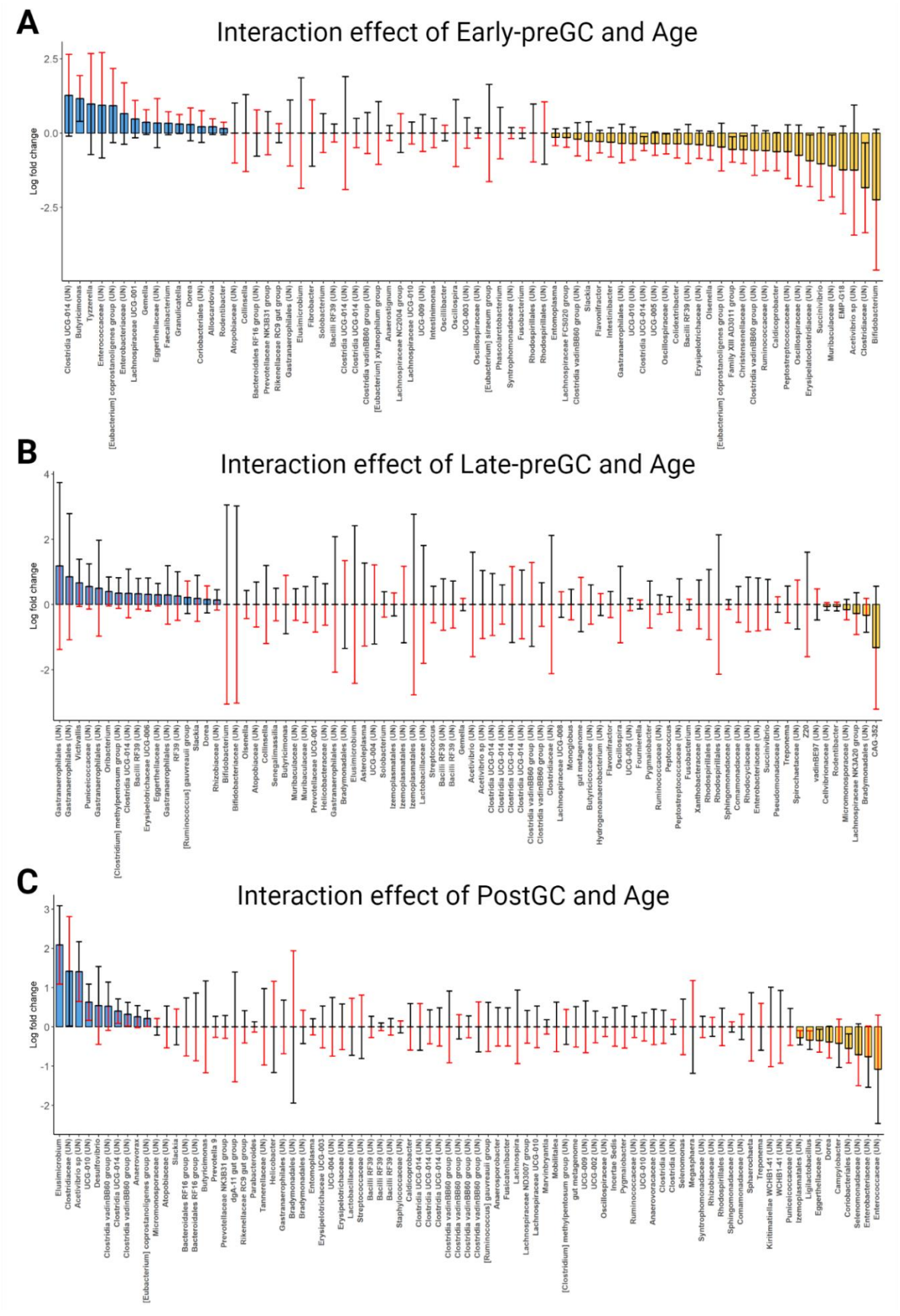


Figure 4 Effect of different maternal predictors moderated by *Age* at sampling on differential relative abundance of bacterial genera during the rich season. Panel A shows the effect of *Early-preGC* moderated by *Age* (*Early-preGC*Age*), panel B shows the effect of *Late-preGC* moderated by *Age* (*Late-preGC*Age*) and panel C shows the effect of *PostGC* moderated by *Age* (*PostGC*Age*). "UN" refers to undetermined/unclassified genus, and family or order is therefore reported. Colored bars indicate the effect direction (blue = positive, yellow = negative) of an increase in 1SD of specific predictors (*Early-preGC* = 59.3 ng/g; *Late-preGC* = 67.0 ng/g; *PostGC* = 52.7 ng/g) estimated at the mean value of age (mean = 4.7 years), and at the mean value of all the other predictors. Red and black lines indicate the effect estimated at the mean plus1SD (red = 7.1 years)

and at the mean minus1SD (black = 2.3) of age. Case example: Panel A 1st taxon from the right: when subjects are 4.7 years old (mean age), the abundance of *Bifidobacterium* experiences a 2.3 log-fold decrease with an increase in maternal GCs during the early gestation of 59 ng/g (1SD). When the subjects are 2.3 years old (mean age-1SD), the same increase in maternal GCs is associated with an 0.12 log fold increase in *Bifidobacterium* abundance (black whisker). Finally, when the subjects are 7.1 years old (mean age+1SD), the same increase in maternal GCs is associated with a 4.6 log-fold decrease in the abundance (red whisker). When the bar is not plotted, the effect of the same increase in maternal GCs at the mean age is 0. Taxa showing the black head (estimates at 2.3 years) further from 0 than the red head (estimates at 7.1 years) show an effect that is reduced with age.

Finally, 65 out of 319 genera (20.4%) were affected by offspring GCs moderated by age (*OffspringGC*Age*). The effect of *OffspringGC* amplified with *Age* in 6% of genera (positive: 1.3%; negative: 4.7%), reduced in 2.2% (positive: 0.9%; negative: 1.3%), and reversed with *Age* in 12.2% (Figure 5).

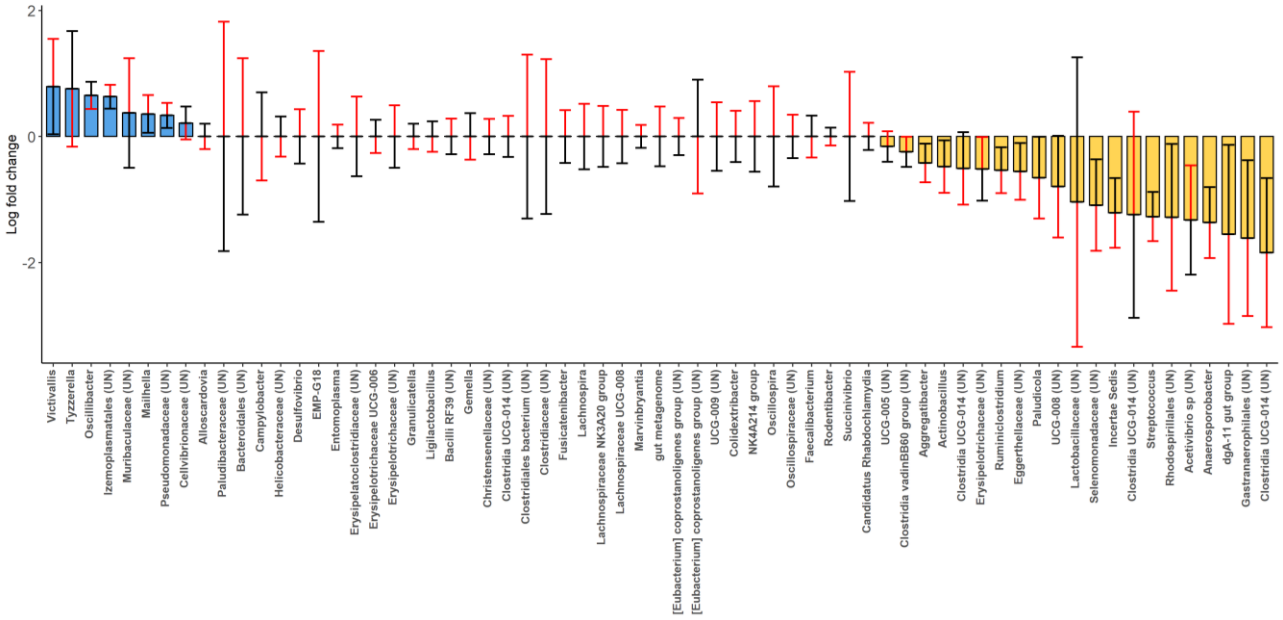


Figure 5 Effect of concurrent *OffspringGC* moderated by *Age* (*OffspringGC*Age*) on differential abundance of genera during the rich season. "UN" refers to undetermined genus, and family or order is therefore reported. Colored bars indicate the effect (blue = positive, yellow = negative) of an increase in 1SD of *OffspringGC* (SD = 130.1 ng/g) estimated at the mean value of age (mean = 4.7 years), and at the mean value of all the other predictors. Red and black lines indicate the effect of *Late-preGC* estimated at the mean+1SD (red = 7.1 years) and at the mean-1SD (black = 2.3 years) of age.

Table 4 – Effect of maternal and offspring GCs on gut bacterial composition

Predictor	Taxa significantly affected	Amplify with age (%)	Attenuate with age (%)	Reverse with age (%)
Early-preGC	74	60.8	0.0	39.2
Late-preGC	84	6.0	21.4	72.6
PostGC	85	5.9	16.5	77.6
OffspringGC	65	32.3	12.3	53.8

Effect of maternal and offspring GCs moderated by age on the gut bacterial composition at the genus level during the rich season. The direction of moderation (amplification, attenuation, reversal) is summarized for significantly affected genera only.

Lean season From a total of 326 genera analyzed, only 11 were significantly affected by maternal GCs during early gestation moderated by age (*Early-preGC*Age*) all of which reversed with age. Late prenatal maternal GCs moderated by age (*Late-preGC*Age*) had a similarly small effect on bacterial composition in terms of number of genera significantly affected (26 out of 326). The effect of *Late-preGC* amplified with *Age* in 2 genera (both positive), reduced with *Age* in 1 taxon (negative) and reversed with *Age* in 11 genera. Postnatal maternal GCs moderated by age (*PostGC*Age*) had a neglectable effect on bacterial composition (2 out of 326 genera), and only 12 genera were affected by offspring GC moderated by age (*OffspringGC*Age*). The effect of *OffspringGC* amplified with *Age* in 1 taxon (positive), attenuate in 1 taxon (negative) and reversed with *Age* in 10 genera (Figure 5).

The *Firmicutes* to *Bacteroidota* ratio We detected seasonal differences in the effect of maternal and offspring GCs on the *Firmicutes* to *Bacteroidota* ratio (*Firmicutes*/*Bacteroidota*). While the full model on the *Firmicutes*/*Bacteroidota* during the lean season revealed no significant effect of all the model predictors (full-null model comparison: full-Model Lean: $\chi2 = 11.95$, df = 10, $p = 0.335$, Supplementary Table 5), the models on the *Firmicutes*/*Bacteroidota* during the rich season revealed an overall effect of all the predictors together (full-null model comparison: full-Model Rich: $\chi2 = 34.01$, df = 10, $p < 0.001$, Supplementary Table 5). We excluded the non-significant interaction *Late-preGC*Age (*$p = 0.177$*)* and ran a reduced model including the main term *Late-preGC*. Also, the reduced model revealed an overall effect of all the model predictors on the *Firmicutes*/*Bacteroidota* ratio during the rich season (reduced-null model comparison: reduced-Model Rich: $\chi2 = 31.73$, df = 9, $p < 0.001$, Table 3). In particular, both *Early-preGC* moderated by *Age (Early-preGC*Age)* and *Late-preGC* as a main term were negatively associated with *Firmicutes*/*Bacteroidota* (Figure 6A and 6C; Table 3), while the predictors associated with postnatal maternal GCs and offspring GCs had a positive effect (Figure 6B and 6C, Table 3).

Table 3 – Effect of model predictors on *Firmicutes* to *Bacteroidota* ratio

Predictor	red-Model Rich (pseudo-R^2 = 0.86)				
	Estimate	**SE**	**95% C.I.**	**LRT**	**P-value**
Intercept	1.165	0.007	1.152 – 1.178	-	-
Early-preGC[1]	-0.020	0.008	-0.036 – -0.005	-	-
Late-preGC[1]	-0.015	0.006	-0.026 – -0.004	5.624	**0.018**
PostGC[1]	0.011	0.008	-0.005 – 0.027	-	-
OffspringGC[1]	0.033	0.008	0.018 – 0.048	-	-
Age[2]	-0.049	0.011	-0.070 – -0.028	-	-
Sex[M][3]	0.004	0.009	-0.013 – 0.021	0.177	0.674
[1]Early-preGC*Age[3]	-0.027	0.008	-0.042 – -0.012	9.066	**0.003**
[1]PostGC*Age[3]	0.035	0.008	0.019 – 0.051	11.955	**0.001**
[1]OffspringGC*Age[3]	0.016	0.008	0.001 – 0.031	3.976	**0.046**

Reduced model explaining the *Firmicutes* to *Bacteroidota* ratio of offspring's gut bacteria during the rich season (red-Model Rich). (1) indicates that the predictor has been $\log_n$-transformed and then z-transformed (mean = 0, SD = 1) to meet model requirements and to increase model interpretability, (2) the predictor has been z-transformed with mean = 0 and SD = 1; (3) coded with "female" as reference category. N = 16.

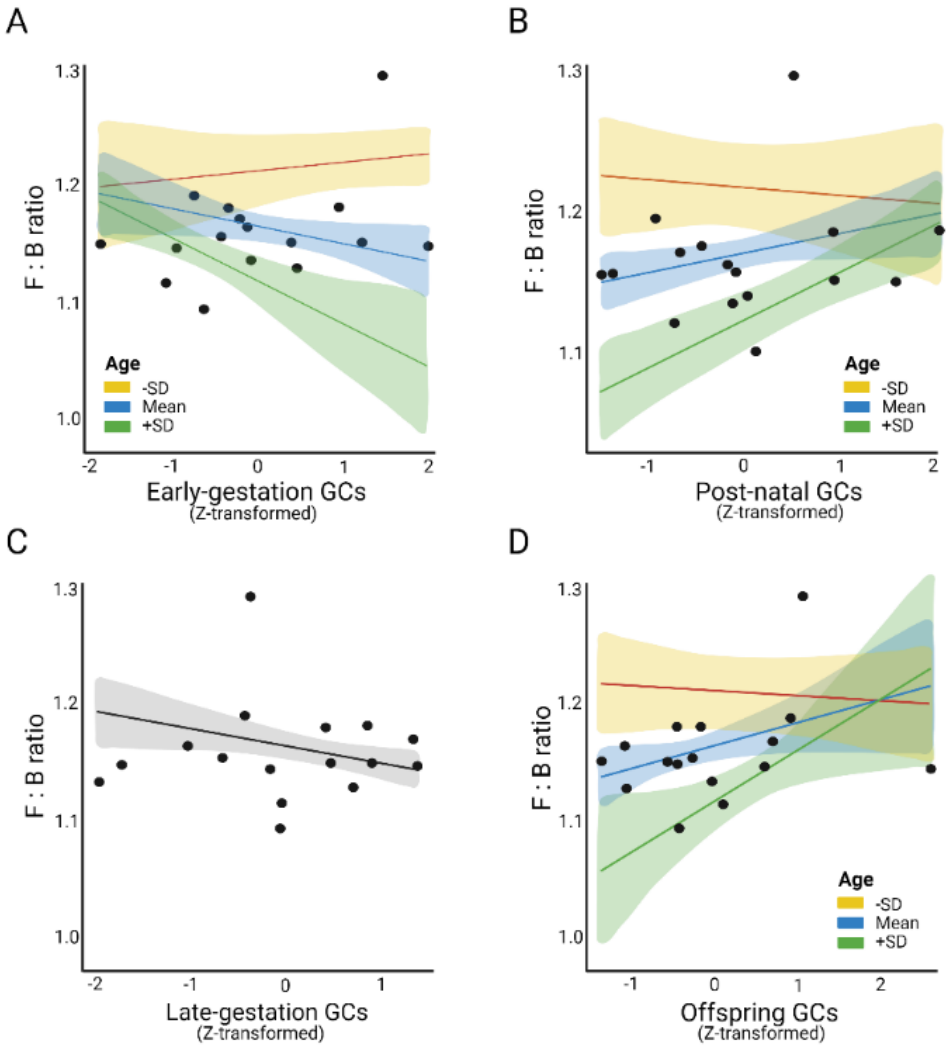


Figure 6 Effect of significant predictors of reduced Model R explaining the variation of *Firmicutes* to *Bacteroidota* ratio (F:B) of offspring's gut bacteria during the rich season. Panel A shows the effect of prenatal maternal GCs during the first half of gestation moderated by offspring's age (*Early-preGC*Age*). Panel B shows the effect of postnatal maternal GCs moderated by offspring's age (*PostGC*Age*). Panel C shows the effect of prenatal maternal GCs during the second half of

gestation (*Late-preGC)*. Panel D shows the effect of offspring GCs moderated by offspring's age (*OffspringGC*Age*). The moderator *Age* is plotted at its mean and ±SD values (4.7±2.4). Results on predictors shown in panels A and C corroborate the overall negative effect of prenatal maternal GCs on offspring F:B. The model response F:B is $\log_n$-transformed to meet model requirements and the predictors *Early-PreGC, Late-PreGC, PostGC,* and *OffspringGC* are $\log_n$-transformed and then z-transformed with mean = 0 and SD = 1 to meet model requirements and to increase model interpretability. Each panel is plotted with all other model predictors at their average value.

Discussion

Cercopithecoid primates and humans show broad similarities in their gut microbiome, even more than the closer related apes and humans [71–73] making macaques a valuable model species for gut microbiome investigations. For example, the microbial butyrate-production pathways of catarrhines including macaques are more similar to those of humans than those observed in lemurs, platyrrhines, and apes after controlling for effects of dietary strategy [71]. The high microbial diversity associated with butyrate pathways of catarrhines and humans implies a more efficient short-chain fatty acid (SCFA) metabolism and may confer higher stability and resilience to perturbations. A higher diversity of microorganisms involved in the degradation of amino acid may confer stability in case of strong variations of diet composition as seen in comparative butyrate-degradation patterns of industrialized and non-industrialized human populations [71]. The ecological and dietary flexibility of macaques is unique among non-human primates and is thought to underlie the widest latitudinal and longitudinal geographical distribution of all primate genera except *Homo* [74–77]. In the following, we first discuss the broad effects of age and season on the gut bacterial composition and then we focus on the timing effects of maternal GC exposure, and on the outcome in terms of known functions of taxa most profoundly affected by maternal GCs in our statistical models.

Seasonal and age differences on gut microbiome composition

Assamese macaques at the Phu Khieo Wildlife Sanctuary (Thailand) can be classified as frugivorous as they mainly consume fruit, pulp, and seeds with no marked age- or sex-related differences in the diet composition of plant parts [52,53]. However, they spend a considerable part of their feeding time opportunistically and slowly foraging for animal matter and they regularly consume large amounts of mollusks from aquatic snails, spiders, ants, caterpillars, termites but also and less regularly small snakes and lizards, amphibians, birds and bird eggs, and even small mammals including different species of rats and squirrels [53]. It has been shown that Assamese macaques from Phu Khieo Wildlife Sanctuary consume at least 165 known plant items belonging to 118 plant species with a moderate overlap of the most common items between years [52]. As environmental conditions in forests of south-east Asia are highly unpredictable, predictability of food and fruit abundances are also very low.

Our study species, therefore, evolved to live in a highly unpredictable environment [54] and although the main part of its diet is based on plant parts, their gut microbiome must endure unpredictable variation in food type ingested which inevitably has to rely on and may benefit from higher gut microbiota diversity.

Among primates, age-related patterns of microbial richness variation are diverse, and the wild Assamese macaques investigated in this study appear to be more similar to humans than other genetically-closer species like chimpanzees [78]. Our results showed an increase in bacterial richness from infants via juveniles to adults, and are in line with studies on vervet monkeys, rhesus macaques, and humans [79–81] but contrast with observations in wild chimpanzees, common marmoset, and mouse lemurs [78,82,83].

Across age and seasons, the sum of the relative abundance of *Lachnospiraceae, Prevotellaceae, Spirochetaceae, Ruminococcaceae,* and *Oscillospiraceae* was approximately 50%, making these families the most abundant ones in wild Assamese macaques. These groups comprise cellulolytic and fermentative taxa that can metabolize complex plant polysaccharides and degrade fibers. The higher abundance of *Prevotella* is a common trait of folivore/frugivore species and produces twice more propionate than bacterial communities dominated by *Bacteroides* [84]. As distinctive traits of most macaque species, also wild Assamese macaques revealed a high abundance of *Spirochetaceae* from the *Treponema* lineage, a genus whose functionality in the gut is still unexplored. Yet, many *Treponema* are fiber-degrading bacteria proposed to give a substantial contribution to short-chain fatty acid (SCFA) production together with *Prevotella* by degrading xylan, xylose, and carboxymethylcellulose [85]. It is absent in humans living in urban environments but inhabits the gut of individuals from rural communities consuming termites [86].

Across seasons, the relative abundance of the top 20 taxa at the family or genus level did not differ markedly except for some taxa (Figure 7): the rich season showed a higher relative abundance of *Blautia* and *Roseburia* (*Lachnospiraceae*), *Prevotellaceae, Bifidobacteriaceae, Faecalibacterium,* and *Catenibacterium,* while the lean season showed higher relative abundance of *Spirochetaceae, Rikenellaceae, Oscillospiraceae* and *Clostridiaceae* (Figure 7).

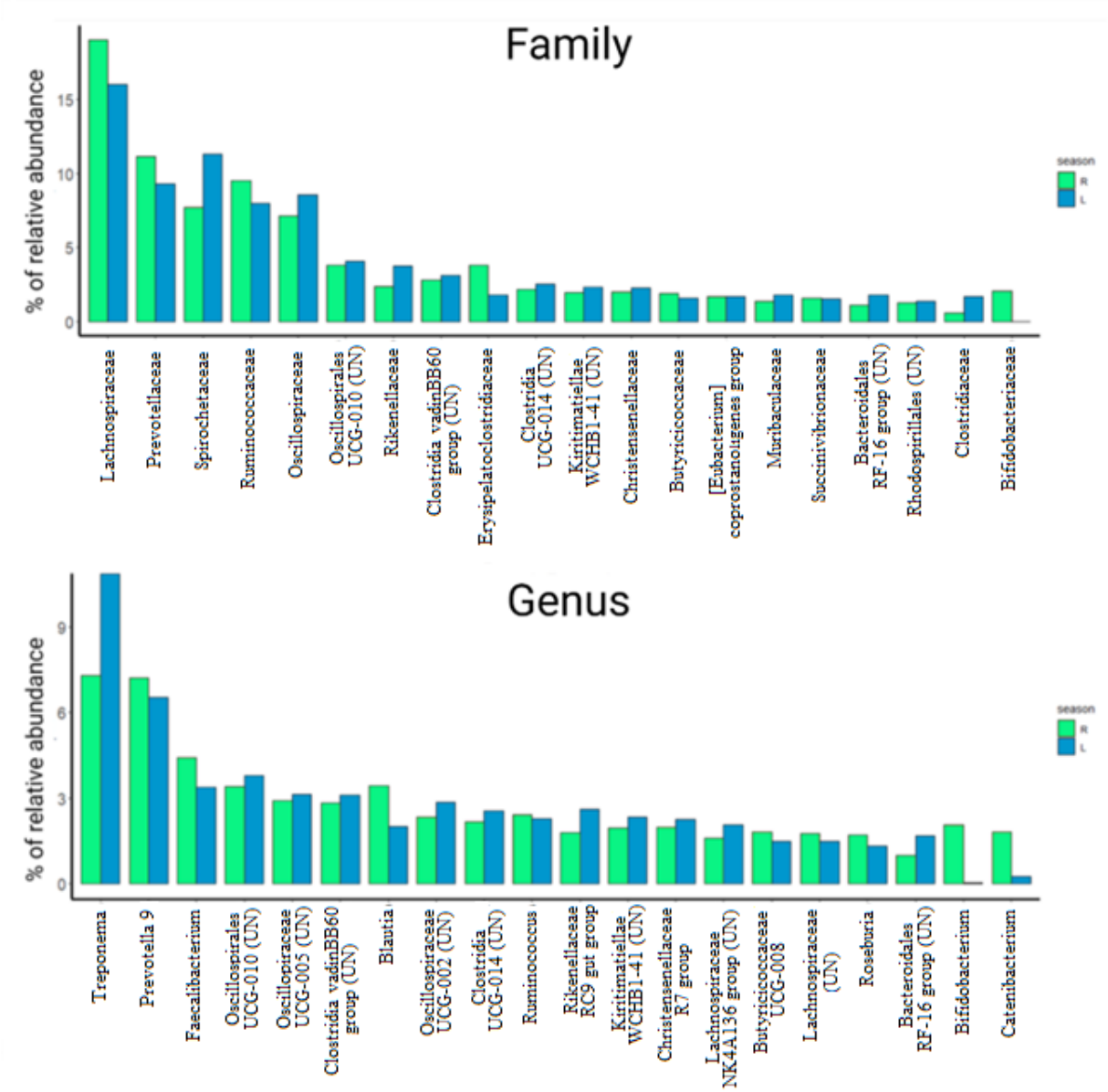


Figure 7 Relative abundance of the 20 most abundant (top) families and (bottom) genera observed in fecal samples of wild Assamese macaques collected during the rich (R, green bars) and the lean season (L, blue bars) at the Phu Khieo Wildlife Sanctuary (Thailand).

Typically, members of *Cercopithecidae* show low relative abundance and diversity of *Bifidobacterium* when compared with other primates [87,88]. Yet, despite host-phylogenetic differences, *Bifidobacterium* is strongly affected by diet [88]. It is highly abundant in the gut of breastfed humans and nursing non-human primate infants and has a key role in the digestion of complex carbohydrates from milk [78,89]. In the rich season of this study, when infants were still highly dependent on milk, *Bifidobacterium* relative abundance in wild Assamese macaques was highest in infants and decreased with age, it had a relative abundance of 3% across all age classes, but was almost absent in samples from the lean season. Higher abundance during the rich season may be driven by hosts consuming fruits and enriching their diet with animal matter like Assamese macaques having a higher abundance

of *Bifidobacteriaceae* when compared with folivores, frugivores, and omnivores [87,88]. Future investigations should focus more on direct quantification of animal matter consumption during the rich and the lean season to clarify seasonal variation of *Bifidobacterium's* relative abundance.

We observed seasonal variation also in the abundance of *Catenibacterium* which was higher during the rich season. Organisms belonging to this taxon can harvest a high quantity of energy through the fermentation of carbohydrates and produce several SCFAs like butyrate, lactate, and acetate [90]. *Catenibacterium* has a higher relative abundance in wild long-tailed macaques when compared with captive individuals [91] and its increase is associated with higher energy storage in humans and rodent models. Thus, members of this genus can have a relevant role in the energy accumulation strategy of wild Assamese macaques which must be maximized during the rich season, the best period of the year for energy storage.

The timing effect of pre- and postnatal maternal GCs on gut bacterial composition

The effect of maternal stress on the programming of physiological phenotypes is a critical factor strongly influenced by the timing of the occurrence [1,47,54,92–96]. We tested for the first time in a wild and long-lived species the effect of maternal GCs on bacterial diversity and composition according to three sensitive time windows: early gestation, late gestation, and postnatal maternal GCs during lactation. The effect of maternal GCs on gut bacterial alpha diversity varied according to the timing of the occurrence. Analyses revealed consistently a negative effect of prenatal maternal GCs during early gestation (*Early-preGC*) on gut bacterial diversity, but no significant effects from maternal GCs exposure during late gestation (*Late-preGC*) or postnatally during lactation (*PostGC*). Our results on the effect of naturally-driven maternal GCs in wild individuals are also in line with studies on primates and rodents in captivity reporting a reduction in infants' microbial diversity associated with experimentally induced stress during gestation [4,47,50]. Fetal exposure to maternal GCs affects HPA axis development and changes gut permeability by perturbing barrier functionality with consequent alteration of typical microbial colonization of the gut [2,4–9,11,12,97].

The composition of gut bacteria during the lean season was only very weakly associated with variation in our predictors. During the rich season, however, bacterial composition was considerably changed with changing levels of maternal GCs and offspring age with a quarter of taxa being differentially abundant. While the proportion of taxa significantly affected was roughly similar for all pre- and postnatal phases, effects either faded with age or reversed with age in response to Late-preGC or PostGC. In response to Early-preGC the effects intensified with

age (positively or negatively) in the vast majority of differentially abundant taxa. Thus, timing of GC exposure had a pronounced effect on the persistence of a long-term gut bacterial signature. Our results on the timing effect of prenatal maternal GCs are in line with other studies that highlight the important role of maternal GC during early as opposed to late gestation in programming morphological or physiological phenotypes in long-lived primates [1,44,54] and expand the list of potentially detrimental effects programmed during the very first phases of fetal development.

Long-term signature of early prenatal maternal GCs

Early preGCs were not only associated with reduced gut bacterial richness all the way into adulthood, but also with a previously unstudied reduction or increase in the relative abundance of 14% of the total genera that amplified with age. We observed a reduction in the abundance of *Bifidobacterium, Acetivibrio, Succinivibrio, Olsenella,* and *Slackia,* and this reduction intensified with age. Typically, *Bifidobacterium* abundance decreases with age in humans and non-human primates. The presence of this health-promoting organism in the gut inhibits pathogen attachment to intestinal cells, promotes protection against enterocolitis and acute diarrhea by slowing down the replication of enteric pathogens, and is typically involved in folate biosynthesis and antioxidant production in human and non-human primates [81,87,88]. Long-term physiological stimulation of the innate immune system can be detrimental with age and can lead to inflammaging processes [98]. Our results suggest that prenatal maternal GCs effects on gut bacteria mimics the inflammaging process which would corroborate findings in rhesus macaques showing a signature of inflammaging in differential gene expression after exposure to the ecological effects of a recent hurricane [99]. The gut microbiome has a pivotal role in regulating these processes and an increase in the abundance of *Bifidobacterium* has been associated with improved health and longevity, and with a reduction or even reversal of dysfunctions driven by inflammatory states associated with age [98]. During the rich season, older subjects were affected more negatively by early prenatal maternal GCs than younger ones. Thus, prenatal maternal GCs may accelerate the typical reduction in *Bifidobacterium* associated with age [100] in Assamese macaques with consequent long-term dysbiotic states in adult subjects which may benefit less from the health-promoted effect of this taxon. The wider age window for offspring in this study from infants to adults may explain discrepancies between these findings and a study on captive rhesus macaques confined to only infant offspring where not the early but the late prenatal maternal exposure to stress was associated with decreased relative abundance of Bifidobacterium [50].

Furthermore, we observed a reduction in the genus *Oscillospira* and one unclassified member of *Oscillospiraceae* with increasing early prenatal maternal stress and age. *Oscillospira* is one of the most abundant genera observed in many primate species [101–106] and humans as well, and it has been recently proposed as a potential probiotic [107]. It is a slow-growing butyrate-producer that ferments complex plant carbohydrates, it can use glycans of the host as an energy source and its abundance is negatively associated with pro-inflammatory molecules in rodent models [108] and with inflammatory disorders in humans [109–111], it is reduced in non-human primates with infections, in captivity or in individuals with higher inflammatory responses to cerebral infarction [27,36,105]. *Oscillospira* increases with age in several macaque species [103,104,112] and it is positively associated with bacterial diversity in humans [110]. Interestingly, prenatally stressed wild Assamese macaques showed lower *Oscillospira* abundance and richness, the same pattern observed in non-healthy humans [113,114], and in captive or inflamed non-human primates [27,36,105], and lacked the typical increase observed with age in healthy individuals of other macaque species [103,104,112]. These results suggest the role of maternal GCs in altering the typical colonization of the abundant *Oscillospira,* with potential long-term detrimental effects on prenatally stressed subjects.

Additionally, the bacterial signature of increased early prenatal maternal GCs was characterized by the increase in the relative abundance of several pro-inflammatory organisms that amplified with age. We observed an increased abundance of a member of the *Enterobacteriaceae* and *Enterococcaceae*, aerotolerant taxa whose overgrowth is promoted by inflammation [115], *Tyzzerella*, a typical pro-inflammatory bacterium which increases intestinal permeability and correlates with circulating inflammatory outcome and anxiety-like behavior [116–118]. Furthermore, early prenatal GC exposure was associated with an increase in the abundance of the pathobiont *Gemella,* a genus with Gram-positive facultative anaerobic organisms involved in gut inflammation [119], associated with increased risks to all type of diabetes due to production of endotoxins inducing inflammatory states [120,121], and able to cause several diseases (e.g., endocarditis, sepsis, allergies, and asthma)[122].

Interestingly, a genus that changed against the pattern of decreased anti-inflammatory and increased pro-inflammatory taxon abundance was *Butyricimonas* — a butyrate-producer that increased in relative abundance with Early-preGC and age. Butyrate reduces inflammation in the gut, helps in maintaining its integrity [123], and protects against infections [124]. Butyrate-producers therefore contribute to the gut homeostasis by increasing the production of mucin which improves tight junction integrity [125]. Such findings could indicate a potential activation of the host immune function in prenatally stressed Assamese macaques which may suffer from a

reduced microbial richness and potential associated metabolic pathways and may rely mainly on fewer bacterial species to digest plant and animal matter, to harvest and store energy, and to produce SCFAs. According to life-history theory, resource diversification may be adaptive or maladaptive depending on the environmental context [126]. In an unpredictable environment, like the ones Assamese macaques live in, putting all eggs in one basket represents a high risk, which can only be clarified by studies on the mediation role of the gut microbiome on the effect of prenatal maternal stress on fitness. Despite this one outlier, patterns of differential abundance associated with prenatal GC exposure are suggestive of inflammatory states exacerbating with age.

Other marked changes that amplified with offspring age when Early-preGCs increased were decreased abundances of taxa with metabolic functions like energy harvesting and storage. *Acetivibrio* is known to produce SCFA from fibers [127], and *Succinivibrio* is a glucose-fermenting organism promoting cellulose and hemicellulose digestion, producing succinic and acetic acids and helping in fatty acids metabolization which can increase the efficiency in energy utilization [128,129]. We also observed a significant reduction in *Olsenella*, *Collinsella*, and an uncultured member of the *Erysipelotrichaceae*, all taxa associated with energy storage in a high-fat diet [72,130–134] and with increased fruit consumption in gorillas and humans [135].

The gut bacteria of early prenatally challenged offspring was further characterized by a significant decrease in *Firmicutes*/*Bacteroidota* ratio. Their relationship is associated with lipid metabolism and an increase in their ratio is indicative of a more efficient capability of energy harvesting, and a greater provision of beneficial SCFAs [91,136]. A recent study investigating oral and gut bacteria, and body-fat indices in wild versus captive long-tailed macaques revealed that wild subjects harbored higher gut and oral diversity, higher *Firmicutes*/*Bacteroidota* ratio, and had higher body-fat indices when compared with captive individuals [91]. Our results suggest that prenatally stressed individuals may have an overall reduced capability of energy harvesting similarly observed in captive and leaner long-tailed macaques.

Being relaxed-income breeders, Assamese macaques accumulate fat during the pre-mating season and rely on the rich season to store the fat necessary to energetically support the next gestation or the current lactation [51]. Early lactating females and those, that are neither gestating nor lactating follow an energy-conserving strategy by storing as much fat as possible during the rich season [51] to ultimately increase their reproductive success. Indeed, fruit availability at Phu Khieo Wildlife Sanctuary modulates conception rates, the peak of the energetically costly lactation period overlaps with the peak of fruit availability, and females exhibit poor physical condition during

lactation [52]. Typically, females in our study population need time to recover from previous reproductive effort and skip reproduction in a given year if they gave birth late in the previous birth season [52]. As the best period to store energy is during the rich season, prenatal maternal stress can negatively impact the energy storage strategy during the fundamental step grounding Assamese macaques' reproductive success by decreasing the relative abundance of several bacterial taxa involved in energy storage and SCFAs production. In summary, the long-term signature observed in the gut bacterial community and associated with the exposure to maternal GC early during gestation (Figure 8) affects the balance of pro-and anti-inflammatory taxa as the differential abundance of taxa involved in energy storage and harvesting.

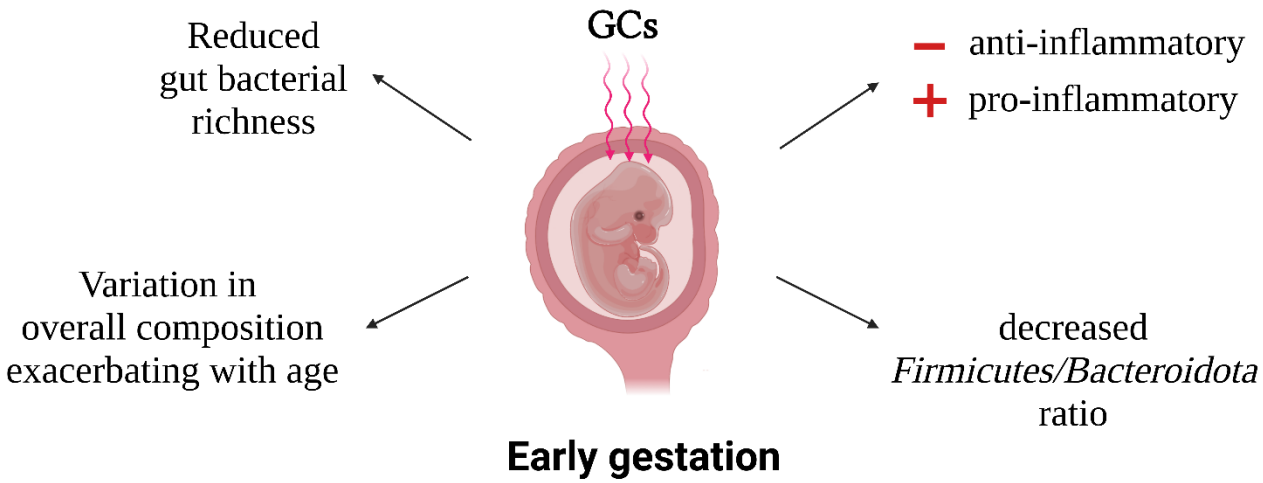


Figure 8 Microbial signature of maternal stress during early-gestation observed across infant, juvenile, and adult age in wild Assamese macaques during the rich season. Exposure to maternal GC late during gestation or after birth had much weaker or less stable effects. Made with Biorender.com

It seems unlikely that in this study the effects of early maternal GC exposure on gut bacteria were mainly mediated by increased HPA axis activity in challenged offspring. Both the early developmental and the concurrent offspring GC levels were included in all the statistical models. Variation inflation factor analyses suggest that their possible covariation in this dataset does not affect the significance of Early-preGC as a predictor. Offspring GC did not explain residual variation in gut bacterial community diversity and its effects on community composition were less broad on the phylum and family level and less stable across offspring age at the genus level. Thus, we believe that early prenatal maternal effects on gut bacteria are more likely to be mediated by alterations of gut microanatomy, physiology, and immunology of the epithelial wall including changes in permeability.

Offspring GCs and gut bacteria

Measures of offspring GCs were not associated with gut bacterial species richness measured from the same fecal sample in wild Assamese macaques which corroborates results on wild gorillas [24,72] and Verreaux's sifakas [137], but contrasts with other studies showing a negative effect in red squirrels [138,139]. Quantitatively, offspring GC had an age-moderated effect on genus composition during the rich season only slightly smaller than prenatal and postnatal maternal GCs. However, the effect was less stable and less pronounced than Early-preGC. Focusing on the effects that amplified with age, increasing seasonal offspring GC levels were associated with a higher relative abundance of the genus *Victivallis*, and a lower abundance of unclassified members of *Clostridia UCG-014* and *Lactobacillaceae*, *Anaerosporobacter*, *Streptococcus*, *Paludicola*, and *Ruminiclostridium.* Unfortunately, the information on metabolic function and potential pathogenicity of most of these organisms is still unexplored. *Ruminiclostridium* can secrete SCFAs, in the herbivore black goat it is considered a potential beneficial bacterium positively involved in growth regulation and gut permeability and helps in maintaining homeostasis and morphology of intestine epithelial cells [140]. *Streptococcus* is a well-known genus with several potential pathogens but also beneficial species. Together with *Bifidobacterium*, members of the *Streptococcus* genus are commensal and are the major producers of lactic acid and acetate in humans with protection against enteropathogenic infection and inflammation reduction [141,142]. *Streptococcus* is associated with increased degradation of fibers and increased production of SCFAs [143]. In obese humans, a reduction in beneficial *Streptococcus* was observed in subjects with a higher intake of dietary fructose [144]. A lower presence of *Streptococcus* could be associated with a lower production of beneficial SCFA and fiber-degraders, or a lower abundance of potential pathogens. Finally, higher offspring GC levels were associated with higher *Tyzzerella* in infants and juveniles but not in adults suggesting an increase of a potentially pathogenic organism in young subjects with higher GCs which fades with age. However, pathogenicity confirmation requires genomic data with higher resolution at the strain-level and was not part of this study.

GCs are metabolic hormones providing information on energy mobilization in response to energetic challenges. The stress response characterized by the release of GCs is an adaptive response to maintain physiological homeostasis during adverse conditions [145] including energy deficits [51]. During the rich season, higher offspring GCs levels were associated with higher *Firmicutes*/*Bacteroidota* ratio suggesting higher energy harvesting capability [146]. As the rich season represents the best time to store energy for wild Assamese macaques, an energy deficit is possible yet unlikely. In the same study population and in the very same individuals,

prenatal maternal GCs induced recalibration of the HPA axis activity of the offspring with long-term hyperactivation and higher allostatic load in infants, juveniles, and adults (see Chapter 2). If prenatally stressed individuals sustain constantly a hyperactivity of the HPA axis, perhaps a gut microbiota with enhanced capability of energy harvesting may serve to balance some of these costs. Today, there is need for a better understanding of how variation in gut bacterial composition can impact energy balance, and future studies could focus on the investigation of metabolic markers to disentangle the role of the gut microbiota in mediating potential energetic constraints and adaptive strategies associated with early adversity.

Conclusion

With this study, we added ecological validity to models tested in captivity and under controlled environmental contexts. Using multivariate regression models and ANCOM-BC approach on a cross-sectional sample, we assessed for the first time in a wild long-lived animal species the effect of maternal GCs on gut bacterial diversity and composition of infant, juvenile and adult offspring. Timing of the exposure was crucially associated with variation in prenatal effect, and early gestation was confirmed as a fundamental sensitive period in shaping offspring physiological phenotypes with persisting long-term effects. From infancy to adulthood, we observed a bacterial signature and dysbiotic states of prenatal maternal stress characterized by an overall reduction in bacterial richness, an imbalance in the ratio of the detrimental to beneficial organisms with a broad reduction in the abundance of several SCFA-producer organisms, and an increase of potentially pro-inflammatory organisms. The effect of maternal GCs during early gestation was more evident during the rich season, a pre-mating time window important for energy accumulation in Assamese macaques and necessary to support energetically the current lactation or the upcoming reproduction. With the caveat of being a correlational study these effects seem to be rather independent instead of being mediated by offspring GC levels. Interestingly, higher offspring GC levels were associated with a bacterial community with potentially increased energy harvesting capability and with no negative effect on richness. These results highlight the adaptive potentiality of the gut bacterial community in regulating metabolic requirements of increased energy allocation to maintenance function of the stress-response physiology. Finally, the bacterial signature under the effect of prenatal stress was characterized by a potential reduction in resource diversification with a higher potential risk for detrimental health and reduced fitness in an unpredictable environment.

Declarations

Ethics approval and consent to participate

Our study was based on non-invasive collection of fecal samples, did not involve experimental work, and adhered to the ASAB/ABS Guidelines for the Use of Animals in Research (https://www.asab.org/ethics). It has been approved by the Thai National Research Council and the Department of National Parks, Wildlife and Plant Conservation Thailand (permits 0002.3/2647 April 2nd 2009, 0002/17 January 2nd 2013, 0002/2424 April 23th 2014, 0002/470 January 26th 2016, 0002/4139 June 9th 2017, 0002/2747 May 4th 2018, 0402/2798 October 4th 2019).

Consent for publication

Not applicable

Availability of data and materials

The raw 16S RNA gene sequencing data are available in the BioProject database (ID: PRJNA795139) of the NCBI repository http://www.ncbi.nlm.nih.gov/bioproject/795139.

Competing interests

The authors declare that they have no competing interests.

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Contributions

Conceptualization, SA, RD, JO, OS; data curation, SA and DS; materials, SS; formal analysis, investigation, and visualization, SA and DS; methodology, all authors; writing—original draft, SA, OS; writing—review and editing, all authors; funding acquisition, RD, OS, and JO; supervision, OS, DS, RD, and JO. The authors read and approved the final manuscript.

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Supplementary Material of:

The long-term gut bacterial signature of a wild primate is associated with a timing-effect of pre- and postnatal maternal glucocorticoid levels

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Amplification of 16S rRNA genes and sequencing: full procedure

Each PCR contained 10 µl of 5-fold Phusion GC buffer, 0.2 µl 50 mM MgCl2, 2.5 µl 5% DMSO, 1 µl 10 mM dNTPs, 31.3 µl nuclease free water (Ambion), 1 µl of forward and 1 µl of reverse primers (equivalent to 0.2 mM), 0.5 µl of Phusion High-Fidelity DNA Polymerase (2 U/µl; ThermoFischer Scientific) and 2.5 µl of 20 ng/µl DNA extract for a total volume of 50 µl. We performed PCR in triplicates on a Labcycler Basic (SensoQuest) with an initial denaturation at 98°C, followed by 25 cycles of denaturation at 98°C for 45 sec, annealing at 55°C for 45 sec and elongation at 72°C for 30 sec. The final elongation was at 72°C for 5 min and samples were then maintained at 10°C until further processing. We confirmed the amplification efficiency and purity by visualizing PCR products on agarose gel electrophoresis. We included negative control without DNA template and a positive control with DNA from *E. coli* on all runs.

After pooling amplicon triplicates, the PCR products were used to attach indices and Illumina sequencing adapters using the Nextera XT Index kit (Illumina, San Diego) and the KAPA HiFi HotStart ReadyMix (Roche Diagnostics, Mannheim, Germany). Index PCR was performed using 5 µl of template PCR product, 2.5 µl of each index primer, 12.5 µl of 2x KAPA HiFi HotStart ReadyMix and 2.5 µl PCR grade water. Thermal cycling scheme was: 95 ° C for 3 min, 8 cycles of 30 s at 95 °C, 30 s at 55 °C and 30 s at 72 °C and a final extension at 72°C for 5 min. Products were quantified using the Quant-iT dsDNA HS assay kit and a Qubit fluorometer (Invitrogen GmbH, Karlsruhe, Germany) following manufacturer's instructions. Purification of the indexed products was performed using MagSi-NGS Prep Plus Magnetic beads (Steinbrenner Laborsysteme GmbH, Wiesenbach, Germany) as recommended by the manufacturer, and normalization was performed with the Janus Automated Workstation from Perkin Elmer (Perkin Elmer, Waltham Massachusetts, USA). Sequencing was conducted using Illumina MiSeq platform using dual indexing and MiSeq reagent kit v3 (600 cycles) as recommended by the manufacturer.

Supplementary Table 1 Full models 1a-1c explaining the offspring's gut bacteria alpha diversity (from richness estimators). Significant *P* values of explanatory variables are in bold. R^2 indicates the conditional coefficient of determination. All covariates are log_n-transformed and then z-transformed (mean = 0, SD = 1) to meet model requirements and increase model interpretability. (1) coded with "female" as the reference category, (2) coded with "infant" as the reference category, (3) coded with "lean" as the reference category, (4) coded with the group "MOT" as the reference category.

	Full model 1a (ObservedASVs) (R^2 = 0.34)					**Full model 1b (ACE) (R^2 = 0.41)**					**Full model 1c (PD) (R^2 = 0.32)**				
Predictors	**β**	**SE**	**CI 2.5-97.5%**	**Z**	**P-Value**	**β**	**SE**	**CI 2.5-97.5%**	**Z**	**P-Value**	**β**	**SE**	**CI 2.5-97.5%**	**Z**	**P-Value**
(Intercept)	1084.03	41.66	1002.38 – 1165.68	26.02	**<0.001**	1397.05	53.65	1291.90 – 1502.20	26.04	**<0.001**	69.90	2.40	65.19 – 74.61	29.09	**<0.001**
Early-preGC	-32.44	22.38	-76.30 – 11.42	-1.45	0.147	-55.59	28.82	-112.07 – 0.89	-1.93	0.054	-2.79	1.29	-5.32 – -0.26	-2.16	**0.031**
Late-preGC	30.90	23.17	-14.52 – 76.32	1.33	0.182	35.93	29.84	-22.56 – 94.41	1.20	0.229	1.00	1.34	-1.62 – 3.62	0.75	0.453
PostGC	33.03	32.18	-30.05 – 96.10	1.03	0.305	55.93	41.44	-25.30 – 137.15	1.35	0.177	2.17	1.86	-1.47 – 5.81	1.17	0.242
OffspringGC	-34.27	22.33	-78.04 – 9.51	-1.53	0.125	-42.37	28.76	-98.74 – 14.00	-1.47	0.141	-0.89	1.29	-3.41 – 1.64	-0.69	0.492
[1]Sex [M]	23.25	23.30	-22.41 – 68.92	1.00	0.318	38.18	30.00	-20.63 – 96.98	1.27	0.203	0.33	1.34	-2.30 – 2.97	0.25	0.805
[2]Age [adu]	83.07	54.64	-24.02 – 190.16	1.52	0.128	154.95	70.36	17.05 – 292.86	2.20	**0.028**	4.79	3.15	-1.39 – 10.96	1.52	0.129
[2]Age [juv]	129.73	57.41	17.21 – 242.25	2.26	**0.024**	214.15	73.93	69.25 – 359.04	2.90	**0.004**	12.08	3.31	5.59 – 18.57	3.65	**<0.001**
[3]Season [Rich]	-150.86	25.74	-201.31 – -100.40	-5.86	**<0.001**	-231.61	33.15	-296.59 – -166.64	-6.99	**<0.001**	-7.60	1.48	-10.51 – -4.69	-5.12	**<0.001**
[4]Group [MST]	-115.78	34.37	-183.14 – -48.41	-3.37	**0.001**	-140.16	44.26	-226.91 – -53.41	-3.17	**0.002**	-4.70	1.98	-8.59 – -0.82	-2.37	**0.018**
[4]Group [SST]	-137.74	41.22	-218.52 – -56.95	-3.34	**0.001**	-173.22	53.08	-277.25 – -69.19	-3.26	**0.001**	-4.89	2.38	-9.55 – -0.23	-2.06	**0.040**
Early-preGC*Age[adu]	-49.26	73.03	-192.39 – 93.87	-0.67	0.500	-41.63	94.04	-225.94 – 142.69	-0.44	0.658	-3.43	4.21	-11.69 – 4.82	-0.82	0.415
Early-preGC*Age[juv]	60.01	45.52	-29.21 – 149.23	1.32	0.187	95.93	58.62	-18.96 – 210.82	1.64	0.102	3.81	2.63	-1.34 – 8.95	1.45	0.147
Late-preGC*Age[adu]	-50.00	35.96	-120.48 – 20.48	-1.39	0.164	-67.92	46.31	-158.69 – 22.84	-1.47	0.142	-3.32	2.07	-7.39 – 0.74	-1.60	0.109
Late-preGC*Age[juv]	-60.98	39.51	-138.42 – 16.46	-1.54	0.123	-73.95	50.88	-173.68 – 25.77	-1.45	0.146	-3.15	2.28	-7.61 – 1.32	-1.38	0.167
PostGC*Age[adu]	14.92	50.31	-83.69 – 113.54	0.30	0.767	5.51	64.79	-121.48 – 132.49	0.09	0.932	1.22	2.90	-4.47 – 6.91	0.42	0.675
PostGC*Age[juv]	9.83	39.94	-68.45 – 88.11	0.25	0.806	1.92	51.43	-98.88 – 102.73	0.04	0.970	-1.46	2.30	-5.97 – 3.06	-0.63	0.527
OffspringGC*Age[adu]	39.06	40.58	-40.47 – 118.60	0.96	0.336	36.21	52.25	-66.21 – 138.63	0.69	0.488	0.65	2.34	-3.94 – 5.24	0.28	0.781
OffspringGC*Age[juv]	35.32	35.61	-34.47 – 105.11	0.99	0.321	38.79	45.85	-51.08 – 128.67	0.85	0.398	2.82	2.05	-1.21 – 6.84	1.37	0.170

Supplementary Table 2 Full model 1d-1e explaining the offspring's gut bacteria alpha diversity (from richness-evenness estimators). Significant *P* values of explanatory variables are in bold. R^2 indicates the conditional coefficient of determination. All covariates are $\log_n$-transformed and then z-transformed (mean = 0, SD = 1) to meet model requirements and increase model interpretability. [(1)] coded with "female" as the reference category, [(2)] coded with "infant" as the reference category, [(3)] coded with "lean" as the reference category, [(4)] coded with the group "MOT" as the reference category.

	Full model 1d (Shannon) (R^2 = 0.18)					Full model 1e (inverse Simpson) (R^2 = 0.15)				
Predictors	**β**	**SE**	**CI 2.5-97.5%**	**Z**	**P-Value**	**β**	**SE**	**CI 2.5-97.5%**	**Z**	**P-Value**
(Intercept)	5.83	0.12	5.60 – 6.06	49.78	**<0.001**	125.34	13.13	99.60 – 151.08	9.54	**<0.001**
Early-preGC	0.03	0.06	-0.09 – 0.15	0.47	0.635	13.49	7.06	-0.34 – 27.31	1.91	0.056
Late-preGC	0.07	0.07	-0.06 – 0.20	1.04	0.297	11.13	7.31	-3.19 – 25.45	1.52	0.128
PostGC	0.00	0.09	-0.18 – 0.18	0.00	0.997	-8.09	10.15	-27.98 – 11.80	-0.80	0.425
OffspringGC	-0.13	0.06	-0.25 – -0.00	-2.01	**0.044**	-15.62	7.04	-29.42 – -1.81	-2.22	**0.027**
[1]Sex [M]	0.00	0.07	-0.13 – 0.13	0.04	0.967	-3.82	7.35	-18.22 – 10.58	-0.52	0.603
[2]Age [adu]	0.03	0.15	-0.27 – 0.33	0.22	0.827	-14.74	17.23	-48.50 – 19.03	-0.86	0.392
[2]Age [juv]	-0.03	0.16	-0.35 – 0.28	-0.21	0.837	-47.58	18.10	-83.06 – -12.11	-2.63	**0.009**
[3]Season [Rich]	-0.25	0.07	-0.40 – -0.11	-3.51	**<0.001**	-3.08	8.12	-18.98 – 12.83	-0.38	0.705
[4]Group [MST]	-0.29	0.10	-0.48 – -0.10	-3.03	**0.002**	-23.76	10.84	-45.00 – -2.52	-2.19	**0.028**
[4]Group [SST]	-0.37	0.12	-0.60 – -0.14	-3.18	**0.001**	-40.48	13.00	-65.95 – -15.01	-3.12	**0.002**
Early-preGC*Age[adu]	-0.16	0.21	-0.56 – 0.25	-0.76	0.445	-30.36	23.02	-75.49 – 14.77	-1.32	0.187
Early-preGC*Age[juv]	0.02	0.13	-0.23 – 0.27	0.16	0.872	-16.29	14.35	-44.42 – 11.84	-1.14	0.256
Late-preGC*Age[adu]	-0.04	0.10	-0.24 – 0.16	-0.41	0.679	-9.64	11.34	-31.86 – 12.58	-0.85	0.395
Late-preGC*Age[juv]	-0.11	0.11	-0.32 – 0.11	-0.96	0.337	10.94	12.46	-13.48 – 35.35	0.88	0.380
PostGC*Age[adu]	0.01	0.14	-0.26 – 0.29	0.09	0.928	7.09	15.86	-24.00 – 38.18	0.45	0.655
PostGC*Age[juv]	0.13	0.11	-0.09 – 0.35	1.17	0.241	17.95	12.59	-6.73 – 42.63	1.43	0.154
OffspringGC*Age[adu]	0.16	0.11	-0.06 – 0.38	1.40	0.162	20.23	12.79	-4.84 – 45.30	1.58	0.114
OffspringGC*Age[juv]	0.13	0.10	-0.06 – 0.33	1.31	0.190	-2.32	11.23	-24.32 – 19.69	-0.21	0.837

Supplementary Table 3 Reduced models 1a-1c explaining the offspring's gut bacteria alpha diversity (from richness estimators). Significant *P* values of explanatory variables are in bold. The model was tested via model comparison with null models: Model 1a: $\chi2 = 72.85$, df = 11, $p < 0.001$; Model 1b: $\chi2 = 91.98$, df = 11, $p < 0.001$; Model 1c: $\chi2 = 66.38$, df = 11, $p < 0.001$. R^2 indicates conditional coefficient of determination. All covariates are $\log_n$-transformed and then z-transformed (mean = 0, SD = 1) to meet model requirements and increase model interpretability. (1) coded with "female" as the reference category, (2) coded with "infant" as the reference category, (3) coded with "lean" as the reference category, (4) coded with the group "MOT" as the reference category.

	Model 1a (ObservedASVs) (R^2 = 0.32)					**Model 1b (ACE) (R^2 = 0.39)**					**Model 1c (PD) (R^2 = 0.29)**				
Predictors	**β**	**SE**	**CI 2.5-97.5%**	**Z**	**P-Value**	**β**	**SE**	**CI 2.5-97.5%**	**Z**	**P-Value**	**β**	**SE**	**CI 2.5-97.5%**	**Z**	**P-Value**
(Intercept)	1046.47	34.87	978.14 – 1114.81	30.01	-	1350.98	45.03	1262.72 – 1439.23	30.00	-	68.21	2.01	64.26 – 72.15	33.88	-
Early-preGC	-27.84	15.28	-57.79 – 2.11	-1.82	0.068	-40.47	19.73	-79.15 – -1.80	-2.05	**0.040**	-2.09	0.88	-3.82 – -0.36	-2.37	**0.018**
Late- preGC	9.87	14.75	-19.03 – 38.78	0.67	0.503	8.11	19.05	-29.22 – 45.44	0.43	0.670	0.00	0.85	-1.67 – 1.67	0.00	0.999
PostGC	25.31	14.89	-3.86 – 54.49	1.70	0.089	36.99	19.22	-0.69 – 74.67	1.92	0.054	1.24	0.86	-0.44 – 2.93	1.45	0.148
OffspringGC	-11.36	13.36	-37.54 – 14.82	-0.85	0.395	-17.42	17.25	-51.24 – 16.39	-1.01	0.313	-0.01	0.77	-1.52 – 1.50	-0.02	0.987
[1]Sex [M]	19.01	22.46	-25.02 – 63.03	0.85	0.397	33.29	29.01	-23.57 – 90.14	1.15	0.251	0.20	1.30	-2.34 – 2.74	0.15	0.879
[2]Age [adu]	137.55	32.13	74.58 – 200.52	4.28	**<0.001**	220.67	41.49	139.34 – 301.99	5.32	**<0.001**	8.62	1.86	4.99 – 12.26	4.65	**<0.001**
[2]Age [juv]	150.26	40.44	71.00 – 229.51	3.72	**<0.001**	251.13	52.22	148.78 – 353.49	4.81	**<0.001**	11.66	2.33	7.08 – 16.23	4.99	**<0.001**
[3]Season [Rich]	-145.82	25.18	-195.17 – -96.47	-5.79	**<0.001**	-225.83	32.52	-289.56 – -162.10	-6.95	**<0.001**	-7.00	1.45	-9.85 – -4.15	-4.82	**<0.001**
[4]Group [MST]	-85.33	28.34	-140.89 – -29.78	-3.01	**0.003**	-101.66	36.60	-173.40 – -29.91	-2.78	**0.005**	-3.11	1.64	-6.32 – 0.10	-1.90	0.058
[4]Group [SST]	-114.88	32.73	-179.02 – -50.73	-3.51	**<0.001**	-147.98	42.27	-230.82 – -65.14	-3.50	**<0.001**	-4.51	1.89	-8.21 – -0.81	-2.39	**0.017**

Supplementary Table 4 Reduced model 1d and 1e explaining the offspring's gut bacteria alpha diversity (from richness-evenness estimators). Significant *P* values of explanatory variables are in bold. The model was tested via model comparison with null models: Model 1d: $\chi2 = 29.26$, df = 15, $p = 0.015$; Model 1e: $\chi2 = 20.08$, df = 11, $p = 0.044$. R^2 indicates the conditional coefficient of determination. All covariates represent average values log-transformed and then z-transformed. All covariates are $\log_n$-transformed and then z-transformed (mean = 0, SD = 1) to meet model requirements and increase model interpretability. (1) coded with "female" as the reference category, (2) coded with "infant" as the reference category, (3) coded with "lean" as the reference category, (4) coded with the group "MOT" as the reference category.

	Model 1d (Shannon) (R^2 = 0.14)					**Model 1e (inverse Simpson) (R^2 = 0.10)**				
Predictors	**β**	**SE**	**CI 2.5-97.5%**	**Z**	**P-Value**	**β**	**SE**	**CI 2.5-97.5%**	**Z**	**P-Value**
(Intercept)	5.71	0.10	5.51 – 5.90	57.83	-	118.64	11.93	95.27 – 142.02	9.95	-
Early-preGC	-0.01	0.04	-0.10 – 0.08	-0.22	0.828	7.22	5.52	-3.61 – 18.05	1.31	0.191
Late- preGC	0.04	0.04	-0.04 – 0.12	0.92	0.358	9.73	5.13	-0.34 – 19.79	1.89	0.058
PostGC	0.02	0.04	-0.07 – 0.10	0.36	0.716	-6.54	5.32	-16.97 – 3.88	-1.23	0.218
OffspringGC	-0.03	0.04	-0.11– 0.05	-0.75	0.455	-7.82	4.46	-16.55 – 0.92	-1.75	0.080
[1]Sex [M]	0.00	0.07	-0.13 – 0.13	-0.01	0.992	-6.16	7.72	-21.30 – 8.98	-0.80	0.425
[2]Age [adu]	0.14	0.09	-0.05 – 0.32	1.45	0.148	2.70	10.97	-18.81 – 24.20	0.25	0.806
[2]Age [juv]	0.02	0.12	-0.22 – 0.25	0.09	0.925	-28.27	13.82	-55.36 – -1.19	-2.05	**0.041**
[3]Season [Rich]	-0.24	0.07	-0.38 – -0.10	-3.37	**0.001**	-5.12	7.89	-20.58 – 10.35	-0.65	0.517
[4]Group [MST]	-0.22	0.08	-0.39 – -0.06	-2.71	**0.007**	-21.75	9.80	-40.97 – -2.54	-2.22	**0.026**
[4]Group [SST]	-0.27	0.10	-0.45 – -0.08	-2.76	**0.006**	-26.49	11.42	-48.88 – -4.09	-2.32	**0.020**

Supplementary Table 5 Full linear models explaining the *Firmicutes* to *Bacteroidota* ratio of offspring's gut bacteria during the lean (Full-Model L) and the rich season (Full-Model R). [(1)] indicates that the predictor has been $\log_n$-transformed and then z-transformed with mean = 0 and SD = 1 to meet model requirements and to increase model interpretability; [(2)] indicates that the predictor has been z-transformed with mean = 0 and SD = 1; [(3)] coded with the group "MOT" as the reference category; [(3)] coded with "female" as the reference category.

Predictor	Full-Model L (pseudo-R^2 = 0.32)					Full-Model R (pseudo-R^2 = 0.91)				
	Estimate	**SE**	**95% C.I.**	**LRT**	**P-value**	**Estimate**	**SE**	**95% C.I.**	**LRT**	**P-value**
Intercept	1.123	0.006	1.112 – 1.134	-	-	1.170	0.007	1.156 – 1.183	-	-
Early-preGC[1]	-0.004	0.005	-0.015 – 0.006	-	-	-0.016	0.008	-0.031 – 0.000	-	-
Late-preGC[1]	-0.002	0.004	-0.010 – 0.006	-	-	-0.018	0.006	-0.029 – -0.007	-	-
PostGC[1]	-0.005	0.005	-0.015 – 0.005	-	-	0.008	0.008	-0.007 – 0.023	-	-
OffspringGC[1]	-0.007	0.004	-0.016 – 0.002	-	-	0.035	0.007	0.021 – 0.049	-	-
Age[2]	-0.001	0.005	-0.010 – 0.008	-	-	-0.042	0.011	-0.064 – -0.020	-	-
Sex[M][3]	0.003	0.007	-0.010 – 0.017	0.205	0.651	0.003	0.008	-0.013 – 0.019	0.157	0.692
[1]Early-preGC*Age[2]	-0.011	0.006	-0.023 – 0.001	2.859	0.091	-0.024	0.007	-0.039 – -0.010	8.333	**0.004**
[1]Late-preGC*Age[2]	0.007	0.005	-0.003 – 0.016	1.849	0.174	0.014	0.009	-0.003 – 0.031	2.275	0.131
[1]PostGC*Age[2]	0.007	0.005	-0.002 – 0.016	2.304	0.129	0.031	0.008	0.016 – 0.047	10.725	**0.001**
[1]OffspringGC*Age[2]	0.001	0.006	-0.010 – 0.012	0.029	0.865	0.017	0.007	0.003 – 0.032	5.034	**0.025**

Supplementary Table 6 Effect of *Early-preGC* in interaction with age on gut bacterial composition during the rich season. Infants are excluded from the analyses. UN = unclassified at the genus level.

Taxon	Early-preGC effect at age = mean	Early-preGC effect at age = mean+1SD	Early-preGC effect at age = mean-1SD
[Bacteroides] pectinophilus group	0.824	1.208	0.441
[Clostridium] methylpentosum group (UN)	0.000	-0.544	0.544
[Clostridium] methylpentosum group (UN)	0.000	0.329	-0.329
[Eubacterium] coprostanoligenes group (UN)	1.503	2.501	0.504
[Eubacterium] coprostanoligenes group (UN)	0.000	0.658	-0.658
[Eubacterium] coprostanoligenes group (UN)	-0.533	-1.009	-0.056
[Eubacterium] ventriosum group	0.000	-0.607	0.607
[Ruminococcus] gauvreauii group	0.000	-0.458	0.458
Acetitomaculum	1.643	2.287	1.000
Acetivibrio	-1.689	-3.042	-0.336
Actinomycetaceae (UN)	0.288	0.577	-0.001
Alloprevotella	0.810	1.522	0.099
Alloscardovia	0.520	0.858	0.183
Anaerobium	0.000	0.544	-0.544
Anaerofustis	0.351	0.629	0.074
Anaerostipes	0.324	0.669	-0.021
Anaerovoracaceae (UN)	-0.559	-1.150	0.031
Anaplasmataceae (UN)	0.257	0.473	0.041
Angelakisella	0.000	0.483	-0.483
Bacilli RF39 (UN)	0.263	0.507	0.020
Bacilli RF39 (UN)	-0.457	-0.800	-0.114
Bacteroidales (UN)	-1.616	-3.820	0.588
Bacteroidales RF16 group (UN)	-0.846	-1.869	0.177
Beijerinckiaceae (UN)	0.270	0.447	0.093
Bifidobacterium	-3.328	-5.936	-0.720
Blautia	0.457	0.946	-0.032
Brachyspira	0.460	1.164	-0.245
CAG-352	1.019	2.456	-0.418
Campylobacter	0.620	1.165	0.075
Candidatus Rhabdochlamydia	0.226	0.425	0.027
Carnobacteriaceae (UN)	0.367	0.658	0.076
Cellulomonadaceae (UN)	0.270	0.447	0.093
Cellulosilyticum	0.000	0.425	-0.425
Christensenellaceae R-7 group	-0.788	-1.396	-0.180
Clostridia UCG-014 (UN)	1.819	2.810	0.829
Clostridia UCG-014 (UN)	0.785	1.354	0.217
Clostridia UCG-014 (UN)	0.213	0.438	-0.012
Clostridia UCG-014 (UN)	-0.243	-0.371	-0.115
Clostridia vadinBB60 group (UN)	0.257	0.473	0.041
Clostridia vadinBB60 group (UN)	0.234	0.498	-0.029
Clostridia vadinBB60 group (UN)	0.000	1.013	-1.013
Clostridia vadinBB60 group (UN)	-0.467	-0.947	0.013
Clostridia vadinBB60 group (UN)	-0.530	-1.126	0.067
Clostridia vadinBB60 group (UN)	-0.543	-0.864	-0.223
Clostridium sensu stricto 5	-1.808	-4.009	0.393
Comamonadaceae (UN)	-0.684	-1.195	-0.173
Coprococcus	1.467	2.958	-0.023
Coriobacteriales (UN)	0.499	0.813	0.184
Corynebacterium	0.257	0.473	0.041
D05-2 (UN)	0.228	0.463	-0.008
Desulfovibrio	0.000	-0.713	0.713
Devosia	0.259	0.428	0.090
Devosiaceae (UN)	0.276	0.552	0.000
dgA-11 gut group	0.000	-1.066	1.066
Dorea	0.680	1.192	0.168
Eggerthellaceae (UN)	0.555	0.802	0.307
EMP-G18	-1.913	-3.644	-0.182
Enterobacteriaceae (UN)	1.335	2.458	0.212
Enterococcaceae (UN)	1.570	2.725	0.415
Erysipelatoclostridiaceae (UN)	0.595	1.550	-0.359
Erysipelotrichaceae UCG-006	0.388	0.755	0.021
Faecalibacterium	0.629	0.987	0.270
Fusobacterium	0.251	0.400	0.102
Gammaproteobacteria Incertae Sedis	0.000	0.395	-0.395
Gemella	0.681	1.071	0.291
gut metagenome	0.430	0.976	-0.117
Helicobacteraceae (UN)	0.214	0.610	-0.181
Incertae Sedis	0.000	-0.337	0.337
Intestinimonas	0.000	-0.540	0.540
Izemoplasmatales (UN)	0.200	0.477	-0.076

Lachnospira	0.334	-0.085	0.753
Lachnospiraceae (UN)	0.454	0.997	-0.089
Lachnospiraceae UCG-001	0.991	1.760	0.222
Lachnospiraceae UCG-007	0.449	0.859	0.039
Lachnospiraceae UCG-009	0.316	0.540	0.092
Lactobacillus	0.000	0.925	-0.925
Megasphaera	0.000	1.404	-1.404
Microbacteriaceae (UN)	0.218	0.431	0.004
Micromonosporaceae (UN)	0.350	0.661	0.039
Mobilitalea	0.000	0.290	-0.290
Muribaculaceae (UN)	-1.013	-1.344	-0.681
Mycobacteriaceae (UN)	0.365	0.643	0.087
Negativibacillus	0.305	0.562	0.049
Neisseria	0.348	0.735	-0.038
Oscillibacter	0.366	0.657	0.074
Oscillospira	-1.172	-2.648	0.303
Oxalobacter	0.000	-0.490	0.490
Oxalobacteraceae (UN)	0.257	0.473	0.041
Paracaedibacteraceae (UN)	0.282	0.426	0.137
Peptococcus	0.000	0.316	-0.316
Peptostreptococcaceae (UN)	-0.778	-1.431	-0.125
Porphyromonas	0.228	0.463	-0.008
Prevotella 9	0.339	0.691	-0.013
Prevotellaceae UCG-003	0.832	1.995	-0.331
Proteobacterium (UN)	0.000	-0.857	0.857
Pseudomonadaceae (UN)	0.256	0.482	0.030
RF39 (UN)	-0.782	-1.380	-0.183
Rhizobiaceae (UN)	0.000	0.247	-0.247
Rhodospirillales (UN)	0.446	0.919	-0.027
Rickettsiella	0.263	0.507	0.020
Rikenellaceae (UN)	-0.400	-1.364	0.563
Rodentibacter	0.451	0.767	0.135
Roseburia	0.000	0.434	-0.434
Rothia	0.373	0.748	-0.001
Ruminococcaceae (UN)	0.298	0.632	-0.035
Ruminococcaceae (UN)	-0.711	-1.277	-0.146
Ruminococcus	0.000	0.438	-0.438
Sphingomonadaceae (UN)	0.325	0.606	0.043
Staphylococcaceae (UN)	0.192	0.501	-0.118
Streptococcaceae (UN)	1.622	3.256	-0.013
Tsukamurellaceae (UN)	0.319	0.536	0.101
Tyzzerella	1.928	3.586	0.270
UCG-002 (UN)	-0.597	-1.207	0.012
UCG-004 (UN)	-0.590	-1.587	0.407
UCG-009 (UN)	-0.463	-1.030	0.104
UCG-010 (UN)	0.282	0.426	0.137
UCG-011 (UN)	0.000	-0.654	0.654
Bacteroidaceae (UN)	1.196	2.234	0.159
Ureaplasma	0.628	1.292	-0.037
Yersiniaceae (UN)	0.263	0.507	0.020

Chapter 4 - Parallel laser photogrammetry

Growth trajectories of wild Assamese macaques (*Macaca assamensis*) determined from parallel laser photogrammetry

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Abstract

Socioecological factors are associated with life history patterns and growth trajectories among primates. Under certain conditions, selection may favor a temporal decoupling of growth and major life history events such as sexual maturation or natal dispersal. Yet, empirical tests of these associations in wild populations remain scarce owing to the lack of non-invasive methods to capture growth trajectories. In this study, we first compared two non-invasive methods of digital photogrammetry. Then we used parallel laser photogrammetry to investigate forearm growth of wild Assamese macaque males and females in their natural habitat at Phu Khieo Wildlife Sanctuary, Thailand to test life-history and socio-ecological hypotheses. Across 48 males and 44 females, we estimated growth trajectories and pseudo-velocity curves by applying quadratic plateau models and non-parametric LOESS regressions. We assessed the development of sexual dimorphism by comparing the sexes at five different ages. Females had completed 96% of their growth at the age at first birth (5.9 years) and ceased growing at 7.1 years of age. Males, in contrast, grew until well after their average age of natal dispersal: they reached 81% of their size at the age of natal dispersal (4.0 years), and ceased growing only at 9.0 years of age, much later than females. Sexual dimorphism in forearm length was driven by an extended growth period in males, which is expected for males dispersing between multimale-multifemale groups and not facing the risk of being ousted by other larger males. Our results contradict the *neonatal investment hypothesis* that predicts a desynchronization of investment in growth and reproduction only in female baboons, but not other papionins producing cheaper neonates. Furthermore, male Assamese macaques do not delay natal dispersal until they are fully grown, in accordance with predictions of the *male career framework* for species with low to medium level of direct competition.

Keywords: Development, sexual dimorphism, life history, neonatal investment hypothesis, digital photogrammetry, parallel lasers, male career framework

Introduction

Darwinian fitness can be enhanced by growing fast to mature early, reproducing at higher rates than others, producing higher quality offspring, and surviving better to enjoy a long reproductive life span (Stearns 1976). The central tenet of life history theory is that resource allocation trade-offs keep individuals from realizing this life history strategy (Stearns 1989). As a result, none of the fundamental processes in life history may always work at the species' maximum capacity (Law 1979). This is evident from the observation that growth velocity can change dramatically during species-typical age-related growth spurts or individualized catch-up growth after facing a period of resource restriction (Dimitriew 2011). Somatic growth is energetically costly and therefore predicted to be traded off against other energy-consuming processes, the most prominent being reproduction (Roff 1992). Such trade-offs are particularly important in primates, because of their low total energy expenditure compared to other placental mammals of similar mass which constrain available energy (Pontzer et al. 2014). As a consequence, according to the *neonatal investment hypothesis* proposed by Leigh

and Bernstein (2006) female primates investing simultaneously in their own somatic growth and in offspring production may not be able to produce high quality precocial neonates and therefore need to complete as much growth as possible before reproduction. Put the other way around, the more altricial the young of a species, the more overlap is predicted for late somatic growth and first reproduction (Leigh & Bernstein 2006).

This hypothesis on neonatal investment was supported by comparisons of olive baboons (*Papio hamadryas anubis*) with other primates of the tribe papionini (Disotell et al. 1992): rhesus macaques (*Macaca mulatta*), mandrills (*Mandrillus sphinx*), and mangabeys (*Lophocebus spp.*, C*ercocebus ssp.*, Leigh & Bernstein 2006). Baboon neonates are regarded as rather precocial because they exhibit a prenatal growth spurt in brain size during the last trimester of gestation and therefore are born with relatively larger and costlier to produce brains than other papionines including rhesus macaques (Leigh & Bernstein 2006). This maternal investment in fetal development is hypothesized to be responsible for a shift in onset of female reproduction that occurs relatively late in baboons when all teeth have erupted and somatic growth is much advanced and almost completed which effectively desynchronizes growth and reproduction. Based on pre- and postnatal brain growth, mangabeys, mandrills, and macaques are thought to produce cheaper offspring that allow for simultaneous investment in offspring and maternal growth (Leigh & Bernstein 2006).

A recent study provided comparative data on wild geladas (*Therophitecus gelada*) which produce cheap offspring: neonatal body and brain size are small perhaps resulting from energetic constraints from a herbivorous diet and mothers readily terminate pregnancies in face of increased infanticide risk (Roberts et al. 2012; Lu et al. 2016). Contradicting the neonatal investment hypothesis, gelada females completed 97% of their growth in body length at first birth, i.e. they desynchronize growth and reproduction just like baboons. Here, we follow analytical methods laid out in that study (Lu et al. 2016) and contribute another data set on growth and reproduction in a wild papionin primate, the Assamese macaques (*Macaca assamensis)*.

We assess whether sexual size dimorphism develops over ontogeny via increased growth rates, extended growth periods, or their combination, and whether this pattern depends on the socio-ecology of the species that determines the type and strength of external mortality risks (Shea 1986; Leigh 1995). For species living in one-male-multifemale groups where male immatures face aggressive expulsion from their natal group upon breeder male replacement followed by a period of life outside of bisexual groups it is predicted that males exhibit increased growth rates relative to females early in development (Shea 1986; Leigh 1995). Conversely, males in multimale-multifemale societies like Assamese macaques are predicted to extend their growth period and either outgrow females after dispersal or delay dispersal until there are fully grown.

We further explore in our study species sexual dimorphism in growth of a linear dimension in relation to life history events. Several aspects of primate male life history have been described to co-vary and form a continuum of male career trajectories (*male-career-framework*, van Noordwijk & van Schaik 2004). Careers vary in the mode of acquiring social status (challenge for alpha position, cooperative strategies, or queuing), decisions about the mode of transfer between groups (individual or joint migration, targeting groups with weakest alpha or with most females or most female-biased sex ratio), and notably about the timing of natal

dispersal in relation to competitive ability, i.e. body size. At the highest levels of contest competition and paternity concentration in the alpha male, males attain top social status by direct challenge and therefore migrate individually into the group with the weakest alpha male and time their natal dispersal with their maximum physical fighting ability (e.g., crested macaques, *Macaca nigra*, Marty et al. 2015, 2016). At the other extreme at low contest competition and low male reproductive skew, tenure in a social group may determine dominance rank. Males may queue but do not fight for status, they target groups with a maximum number of mating partners and often transfer jointly especially during natal dispersal that occurs well before males reach their full fighting ability (van Noordwijk & van Schaik 2004). In Assamese macaques, males migrate before reproducing and females are philopatric. The study population exhibits low to medium male contest competition at 29% alpha male paternity (Sukmak et al. 2014).

With this study we aim to first establish parallel laser photogrammetry as a method of measuring size in wild Assamese macaques. We present sources of measurement error and their relationship to inter-individual differences in the dimension measured as well as a comparison with an alternative photogrammetric method. The second aim is to test the *neonatal investment hypothesis.* Given that congeneric rhesus macaques produce cheap neonates and that the hypothesis is set up to explain differences between baboons and all other papionins, we predicted substantial overlap between maternal growth and reproduction, i.e. that females continue to grow long after they had their first infant. As our third aim we test predictions about the ontogeny of sexual dimorphism and from the *male-career-framework*. We predicted that males grow longer but not faster than females and that due to their low-medium level of direct male competition, male Assamese macaques disperse from their natal group well before they are fully grown.

Methods

Study population and demography

Data have been collected on a population of Assamese macaques that has been studied since 2005 and lives in its natural habitat in Phu Khieo Wildlife Sanctuary (16°5'-35'N, 101°20'-55'E, part of a >6500km² system of connected protected forests) in Northeast Thailand. Phu Khieo Wildlife Sanctuary has the highest protection status Thailand offers for the conservation of plants and wildlife. The terrain at the local study site Huai Mai Sot Yai (16°27′ N, 101°38′ E) is hilly at 600-800m a.s.l. and the habitat comprises mainly hill and dry evergreen forest (Borries et al. 2002). The study population is frugivorous with the main part of their plant diet comprising fruit, pulp, and seeds, and a considerable part of their feeding time budget devoted to slow and low intake foraging for animal matter (Schülke et al. 2011; Heesen et al. 2013, Touitou et al. 2021a). Individuals measured for this study lived in four neighboring social groups with overlapping home-ranges.

Age at weaning is approximately 12 months based on last observations of nipple contact (Ostner et al. 2013; Berghänel et al. 2016). For assessment of age at natal dispersal from the demographic records of the long-term project, we only used individuals that were born into our study groups after habituation to human observers

was accomplished with birthdates often known to the day (median precision ±1.2, mean 10.7, maximum 60 days). The first study group we followed since 2006 split in two in 2011 and the second one we followed since 2012 split in 2014; all four resulting groups have been followed since. These group splits were relevant when determining the average age at natal dispersal for males. We once calculated average age at natal dispersal very conservatively only across those males that did not experience a group split before their first dispersal. Since both groups split along matrilines (De Moor et al. 2020), if males left their mother in the course of a group split, they also left all of their maternal female kin. Therefore, we can interpret a group split as a natal dispersal event if an immature ended up in another sub-group than its mother which we did for our second calculation. The mean age in years at natal dispersal was the same when calculated including (4.0 ± 1.2 years mean ± SD, N=41) or excluding (4.0 ± 1.20) eleven males that experienced a group split the way described above.

Reproduction was seasonal with 79% of 201 births recorded between 2006 and 2019 occurring in the three months from April through June (Touitou et al. 2021a; unpubl. data). Female age at first reproduction was assessed from live birth events and averaged 5.9 ± 0.5 years (mean ± SD, range 5.0 - 7.0 years) in 40 first-time mothers born into the study groups after habituation with mother birth dates often known to the day (median 2.5, mean 13 days) and offspring birth dates estimated with a precision of 21 days on average, median 11 days. For some of the adults and first births in the sample, only the year of birth was known; for this analysis their birth day was estimated at the middle of the birth season, i.e. May 15th, of the respective year with a precision of 60 days.

Laser device set-up and sampling regime

We took pictures with a Nikon D7100 camera and an AF-S Nikkor 18-200 G II EO lens with a resolution of 6000 x 4000 pixels at distances between 0.54m and 6.71m (mean 2.20m) from the object measured for 375 photos with a digital laser range finder (Bosch PLR 25, Bosch, Gerling, Germany). A custom-made laser box with three green lasers (DD532-1-3(16x60), Picotronic, Koblenz, Germany), situated in an aluminum block and powered by 6 AA rechargeable batteries was mounted under the camera and used to project a size standard onto the object to be measured. The three lasers were oriented in an L shape with 2cm length on each arm and thus formed a right isosceles triangle (Galbany et al. 2016; Fig. 1). The idea was that if the object to measure was large enough, all three laser dots could be projected onto it and most deviations from the object dimension to be measured being perpendicular to the camera-object axis will result in relative distances between projected laser dots deviating from the right isosceles triangle formed by the lasers (Fig. A1). Yet, even if the object was too small to accommodate all three laser dots, as in infant Assamese macaques, the set-up will allow measuring objects that were oriented roughly horizontally or vertically without turning the laser-mounted camera.

The choice of dimension used to measure body size was constrained by substrate use. Body length is best measured as head-rump-length or shoulder-rump length if the animal stands on a horizontal surface (Lu et al. 2016), typically on the ground (but see Rothman et al. 2008). Given that Assamese macaques are highly arboreal (Schülke et al. 2011) and rarely stand on horizontal surfaces, we chose to measure the length of the

lower arm from wrist to elbow (Turnquist & Kessler 1989; Berghänel et al. 2015). As a result of that choice and because of our particular interest in immature growth, the distance between lasers had to be rather small (2cm) to fit on the lower arm also of the youngest infants (see Fig. 1 for typical postures targeted by photographers). We ensured that the assumption of parallel orientation of lasers was not violated by controlling the 2cm reference distance between lasers projected on photos of graph paper at different camera-object distances. These data are referred to as the parallel laser data set in the following.

Lower arm length was also measured for the data from Berghänel et al. (2015) who used a slightly different method. Instead of parallel lasers, we used a Nikon D5000 camera and a digital laser range finder (Bosch PLR 50) to synchronously take the picture and measure the distance between the camera and the object. Length of the lower arm was then calculated using the intercept theorem by multiplying the number of pixels in the picture (determined with ImageJ 1.44p, National Institutes of Health) with the distance. These data are referred to as the distance meter data set in the following.

The sampling regime was similar for both the parallel laser data set and the distance meter data set. To eventually generate one size estimate for an individual of a given age, several photos were taken within a 1-2 weeks period. Juvenile and adult individuals of different ages were measured during only one of these periods, whereas the 2011, 2012, and 2018 birth cohorts were each measured repeatedly in a longitudinal sampling design at several of these periods scattered across their first year of life. We excluded photos of poor quality (exposure, focus, obvious parallax) and with size estimates deviating more than two standard deviations from the mean across photos taken from the same individual within one period (Between photograph error; see below). After quantifying different types of measurement error, we further excluded all repeated measurements of the same individual at different ages. We used only one size estimate per individual (mean over a maximum of six photos) for estimating growth trajectories and sexual size differences from this cross-sectional sample. Because of these steps in excluding photos, sample size differs for different analyses and descriptive statistics across this paper.

With parallel lasers projected on the monkey, we collected a total of 1826 photos (1748 between 2016 and 2018 and 78 in 2021) from 48 males and 44 females. For descriptive purposes we classified ages into infant (0-1 year, 16 males, 8 females), juvenile (1-6 years, 19 males, 15 females) and adult (>6 years, 13 males, 21 females; intervals included the upper boundary value). Please note that we used one cut-off for adult males and females here although males mature more slowly and reach adulthood only with 7.4 years of age on average (N=18) and are therefore classified differently in our other publications. Error estimates were derived from 782 pictures (5.35 ± 0.72 per subject; mean ± se) remaining after excluding low quality and outlier pictures.

The full distance meter data set comprised 1571 photos from 30 males and 31 females. We classified ages with the same criteria (infants: 6 males, 6 females; juveniles: 14 males, 16 females; adults: 10 males, 9 females).

Estimating size from photographs

We used ImageJ software (version 1.52a; Schneider et al. 2012) to measure: (i) the 2cm reference distance between two lasers in pixel number (D), (ii) the subjects' forearm length in pixel number (L_{pxl}) from the elbow to the wrist, and then (iii) we used the 2cm reference distance to transform the forearm length from pixel to centimeters (L) with the following formula: $L = \frac{2 * L_{pxl}}{D}$. The multiplier of L_{pxl} value (here 2) is derived from the reference distance in centimeters (Figure 1).

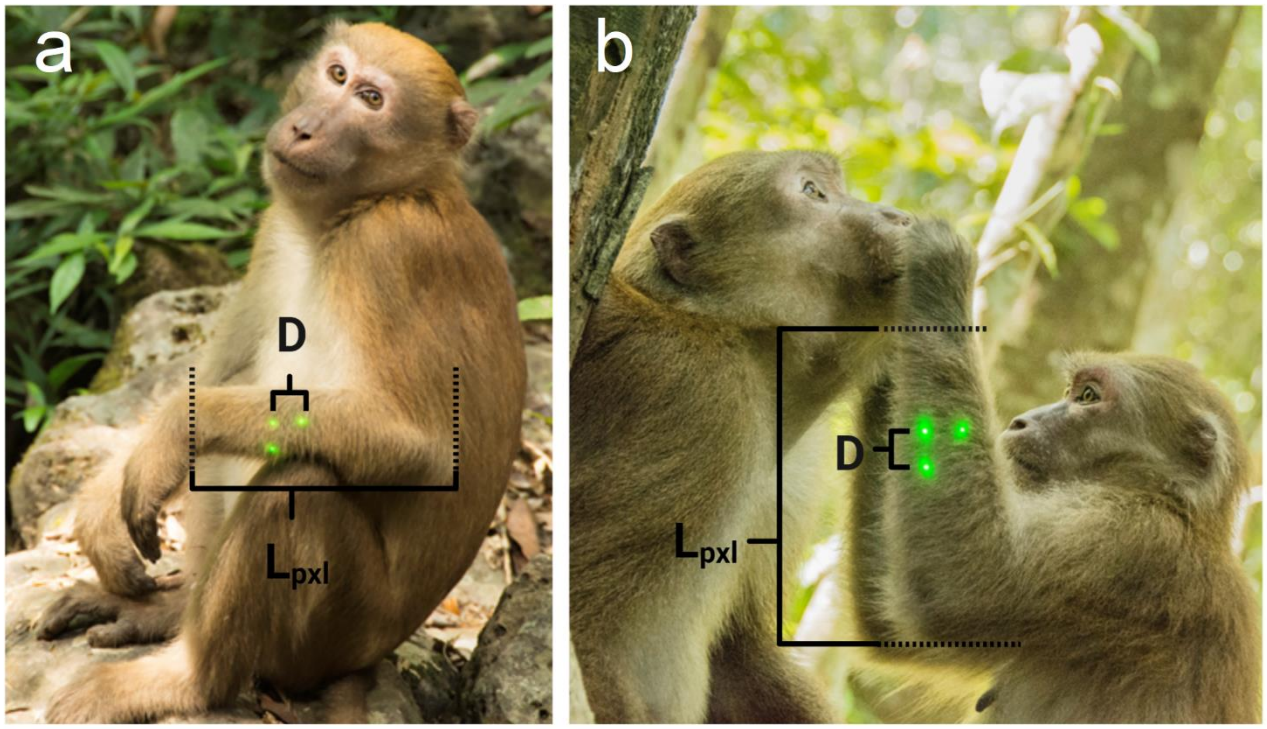

Fig. 2 Adult Assamese macaques (*Macaca assamensis*) and laser dots projected on their forearm in (a) horizontal and (b) vertical position. Panel a shows a male resting in a typical posture with flexed elbow and wrist. Panel b shows a female grooming a male with the elbow flexed. Reference distance D = 2 cm distance between lasers measured in the digital picture as pixel number. L_{pxl} = forearm length measured as number of pixels in picture and transformed to length in cm (L) from reference distance (D). The photographs were taken by Simone Anzà at Phu Khieo Wildlife Sanctuary (Thailand).

Remote measuring with parallel laser has been shown to be highly accurate (Bergeron, 2007; Rothman et al., 2008; Deakos, 2010; Barrickman et al., 2015; Galbany et al., 2016; Galbany et al., 2017), yet potential error sources associated with human measurement of digital photographs can affect precision (Barrickman et al., 2015; Lu et al., 2016; Galbany et al., 2016; Galbany et al., 2017). To test the quality of our method, we assessed three types of error in measurement repeatability: (i) *Repeated measurement error*, (ii) *Between photograph error*, and (iii) *Between measurer error*. Two observers, or measurers (S. Anzà and an assistant, Pearl Väth), performed all digital measurements. Error was expressed as the coefficient of variation (CV) in % of the absolute differences. We calculated the *Repeated measurement error* from 555 pictures each measured twice by the same observer (Pearl Väth). We estimated the *Between photograph error* from different photographs of the same animal shot at different days. We calculated this error twice, once per each measurer (709 and 627 pictures from a total of 782) and also report the average for those pictures measured by both observers (*Between*

photograph error mean: 585 pictures). We estimated the *Between measurer error* by comparing measurements from the same photograph between the two observers (N=555). We excluded from further analyses all pictures with a *Between photograph error* larger than 2 standard deviations. We compared our results with the *Between photograph error* (N=1571) and the *Between measurer error* (N=179) estimated from the distance meter dataset; Table A4). We provide detailed information on types of error and specific age-related tables in the results section.

Growth trajectories and pseudo-velocity curves

We estimated growth trajectories separately for 48 males and 44 females. Date of birth was known (40 males, 38 females) or estimated to the year of birth with the middle of the birth season set as birth date (8 males, 6 females). We built quadratic plateau models and ran local polynomial regressions (Locally Estimated Scatterplot Smoothing – LOESS) using the software RStudio 1.3.1093 (RStudio Team, 2021; with R 4.0.4 - R Core Development Team, 2021) and packages *stats* (version 4.0.3), *easynls* (version 5.0), *nlstools* (version 1.0-2; Baty et al. 2015), *rcompanion* (version 2.4.0; Mangiafico 2016). While LOESS fits consecutive segments using subsets of data and therefore does not require any specified function, quadratic plateau models assume the data follow a quadratic curve up to a critical value, and then settle into a plateau. The critical value can be interpreted as age at growth cessation (Leigh, 1994; Leigh & Terranova, 1998; O'Mara et al., 2012; Lu et la., 2016).

$$\mathrm{Y} = \mathrm{a}x^2 + \mathrm{b}x + \mathrm{c} \quad \text{for} \quad x \leq -\frac{\mathrm{b}}{2a}$$

$$\mathrm{Y} = -\frac{b^2}{4\mathrm{a}} + \mathrm{c} \qquad \text{for} \quad x > -\frac{\mathrm{b}}{2a}$$

Where c = neonatal size, $-b^2/4a + c$ = adult body size and $-b/2a$ = critical value (age at reaching adult body size). Next, we estimated 2.5%-97.5% confidence intervals around the four parameters (a, b, c, critical value) with 999 bootstrap iterations, and used the predicted values as estimates of the percentage of growth at specific life-history milestones. We compared the quadratic plateau models built from the parallel laser data set with models built in the same way from the distance meter data set using only cross-sectional data (each individual only contributing one data point; 323 photos from 61 individuals; Males: 6 infants, 14 juveniles, 10 adults; Females: 6 infants, 16 juveniles, 9 adults; Berghänel et al., 2015). Previous work on growth trajectories in geladas showed that the quadratic plateau model performs well on a global level but poorly fits neonatal size (Lu et al., 2016). We therefore excluded the information on predicted size at birth.

We estimated pseudo-velocity curves of growth in forearm length (Coelho et al., 1985; Lu et al., 2016; Galbany et al., 2017) by dividing the difference in successive predicted values by the difference in successive age values, and smoothing the resulting values using LOESS curve fitting (Setchell et al., 2001) for both sexes separately. The degree of smoothing can be set by adjusting the time span over which it is applied. We chose 0.6 as a compromise between precision and confidence. The effects of smoothing on pseudo-velocity curves

is demonstrated in Figure A2. Then, we estimated sex-related differences in growth by comparing residual differences in size for age.

To assess sexual dimorphism in body size, we compared forearm lengths between males and females at different ages (0-1, 1-3, 3-5, 5-7, and >7years old, intervals including the upper boundary value). Two-year blocks were chosen arbitrarily starting at weaning. We performed Mann-Whitney-U-tests using the function *wilcox.test* of the R base package *stats*, and Benjamini-Hochberg correction for multiple testing.

Results

Precision of photogrammetry

Several sources of error in the measurement of forearm length were assessed (Table 1). The error from the same person repeatedly measuring size from the same photo (*Repeated measurement error*) was rather small with 2.67%. The error from measuring the same individual from several different photos taken within a 2-week window (*Between photograph error*) was 3.26% to 4.66% depending on how information from different observers were integrated. Two observers measuring the same photo (*Between measurer error*) disagreed by 5.29%. All these sources of error were sensitive to the size of the object as evident from errors declining from estimates for adult monkeys only (e.g. mean across observers of *Between photograph error* 2.90%), via juveniles (3.51%) to infants where the absolute forearm length was the smallest and the measurement error the largest (5.30%, Tables A1-A3).

In the distance meter data set, measurement errors were similar both the *Between measurer error* (mean across age classes = 4.44%) and the *Between photograph error* (mean across age classes = 3.67%). The *Between photograph error* also decreased with age (infants = 4.37%, juveniles 3.58%, adults 3.06%, Table A4) showing the same sensitivity to object size.

The inter-individual variation in the dimension of interest was at its maximum in infants (16.61%), decreased with age but stabilized at around 7% from age 4 onwards (Tab. 3, parallel laser photogrammetry). Thus, inter-individual variation in lower arm length was much larger than any of the measurement errors.

Growth trajectories

Estimates of age at growth cessation from the best fit quadratic plateau models of growth in females and males (Table 2 and Figure 2) suggested that females stopped growing earlier than males (non-overlapping confidence intervals of critical values). More precisely, females grew until they were 7.1 years of age whereas males continued to grow for two more years until they were 8.9 years old. As a consequence, females had completed 96% of growth at 5.9 years, the age at first birth (compared to 91% for males at this age). At 4.0 years, the average age of male natal dispersal, males had finished 80% of growth (females 88%). Males went through 96% of their growth until they reached adulthood at the average age of full canine size development and full

testicular enlargement (7.2 years, N=18 unpubl.data). Very similar patterns were seen when using the distance meter data set from 2011-2012 (Berghänel et al. 2015; Figure A3).

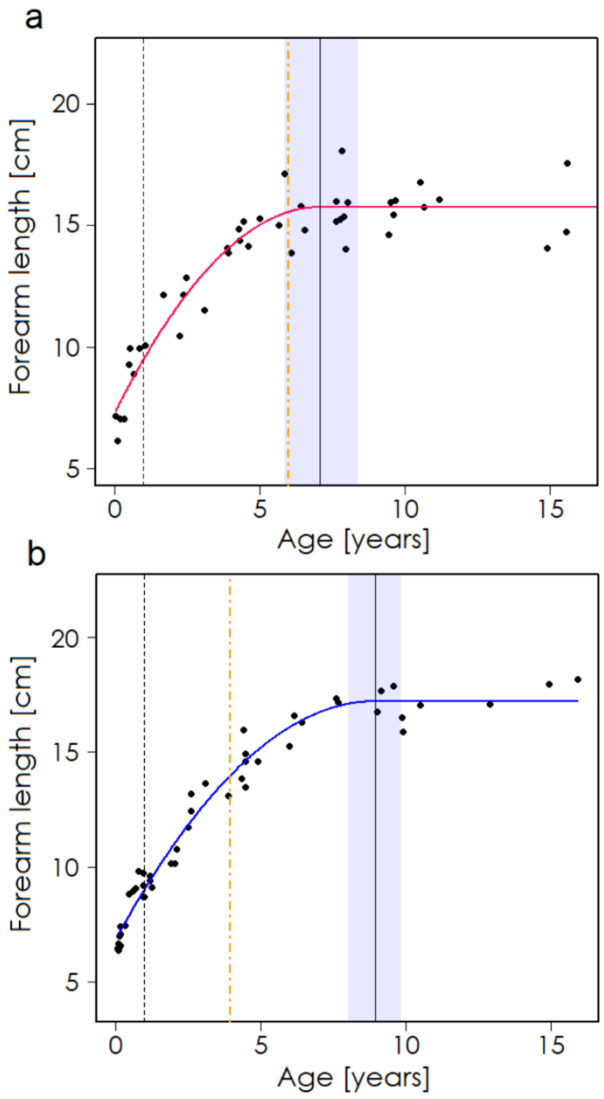

Fig. 2 Growth curves for (a) females and (b) males estimated with separate quadratic plateau models. Dashed black line indicates age at weaning (1.0 year), orange dash-dotted line indicates mean age (a) at first birth (5.9 years) in females (b) at natal dispersal (4.0 years) in males. Solid black line indicates the model estimated age at cessation of growth with the 95% confidence interval shown as the shaded area. First birth coincides with growth cessation whereas natal dispersal occurs years before males stop growing.

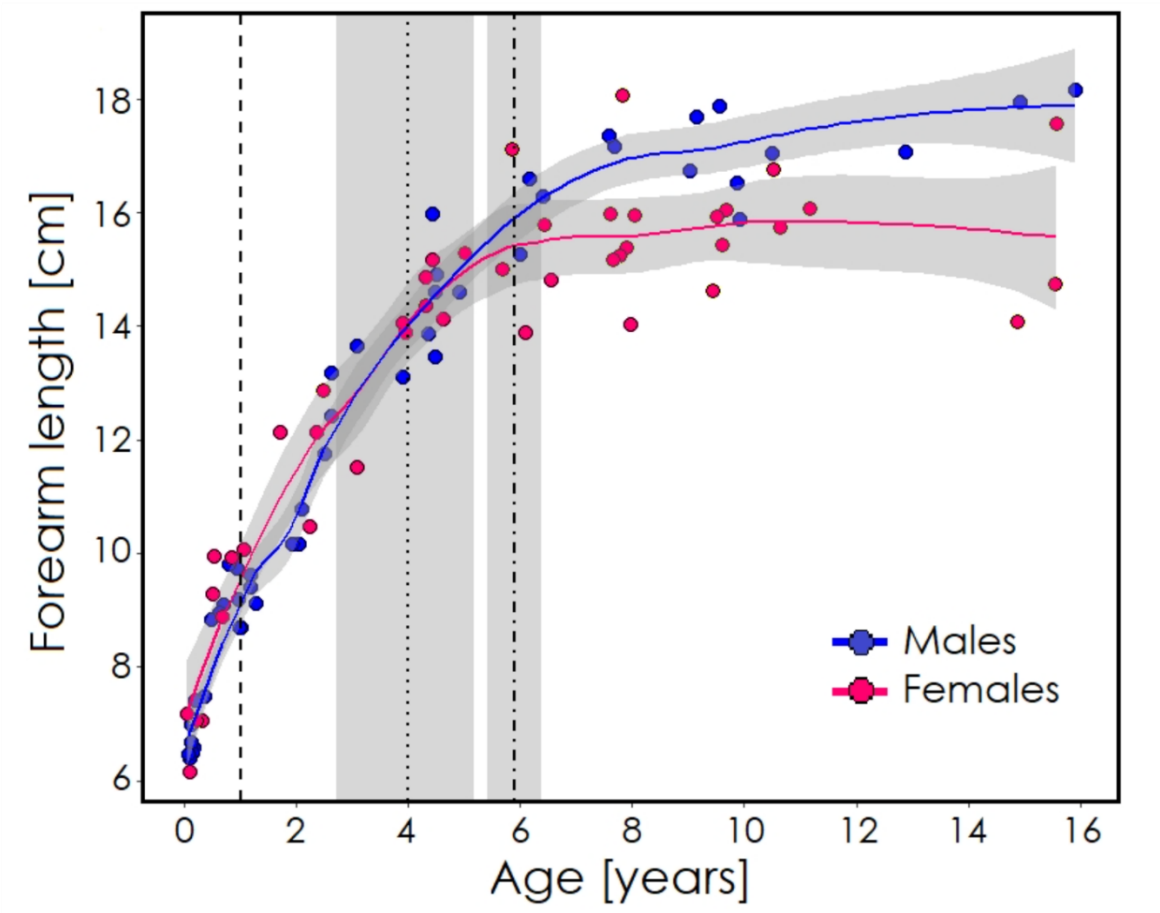

Fig. 3 Growth curves of females and males fitted with non-parametric LOESS regression method, and developmental milestones. Dashed line indicates age at weaning (1.0 year), dotted line indicates mean age at natal dispersal (4.0 years) in males, and dash-dotted line indicates mean age at first birth in females (5.9 years). The grey shaded area around the vertical lines depicts the standard deviation around the age at natal dispersal (SD = 1.23) and the age at first birth (SD = 0.48), respectively. Confidence intervals around the LOESS curve are larger for females (yellow) than males (blue) from age 2 through 10. We selected a span value of 0.60 by visually identifying more conservative curves with less "noise" produced by the local regression method.

Non-parametric LOESS regressions of growth (Figure 3) and pseudo-velocity curves (Figure 4) suggested that female growth rate declined rather steadily from birth (2.8 cm/year) until six years of age (0.3 cm/year) with a reduction in speed of -0.4cm/year per year. From six years onwards, the change in growth velocity was much smaller at -0.1cm/year per year. The pseudo-velocity curve fitted to size data from males has similarities and differences with the one for females. Both sexes show a similarly steep decline in growth rate during the first year of life down to 2.1cm/year in males and 2.3cm/year in females at age 1. The estimated speed of growth for males in their second year of life is associated with considerable uncertainty and appears slightly faster than females. However, the same delay in male growth rate decline is not found in the distance meter data set for males aged 1-2 years (Figure A4) and inspection of the overlaid LOESS curves (Fig. 3) also suggests that growth trajectories of males and females did not differ strongly from birth until age 4-4.5 years. After that, male growth rate declined more slowly than that of females which bottomed out soon after age six.

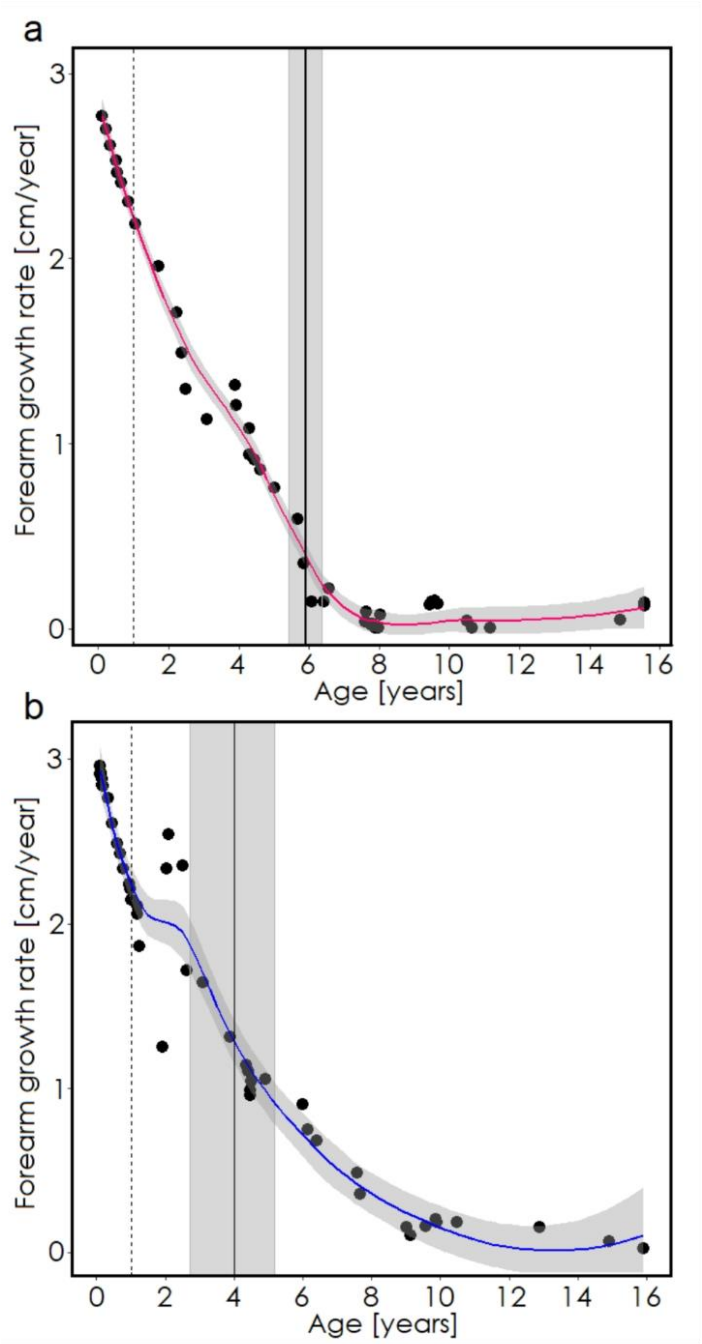

Fig. 4 Pseudo-velocity curve for (a) females and (b) males estimated by non-parametric LOESS regression method, and developmental milestones. Dashed line indicates age at weaning (1.0 year). Solid black line indicates age at first birth for females (5.9 years) and age at natal dispersal for males (4.0 years) with gray shades indicating standard deviation around those measures (0.48 for age at first birth and 1.23 for age at natal dispersal). We selected a span value of 0.60 by visually identifying more conservative curves with less "noise" produced by the local regression method (Figure A2).

As a consequence of these growth trajectories, sexual dimorphism in forearm length was not detected until age 5-7 (Table 3) when males tended to be larger than females (F = 15.3 cm, M = 16.1 cm; adjusted $p = 0.060$). From seven years of age onwards, males had clearly outgrown the females (F = 16.0 cm, M = 17.4 cm, adjusted $p < 0.001$).

Discussion

Photogrammetry

Assamese macaques are highly arboreal in the study population and spend 60% of time away from the ground and low storey of the forest, which makes it difficult to weigh them on a balance (Schülke et al. 2011), especially if the balance cannot be baited with food. Therefore, in this study we used non-invasive parallel-laser photogrammetry to measure growth trajectories from skeletal dimensions (Galbany et al., 2016). In a previous project, we had used distance to object measured with a digital laser range finder to convert pixel number in digital photos to actual size in centimeters (Berghänel et al. 2015, 2016). With the box holding the parallel lasers being mounted under the camera, the new method was less demanding in the field and should eventually allow for computer guided automation of measurements in digital photographs (see Richardson et al. 2022 in this Special Issue).

The repeatability of single measurements was not ideal which partly is owed to the small size of the body part measured (the forearm) which also forced us to use a rather small between-laser distance of 2 cm (25% of body part in infants, 15% in juveniles, 12% in adults). Repeatability increased with increasing size of the animal from infants via juveniles to adults. Other primate studies have used 4 cm laser spacing, measured larger body parts of larger animals, and achieved lower measurement errors around 2cm (Rothman et al. 2008; Barrickman et al 2015; Lu et al 2016). With a laser spacing of 4 cm used to measure a trait of 2-8cm size Bergeron (2007) produced an intermediate error of 3.7%. As a further point in case, in a study using 50 cm laser spacing for morphometrics of whale sharks, measurement error increased the smaller the somatic structure that was measured (Jeffreys et al. 2013). The error in the arm length estimate was similar in the parallel laser data set and the distance meter data set, but we were unable to properly propagate errors for both methods in similar ways; the parallel laser method holds errors from counting pixels between laser dots and from counting pixels on the forearm, whereas with the distance meter method error from counting pixels on forearm have to be considered along with unknown error from camera-object distance measurement (for a more direct comparison between these methods see Galbany et al. 2016). Repeatability of photogrammetric studies will be enhanced when automated computer assisted methods become available for identifying locations of and distances a) between projected laser dots and b) surface landmarks of interest (see Richardson et al. 2022 in this Special Issue).

One important source of error (parallax) results from the camera-object axis not being perfectly perpendicular to the dimension to be measured (Deakos et al. 2010). Possible issues with parallax distortion can be identified, but not easily corrected, if more than two lasers of known position are used and if all points can be projected on a rather plane surface.

Life history

The data presented here suggest that female Assamese macaques living in their natural habitat completed the vast majority of their growth in forearm length before they started to reproduce. With 96% of growth finished

at first birth, wild Assamese macaques were more similar to captive olive baboons (~100%) than to captive mangabeys (90%, Leigh & Bernstein 2006). Together with evidence on the rather close coincidence of growth cessation and first birth in wild geladas (Lu et al. 2016), these results contradict the *neonatal investment hypothesis* (Leigh & Bernstein 2006) which suggests that the production of rather costly offspring selected for a desynchronization of growth and reproduction in the genus *Papio*, but not in other papionins.

Discrepancy between original work and recent tests of the hypothesis concern i) the choice of body size metric, ii) methods to determine age at growth cessation, and iii) food provisioning, and iv) assumptions about the costs of producing neonates. Body size was measured by body mass or crown-rump length by Leigh & Bernstein (2006), yet the gelada study (Lu et al. 2016) used shoulder-rump length and the current study is based on forearm length. The latter two studies are non-contact, photogrammetric studies using parallel lasers which require taking measurements at highly repeatable body postures. The arboreal Assamese macaques studied here are rarely found standing on a rather horizontal surface to be measured. Gelada head position when standing is variable and affects crown-rump length, making the metric less reliable. The shoulder is a more tangible landmark (Lu et al. 2016). If the metrics chosen for the photogrammetric studies followed different growth trajectories than crown-rump length as measured by Leigh & Bernstein (2006), age at growth cessation would be affected and the conclusions regarding the neonatal investment hypothesis would be invalid. Evaluation of this argument is hampered by the lack of repeatable objective metrics of growth cessation (e.g., estimation from a quadratic plateau model) in earlier studies. Comparative growth data are available for provisioned rhesus macaques and wild toque macaques (*Macaca sinica*) and our visual inspection of tabulated (Turnquist & Keller 1989) and plotted data (Cheverud et al. 1992) suggest forearm growth in rhesus and arm growth in toque macaques to cease at the same age as crown-rump growth in the respective data sets. We conclude that our growth trajectories for forearm length of a third macaque species is comparable to trajectories of crown-rump length of other papionins and note that body arm length follow slightly different age trajectories in a large great ape species (*Gorilla gorilla berengei*, Galbany et al. 2017). It remains to be shown whether comparative data on body mass growth in wild baboons and other papionins supports the *neonatal investment hypothesis.*

The second predictor from the neonatal investment hypothesis is age at first reproduction which may be systematically different in the recent studies on wild populations experiencing natural fluctuations in food availability compared to the captive and food provisioned groups studied by Leigh and Bernstein (2006). Female cercopithecid primates experiencing food enhancement show marked shifts towards earlier maturation and onset of reproduction (Strum & Western 1982; Sugiyama & Ohsawa 1982; Borries et al. 2001; Altmann & Alberts 2005; Borries et al. 2013). Females in the study population are under food stress evident from the observation that females have to skip a mating season if they gave birth late in the same year (Heesen et al. 2013). Considerable food stress is suggested also by correlations between food availability and a) the probability to conceive (Heesen et al. 2013) and b) female fecal glucocorticoid levels during gestation with effects on offspring phenotype (Berghänel et al. 2016, Touitou et al. 2021b). At 5.9 years wild Assamese macaque females reproduce considerably later than congeneric provisioned rhesus macaques (4.0 years; Leigh

& Bernstein 2006) which contributes to the separation of somatic growth and reproduction in rhesus macaques. The restricted data available to date, suggest an important role of energy allocation in the scheduling of growth and reproduction in female primates, but conclusive tests of the *neonatal investment hypothesis* have to await the collection of more comparative data, ideally from wild populations.

Our test of the neonatal investment hypothesis rest on the assumption that Assamese macaques are more similar to rhesus macaques and mangabeys than to baboons in pre- and postnatal brain development. Baboons are born with more developed brains which makes gestation particularly costly to females. Comparative data on brain development are not available for Assamese macaques and neither are data on feeding behavior of immatures that could indicate how skilled and constrained they are compared to baboons (Altmann 1998). However, the relationship between ecological seasonality and female reproduction may inform the question how costly reproduction is to female Assamese macaques in the study population. Assamese macaques are mainly frugivorous: the feeding time budget comprises of 59% fruit and seeds, 24% insects, spiders, reptiles, and other animal matter, 13% leaves, and 4% other food items; Heesen et al. 2013). Timing of reproduction relative to seasonal fluctuations in food availability follows a relaxed income breeder strategy (Brockman and van Schaik 2005): females accumulate fat before conception and the birth season includes the peak of food availability. Gestating females trade-off feeding time at the expense of resting, do not conserve energy, and use up their fat stores during gestation (Touitou et al., 2021a). Females have to be in good physical condition to conceive (Heesen et al. 2013), food availability affects conception rate (Richter et al. 2016), and infant mortality is very low (5%; Ostner & Schülke unpubl. data) all of which suggests that females face considerable costs during gestation and invest prenatally in high quality offspring. It remains unclear how these demonstrated investments of Assamese macaque females in offspring quality compare to those of baboons and other papionins. Even if Assamese macaques were more similar to baboons than to rhesus macaques and mangabeys, the desynchronization of growth and reproduction found in geladas still does not fit predictions of the neonatal investment hypothesis.

Sexual dimorphism in body size may develop from sex differences in growth speed, growth duration, or both (Shea 1986, Leigh 1995) and variation in these patterns has been associated with male reproductive strategies and resulting differences in social organization (Leigh 1995). The data set presented here is too sparse to conclusively determine the intricacies of ontogeny of sexual size dimorphism. The pseudo-velocity curves suggest that males may maintain a higher growth rate than females between age one and two for this new data set or between age two and three in the older distance meter data set. Yet, this difference in rate is associated with considerable measurement uncertainty and the one-year shift between the two data sets further suggests these results to be spurious; since both curves were based on cross-sectional data, size differences between age cohorts (Berghänel et al. 2016) may have produced these patterns. The most parsimonious interpretation is that this study provides no evidence of adolescent growth spurts in forearm length of females or males. Instead, sexual dimorphism in forearm size among adult Assamese macaques seems to be owed to an extended growth period following natal dispersal, rather than increased growth rate in males (Leigh 1996; Badyaev 2002). Extended growth periods in males have been described for several dimensions of skeletal growth in wild toque

macaques (Cheverud et al. 1992), crown-rump length in free-ranging mandrills (Setchell et a. 2001), and shoulder-rump length in wild geladas where both sexes go through a small growth spurt around the age of 4 years before females give birth for the first time (Lu et al. 2016). Male growth spurts have been associated with particular risks for males growing up in a one-male – multi female social organization where males can expect to be ousted from their natal group upon take-over by another male (Leigh 1995). Males living in multi male – multi female groups do not face similar risks and may either delay dispersal until they are fully grown or disperse at young age and small size when they do not face much resistance from residents. Thus, multi-male societies are conducive to prolonged male growth periods driving sexual size dimorphism. Notably, the analyses listed above all concern skeletal growth and neglect possible changes in muscle mass. In one-male – multi-female groups, sexual dimorphism in body mass is more pronounced than in one-dimensional size metrics (Leigh 1996). Sexual mass dimorphism results from both increased growth rate and extended growth period in males compared to females in wild and food-enhanced yellow baboons (Altmann & Alberts 2005), food enhanced rhesus (Turnquist & Kessler 1989) and longtailed macaques (Schillaci et al. 2007), but not wild toque macaques (Cheverud et al. 1992) and thus contradict the explanation based on social organization alone.

The *male career framework* offers another socio-ecological explanation for the timing of dispersal relative to growth. As expected for a species with medium to low levels of reproductive skew, male Assamese macaques did not delay natal dispersal until they were fully grown and at their maximum fighting ability. This latter life history strategy is seen for example in male crested macaques, where natal migrants challenge the current alpha of another multi male group and replace him, if successful, immediately after immigration, and sire the vast majority of offspring for about one year until they are replaced themselves (Marty et al. 2015, 2016). Male Assamese macaques leave their natal group at a much younger age and much smaller size. Male dominance rank attainment has a cooperative component (Schülke et al. 2010) and some alpha males were clearly not the largest males in the group (see discussion in Schülke et al. 2014). Thus, body size may be less decisive for dominance rank attainment and reproductive success. Further studies will have to elucidate whether small size facilitates integration into a new group, whether males migrate together with others to further reduce risks, and exactly how they acquire their adult dominance rank in relation to immigration status as predicted from the *male careers framework* (van Noordwijk & van Schaik 2004).

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Declarations

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Conflicts of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Data availability

The data analyzed for this study are available from the open data repository GRO.data of University of Goettingen, Germany.

Authors' contribution

Conceptualization: Oliver Schülke and Julia Ostner; Formal analyses and investigation: Simone Anzà, Andreas Berghänel, Oliver Schülke; Writing original draft: Simone Anzà and Oliver Schülke, Writing review and editing: all authors; Funding acquisition: Oliver Schülke, Supervision: Julia Ostner and Oliver Schülke.

Ethics approval

The study was observational and has been approved by the Thai National Research Council and the Department of National Parks, Wildlife and Plant Conservation Thailand (permits 0002/470 January 26 2016, 0002/4139 June 9th 2017, 0002/2747 May 4th 2018, 0402/2798 October 4th 2019, 402/4707 October 2nd 2020).

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Tables

Table 1 Repeatability of photogrammetric measurements of forearm size; CV refers to coefficient of variation; 1, 2 are observers 1 and 2.

Source of error	N (photos)	Mean CV (%)	Range CV (%)
Repeated measurement[a]	555	2.67	0.00-14.99
Between photograph error – 1[b]	627	4.66	0.93-10.74
Between photograph error – 2	709	3.26	0.49-9.99
Between photograph error (mean 1,2)		3.96	
Between measurer error (1-2)[c]	585	5.29	0.02-17.56

[a] Comparison of two measurements taken by the same person from the same photo.
[b] Averaged across two measurements rounds.
[c] Comparison of measurements of the same photo assessed by two observers.

Table 2 Quadratic plateau model of growth in forearm length of females and males with best fit value of a) quadratic term, b) linear term, and c) intercept. Confidence intervals and p-values were estimated with 999 bootstrap iterations. R^2 refers to adjusted-R^2

Parameter	Best fit value	2.5% C.I.	97.5% C.I.	P-value
females				
a	-0.17	-0.26	-0.11	< 0.001
b	2.40	1.96	3.03	< 0.001
c	7.26	6.46	8.00	< 0.001
critical value (Age at growth cessation)	7.07	5.86	8.39	
R^2	0.89			
males				
a	-0.13	-0.16	-0.11	< 0.001
b	2.33	2.11	2.59	< 0.001
c	6.81	6.47	7.17	< 0.001
critical value (Age at growth cessation)	8.93	8.02	9.82	
R^2	0.97			

Table 3 Development of sexual dimorphism in body size estimated from forearm length. Data suggest that females tend to be larger than males at 1-3 years of age, and males are larger than females from age 5 onwards. Size measured by laser photogrammetry compared between males and females for five different age windows (0-1, 1-3, 3-5, 5-7, and >7 years old.) and tested with Mann-Whitney-U-tests. Raw p-values and p-values adjusted for multiple testing with the Benjamini-Hochberg method are reported.

Age category	Inter-individual variation (CV%)	Sex	Forearm length (mean ± SD)	N	U	P-value	Adjusted p-value
0-1	16.61	F	8.24 ± 0.77	7	67	0.332	0.335
		M	8.12 ± 0.83	15			
1-3	13.20	F	11.66 ± 1.03	6	51	0.070	0.117
		M	10.83 ± 0.92	11			
3-5	7.32	F	14.19 ± 0.39	7	19	0.336	0.336
		M	14.25 ± 0.69	8			
5-7	6.40	F	15.28 ± 0.23	6	0	0.024	0.060
		M	16.05 ± 0.14	3			
7+	7.71	F	16.04 ± 0.35	18	2	< 0.001	< 0.001
		M	17.43 ± 0.35	11			

Appendix I

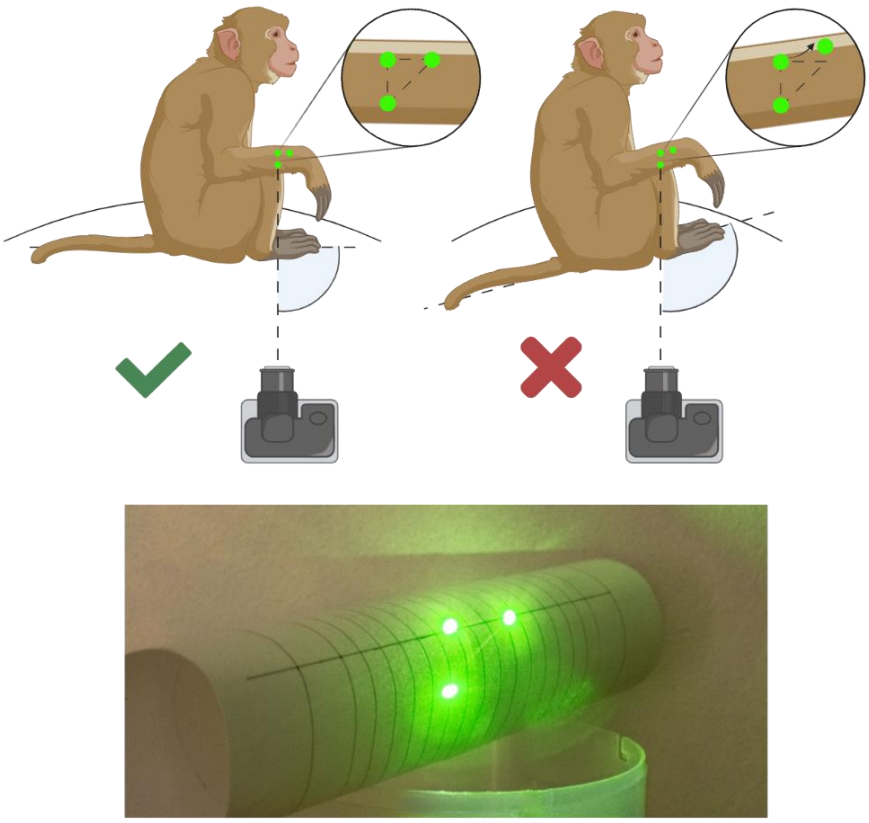

Figure A1 Illustration of how deviation from a perpendicular orientation camera-object axis can be detected with a 3-laser set-up. In most cases such deviation leads to the projected laser dots not forming a right isosceles triangle, either because the triangle is not right or not isosceles or both.

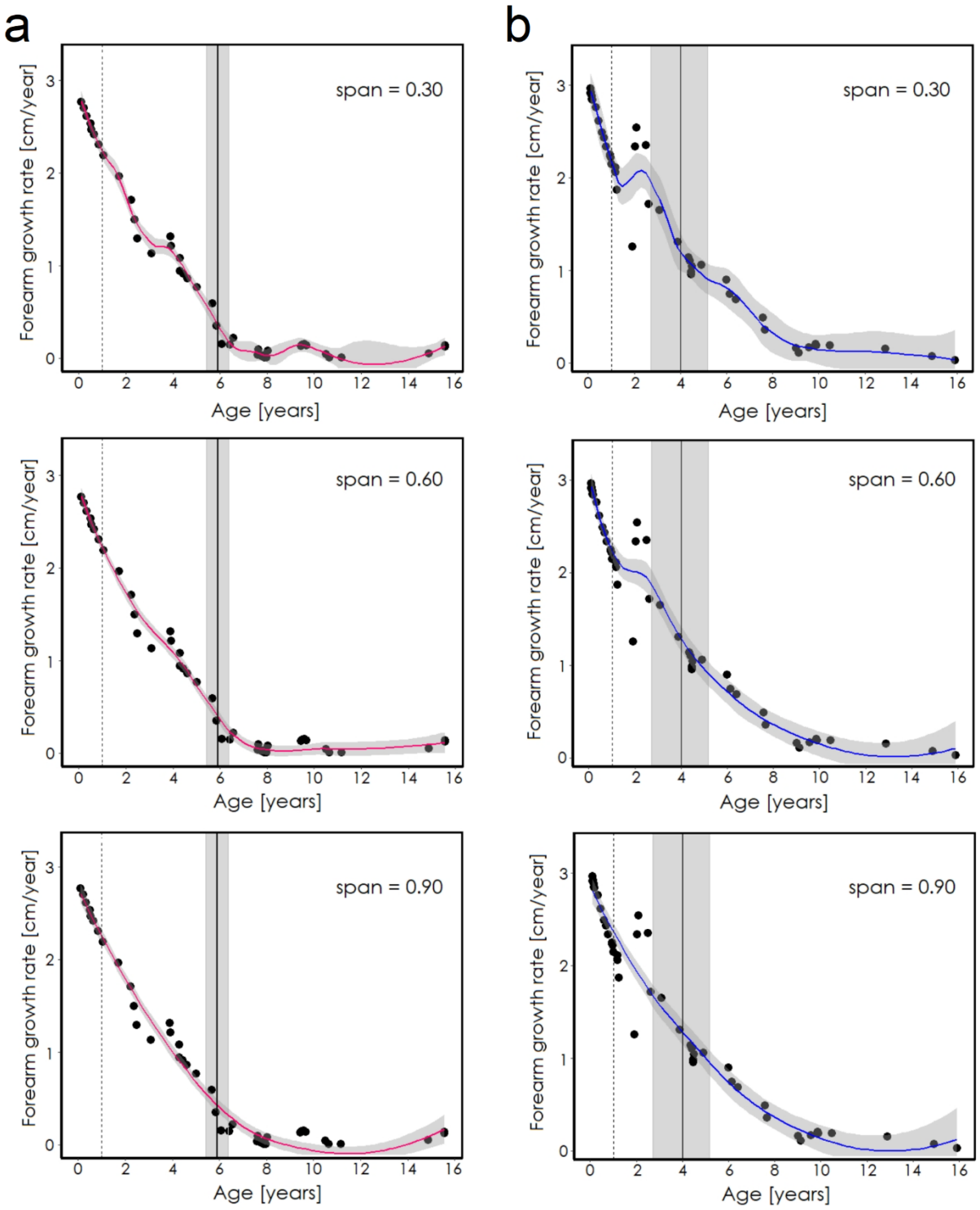

Figure A2 Example of visual inspection of LOESS curves for (a) females and (b) males of wild Assamese macaques plotted with a span value of 0.30, 0.60, and 0.90. The most conservative curves with less "noise" produced by the local regression are the one with span = 0.6 (central plots).

Table A1 Repeatability of photogrammetric measurements of forearm size of adult individuals only (> 6 years old); CV, coefficient of variation; 1, 2 observers 1 and 2

Source of error	N (photos)	Mean CV %	Range CV %
Repeated measurement [a]	127	2.37	0.00-10.49
Between photograph error – 1[b]	163	3.18	1.15-5.76
Between photograph error – 2	169	2.63	0.73-9.98
Between photograph error (mean 1,2)		2.90	
Between measurer error (1-2)[c]	144	5.39	0.04-16.96

[a] Comparison of two measurements taken by the same person from the same photo
[b] Averaged across two measurements rounds
[c] Comparison of measurements of the same photo assessed by two observers

Table A2 Repeatability of photogrammetric measurements of forearm size of juvenile individuals only (1-6 years old); CV, coefficient of variation; 1, 2 observers 1 and 2

Source of error	N (photos)	Mean CV %	Range CV %
Repeated measurement [a]	69	2.25	0.01-12.07
Between photograph error – 1[b]	79	3.99	1.05-12.35
Between photograph error – 2	152	3.02	0.49-12.11
Between photograph error (mean 1,2)		3.51	
Between measurer error (1-2)[c]	73	6.31	0.12-18.74

[a] Comparison of two measurements taken by the same person from the same photo
[b] Averaged across two measurements rounds
[c] Comparison of measurements of the same photo assessed by two observers
[c] Comparison of measurements across two observers

Table A3 Repeatability of photogrammetric measurements of forearm size of infant individuals only (0-1 year old); CV, coefficient of variation; 1, 2 observers 1 and 2

Source of error	N (photos)	Mean CV %	Range CV %
Repeated measurement [a]	359	2.86	0.00-21.62
Between photograph error – 1[b]	385	5.59	1.53-17.20
Between photograph error – 2	388	5.01	0.49-14.99
Between photograph error (mean 1,2)		5.30	
Between measurer error (1-2)[c]	368	5.29	0.02-25.16

[a] Comparison of two measurements taken by the same person from the same photo
[b] Averaged across two measurements rounds
[c] Comparison of measurements of the same photo assessed by two observers

Table A4 Between photograph error and Between measurer error of forearm size estimated from distance meter data set in Berghänel et al. 2015. Infants: 0-1 year old; juveniles: 1-6 years old; adults > 6 years old. CV refers to coefficient of variation.

	Between photograph error			Between measurer error		
Age class	N (photos)	Mean CV %	Range CV %	N (photos)	Mean CV %	Range CV %
Infant	1111	4.37	0.99-25.28	63	3.89	0.24-19.91
Juvenile	370	3.58	0.14-19.55	116	4.99	0.16-16.08
Adult	90	3.06	1.04-7.37			

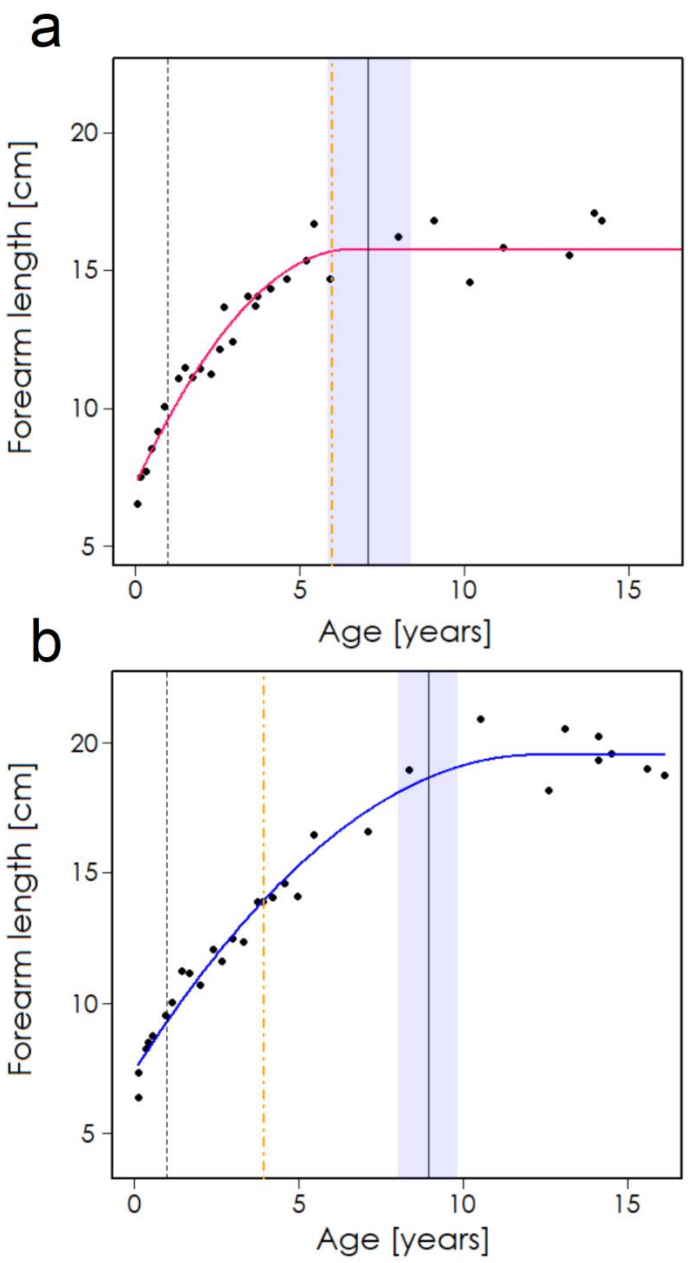

Figure A3 Growth curves for females (a) and males (b) estimated from distance meter data set (Berghänel et al., 2015) with separate quadratic plateau models. Age at weaning and age at first birth are estimated using data from this study. Dashed line indicates age at weaning (1.0 year), orange dash-dotted line indicates mean age at first birth (5.9 years) in females (a) and mean age at natal dispersal (4.0 years) in males (b). Solid line indicates the model estimated age at cessation of growth with the 95% confidence intervals shown as a grey bar. Consistently with this study, also in Berghänel et al., 2015 the first birth coincides with growth cessation whereas natal dispersal occurs years before males stop growing.

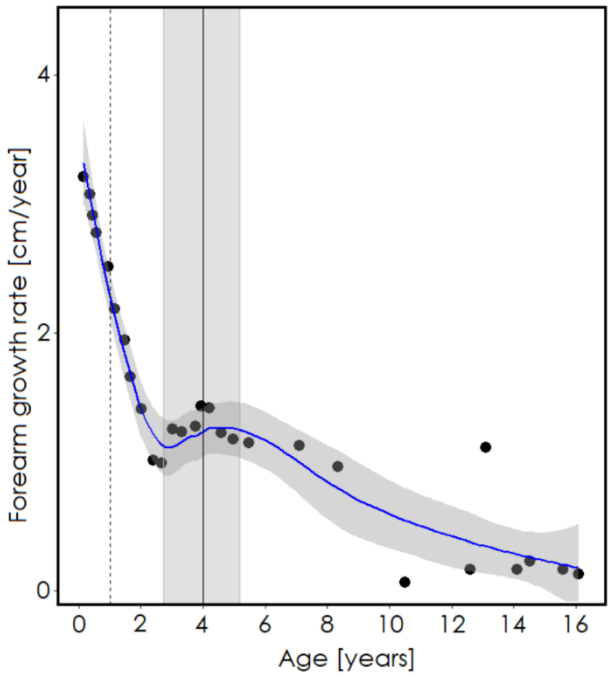

Figure A4 Pseudo-velocity curve of males estimated by non-parametric LOESS regression method, and developmental milestones. Dashed line indicates age at weaning (1.0 year). Solid black line indicates age at natal dispersal (4.0 years) with gray shades indicating standard deviation around those measures (0.48 for age at first birth and 1.23 for age at natal dispersal). We selected a span value of 0.60 by visually identifying more conservative curves with less "noise" produced by the local regression method.

Supplementary Material no. 1

Growth trajectories of wild Assamese macaques (*Macaca assamensis*) determined from parallel laser photogrammetry

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Highlights:

- Three lasers forming a right isosceles triangle were projected to measure forearm length from digital photos
- Measurement errors were much lower than variation in forearm length and decreased with object size
- Females completed 97% of growth by age of first birth effectively desynchronizing growth and reproduction
- Sexual dimorphism in body size developed from extended growth periods in males rather than increased rates of growth
- Males dispersed from natal groups years before they completed growth

This article is part of a thematic collection of articles (Special Issue) of *Mammalian Biology* and covers the following topics and taxa (marked with ☑) addressed in the Special Issue:

Article Type

☑ Original Research ☑ Techniques ☐ Review ☐ Short Communication ☐ Concept Note

Taxon

Terrestrial

☐ Bats *(Order Chiroptera)*
☐ Carnivores : Bears *(Family Ursidae)*
☐ Carnivores : Canids *(Family Canidae)*
☐ Carnivores : Felids *(Family Felidae)*
☐ Carnivores : Hyenas *(Family Hyaenidae)*
☐ Carnivores : Mustelids *(Family Mustelidae)*
☐ Elephants *(Family Elephantidae)*
☐ Primates : Great Apes *(Family Hominidae)*
☑ Primates : Old World monkeys *(Family Cercopithecidae)*
☐ Ungulates : Bovids *(Family Bovidae)*
☐ Ungulates : Deers *(Family Cervidae)*
☐ Ungulates : Giraffes *(Family Giraffidae)*
☐ Ungulates : Horses *(Family Equidae)*
☐ Multiple taxa *(3 or more Families/Orders)*

Marine

☐ Baleen whales : Right whales *(Family Balaenidae)*
☐ Baleen whales : Rorquals *(Family Balaenopteridae)*
☐ Carnivores : Bears *(Family Ursidae)*
☐ Carnivores : Mustelids *(Family Mustelidae)*
☐ Dolphins *(Family Delphinidae)*
☐ Large toothed whales *(Families Delphinidae & Hyperoodontidae)*
☐ Pinnipeds : True seals *(Family Phocidae)*
☐ Porpoises *(Family Phocoenidae)*
☐ Sirenians : Manatees *(Family Trichechidae)*
☐ Multiple taxa *(3 or more Families/Orders)*

Topic

☐ Acoustic ID
☐ Aerial surveys
☐ Analytical innovations
☐ Automated pattern recognition
☑ Behavioural ecology
☐ Camera-trapping
☐ Conservation management
☐ Data management
☐ Demographic parameters
☑ Field methodology
☐ Genetic ID
☐ Health conditions
☐ Other: *(please specify)*
☐ Identification techniques
☑ Life-history
☐ Machine learning
☐ Mark-recapture analysis
☑ Morphometrics
☐ Network analysis
☑ Photogrammetry
☐ Population ecology
☐ Site fidelity & Movement
☑ Social ecology
☐ Software/Package development
☐ Thermal imagery

Chapter 5 - Growth trajectories

Prenatal maternal glucocorticoids during early gestation accelerate growth trajectories of the offspring

Background

According to the predictive adaptive response (PAR) hypothesis, prenatal stress provides the offspring the information on environmental context and reprograms its phenotypes in preparation for the predicted long-term future. Early adversity can translate into developmental constraints and early disadvantaged phenotypes which can lead to disadvantaged states later in life, during adulthood. The more recent version of the PAR, the internal PAR (iPAR), dissociates the adaptive value of developmental plasticity from the prevision of future environmental contexts (Nettle et al., 2013) and proposes that because disadvantaged somatic states are predicted to be negatively associated with life expectancy, in a context of unpredictable environment the offspring is thought to use its own disadvantaged somatic state to recalibrate its own life-history pace and guarantee offspring production under reduced life expectancy (Metcalfe and Monaghan, 2001; Dantzer et al., 2013; Del Giudice, 2014; Hanson and Gluckman, 2014; Nettle and Bateson, 2015; Tung et al., 2016; Berghänel et al., 2016). However, the process of adaptive recalibration necessitates resources that may be traded off with quality-related attributes furnishing benefits that would increase with life span (e.g., immune function, cognitive and physical skill acquisition) (Metcalfe and Monaghan, 2001; Berghänel et al., 2015, 2016; McGowan and Matthews, 2018). Results of previous investigations on prenatal GCs and growth are conflicting with some studies reporting negative or positive effects (Berghänel et al., 2016, 2015; Dantzer et al., 2013; Emack et al., 2008; Hauser et al., 2006; Mueller and Bale, 2006; Patin et al., 2002; Schöpper et al., 2012; Schülke et al., 2019). It has been proposed that such inconsistency may be driven by differences in the timing of the stressor potentially triggering or not the adaptive recalibration according to the different sensitivity of the ontogenetic periods (Schülke et al., 2019). Early but not late stress exposure is associated with DNA methylation in metabolic and growth regions of prenatally stressed individuals (Tobi et al., 2009). Targeting specific time windows is therefore crucial when investigating the effect of early adversity on phenotypic adaptive recalibration.

In this chapter, I tested the specific iPAR prediction that prenatal maternal GC levels are positively associated with accelerated growth as a proxy for life-history pace recalibration under the assumption that a faster life history trajectory is associated with a quicker maturation and a faster reproduction

which maximize fitness under bad early condition. With the prediction that early gestation represents a major sensitive period for growth recalibration, I tested also whether growth trajectories and pseudo-velocity curves were associated with a timing effect of prenatal maternal GCs.

Combining growth trajectory and maternal GCs information

Once validated on a sample size of 92 subjects (48 males, 44 females) the remote laser projection measurement to estimate non-invasively object size (Chapter 4), I combined the information on forearm length previously estimated with maternal GC levels and tested the specific hypothesis of accelerated growth in prenatally stressed infants, juveniles, and adults. The laser setup, sampling regime, and forearm length estimation are fully described in Chapter 4.

Information on maternal glucocorticoids was derived from the immunoreactive 11ß-hydroxyetiocholanolone extracted from fecal samples collected for the long-term project and used for the analyses described in Chapter 2 and Chapter 3. The immunoreactive 11ß-hydroxyetiocholanolone (GC) is considered a major metabolite of cortisol in primate feces (Heistermann et al., 2006) and was extracted from fecal matter using enzyme immunoassay. The assay was performed to assess adrenocortical activity (Fürtbauer et al., 2014; Heistermann et al., 2006; Ostner et al., 2008; Shutt et al., 2012) of mothers during prenatal and postanal phases. The full protocol of fecal samples collection, sample extraction, extract dilution, and assay sensitivity is reported in Chapter 2.

All the analyses performed here include a subset of individuals with known forearm length and prenatal and postnatal maternal GCs levels (n = 45). Full information on the study population and demography is reported in Chapters 2 and 3. The combined dataset includes 21 infants (9 females, 12 males), 17 juveniles (7 females, 10 males), and 7 adults (5 females, 2 males). The individuals' age range in the subset of data was 17-2910 days (mean = 996, SD = 989). Since part of the data collection was longitudinal (3 collection periods for the infants) and part was cross-sectional (1 period for juveniles and adults) I estimated the mean forearm length and corresponding body size index at specific mean age in infants and analyzed 1 datapoint per subject.

Statistical analyses

I performed all statistical analyses using the software RStudio 1.3.1093 (RStudio Team, 2021; with R 4.0.4 - R Core Development Team, 2021) and packages *glmmTMB* (version 1.1.2.3), and *stats* (version 4.0.3). I ran linear mixed models (LMM) with the forearm length measure coded as the response variable, and I included the predictor *Age* in interaction with *Early-preGC*, *Late-preGC*,

PostGC, and *Sex*. Linear growth is expected for an increase in volume and not length, thus I coded the response variable as the cubic value of the forearm length measure, and used this measure as an index of body size (BSI). Finally, I $\log_n$-transformed the BSI and maternal GCs predictors to achieve model requirements. I ran the full model including age as a polynomial of second degree in interaction with maternal GCs measures. I conducted the likelihood-ratio test to obtain p-values for single predictors by applying the function *drop1* (Barr et al., 2013) which performed single-term elimination. I removed from the full model not significant predictor Age^2 and ran reduced models including age as a linear term. I checked model assumptions, homogeneity of variance, normality of residuals, linearity, and collinearity of predictors (all VIF < 2) by using the package *performance* (version 0.8.0). Despite the non-significance of the Age^2 value in the full model, the visual check of the reduced model revealed a potential non-linear effect (Figure 1).

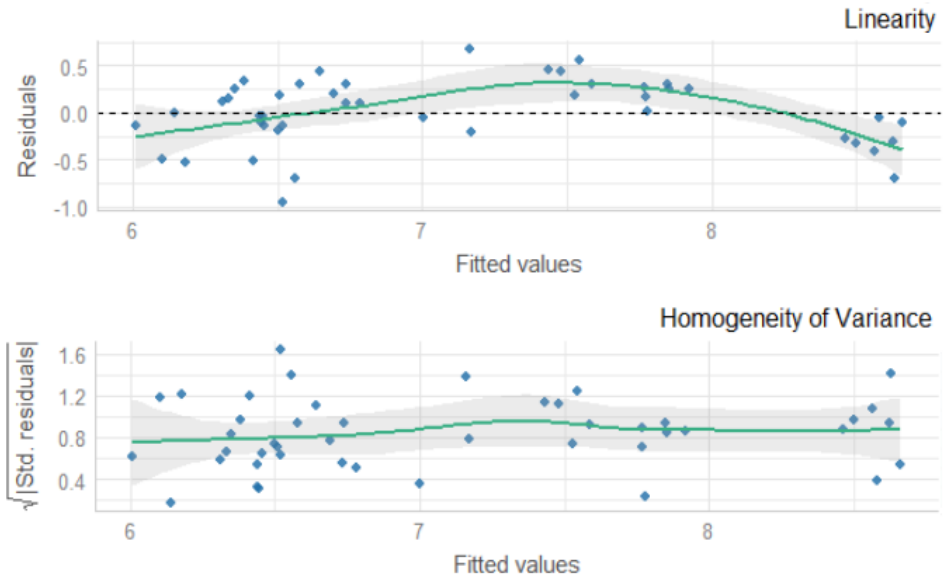

Figure 1 Visual check of model assumptions of (top) linearity and (bottom) homogeneity of variance. The reference line should be flat and horizontal.

Therefore, I integrated the LMM analyses with a non-linear approach by fitting a local polynomial regression (LOESS) which fits a polynomial curve determined by one or more numerical predictors using a local fitting (Cleveland et al., 1992). Since LOESS fit a local polynomial regression, the method did not require the use of the cubic measure of forearm length nor the $\log_n$-transformation of the response. LOESS regressions are fitted locally and may be sensitive to data structure — the parameter alpha, or span, controls the degree of smoothing and can be adjusted accordingly by visual inspection (Cleveland et al., 1992). I used a span value of alpha = 1 to control for local data lacking in the 2-3 and in the 5-7years time windows (Figure 3) and I estimated predicted values of forearm length and growth trajectories moderated by prenatal and postnatal maternal GCs at the (i) mean

value, (ii) mean+1SD value, and (iii) mean-1SD value. Finally, I predicted pseudo-velocity curves of forearm growth moderated by prenatal maternal GCs with LOESS regression and postnatal maternal GCs at the mean value and predicted data for all subjects with known age and forearm length (n = 93).

Results

The LMM on the effect of prenatal and postnatal maternal GCs on body size index while controlling for sex differences (full model) revealed no significant effects of the quadratic age (Age^2) in interaction with maternal GCs and sex (Appendix I - Table 1). Thus, I ran a reduced model including age as a linear predictor (Table 1). The reduced model revealed the significant effect of maternal GCs during early gestation in moderating the effect of age (*Early-preGC*Age*) while all the other predictors did not affect the response significantly (Table 1, Figure 2). Specifically, an increase in early prenatal maternal GCs was associated with an increase in body size index.

Table 1 Linear effect of age (days) in interaction with prenatal and postnatal maternal GCs, and sex on body size index. P-values are obtained with the likelihood ratio test and indicated in bold when significant. The response body size index is $\log_n$-transformed forearm cubic length. (1) $\log_n$-transformed to meet model assumptions. (2) coded with "female" as the reference category. Pseudo-R^2 = 0.89. P-values, degree of freedom, and likelihood ratio of single terms and the intercept are not reported because of very limited interpretation.

Predictor	Estimates	SE	C.I.	df	LRT	P-value
Intercept	7.574	1.370	4.888 – 10.260	-	-	-
[1]Early-preGC*Age	0.000	0.000	0.000 – 0.001	1	7.800	**0.005**
[1]Late-preGC*Age	0.000	0.000	-0.000 – 0.000	1	0.994	0.319
[1]PostGC*Age	-0.000	0.000	-0.001 – 0.000	1	1.310	0.252
[2]Sex[M]*Age	0.000	0.000	-0.000 – 0.000	1	3.089	0.079
[1]Early-preGC	-0.205	0.172	-0.542 – 0.132	-	-	-
[1]Late-preGC	0.087	0.160	-0.226 – 0.399	-	-	-
[1]PostGC	-0.137	0.300	-0.725 – 0.452	-	-	-
Age	-0.001	0.001	-0.003 – 0.001	-	-	-
[2]Sex [M]	-0.136	0.135	-0.401 – 0.129	-	-	-

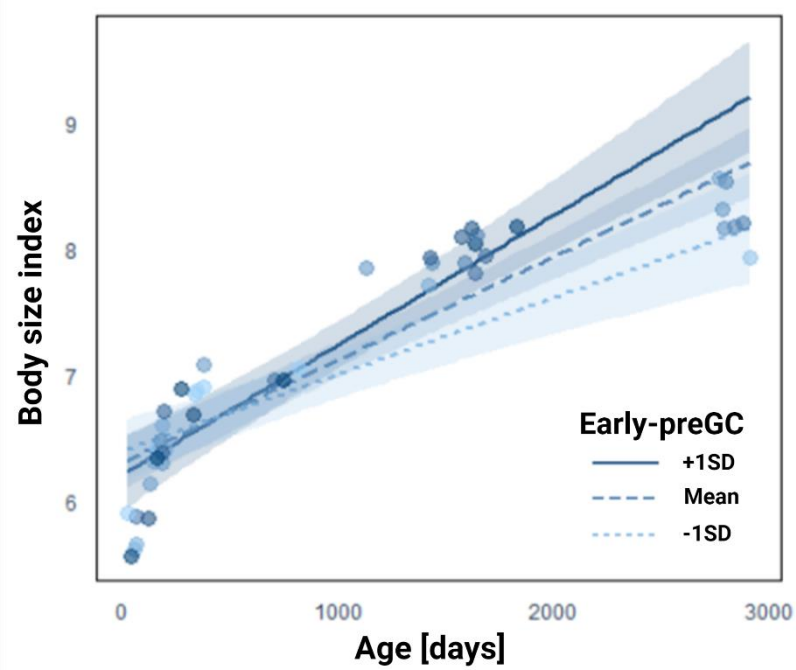

Figure 2 Linear effect of age (days) moderated by prenatal maternal GCs during early gestation (*Early-preGC*Age*). Dashed line = age effect at the mean value of *Early-preGC* (166.57 ng/g); solid line = age effect at the mean+1SD (250.70 ng/g); dotted line = age effect at the mean-1SD (82.46 ng/g). The response and the predictor *Early-preGC* are both $\log_n$-transformed to meet model requirements. The model lines are plotted with all the other predictors at their mean value.

Since a visual check of fitted data versus residuals revealed potential non-linearity after the removal of the not significant Age^2 predictor (Figure 1), I performed polynomial local regression (LOESS) to integrate the analyses with a non-linear approach.

The prediction of the growth curves for early and late prenatal maternal GCs at the (i) mean value, (ii) mean+1SD, and (iii) mean-1SD confirmed the results of the LMM. The comparison of the growth curves and predicted values revealed that increased prenatal maternal GC levels were associated with longer forearm length from infancy to adulthood (Figure 3). Consistently to the linear analyses performed with LMMs, also the LOESS prediction of the growth curves showed a less stable and less pronounced effect of maternal GCs during the second half of gestation and during lactation in moderating age (Figure 3).

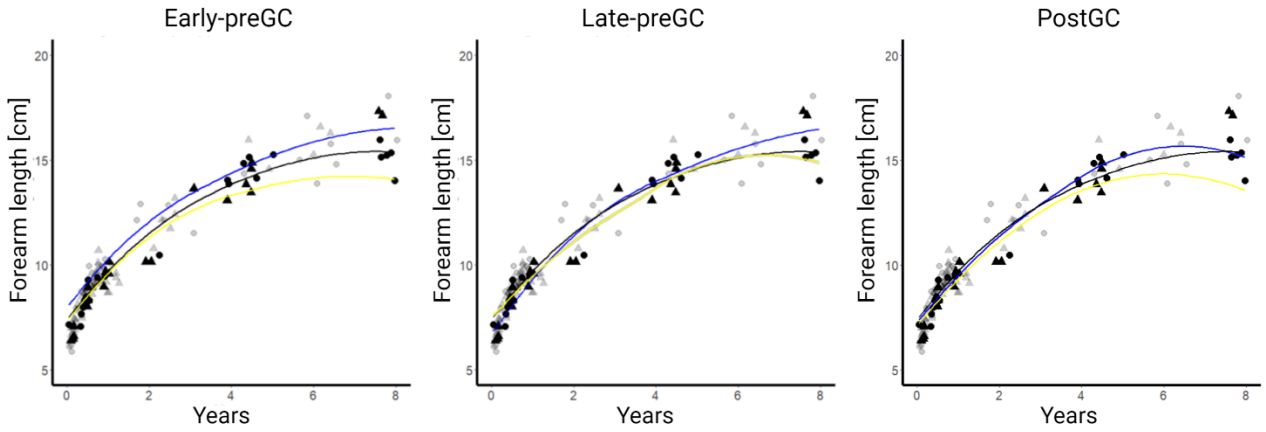

Figure 3 Effect of maternal GCs during (left) early gestation, (middle) late gestation, and (right) postnatal phase, in moderating growth of males and females forearm length. Black line = specific maternal GCs at the mean value, blue line = mean+1SD value, yellow line = mean-1SD value. Triangle = male, circle = female. Data points used to predict and plot local regressions are colored in black. Data points plotted in gray lack the information on maternal GCs and were not used to predict growth but were included for a visual comparison of forearm length variability associated with age. Model lines of different growth trajectories are plotted with all the other model predictors at their average value. All growth trajectories are predicted and plotted with span = 1 to control for local data lacking.

Finally, LOESS regression predicting pseudo-velocity curves moderated by early and late prenatal maternal GCs at the (i) mean value, (ii) mean+1SD, and (iii) mean-1SD of respective maternal GCs value revealed a decrease in pseudo-velocity deceleration associated with early prenatal maternal GCs (Figure 4A). Specifically, approximately at the age of 18 months, the predicted pseudo-velocity curve for early prenatally stressed subjects (blue line) started to diverge from the predicted curve of subjects with prenatal maternal GCs at the mean value (black line) and mean-1SD value (yellow line). Consistently with models on forearm length, late prenatal maternal GCs had a weaker effect in moderating the pseudo-velocity curve of forearm growth (Figure 4B).

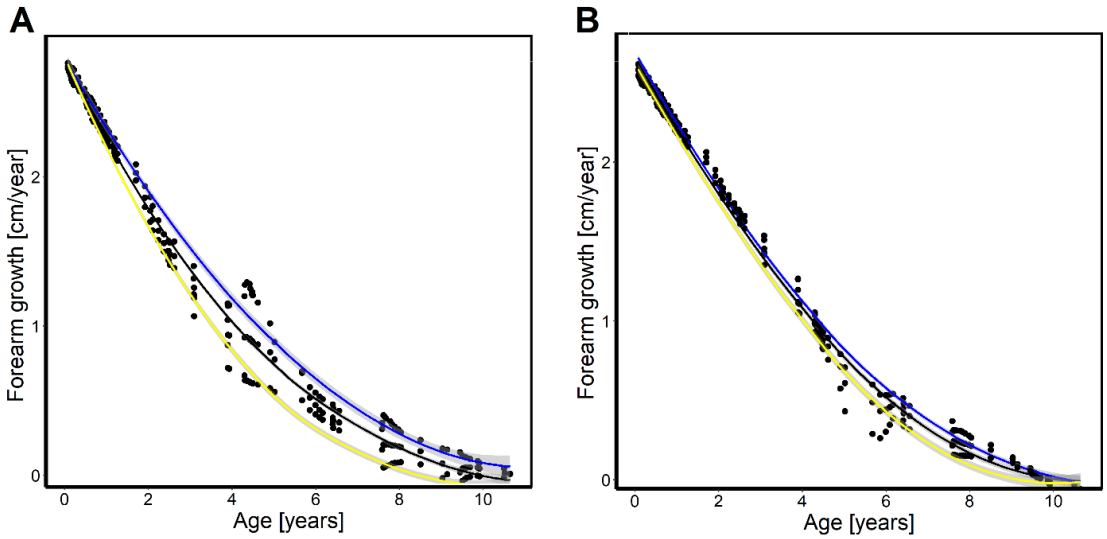

Figure 4 Predicted effect of maternal GCs during (A) early gestation and (B) late gestation in moderating pseudo-velocity curves of forearm growth. Black line = specific maternal GCs at the mean value; blue line = mean+1SD value; yellow line = mean-1SD value. All growth trajectories are predicted and plotted with span = 1. Black dots represent data points of predicted pseudo-velocity for all the subjects with known age and forearm length.

Methodological considerations and conclusions

The analyses on growth trajectories described and validated in Chapter 4 showed the potentiality of the LOESS regression method when compared with the quadratic-plateau model used to estimate growth trajectories. Specifically, the use of the local regression method led to similar results (Chapter 4) while offering the inclusion of predictors without the aprioristic knowledge of the function describing the relations with the response required by the quadratic plateau model (Lu et al., 2016). However, LOESS regression remains a visualization method that does not provide p-values or statistical information on the significance of the model and its application for the hypothesis testing approach remains limited.

In Chapter 4, the method validation suggested an optimal span value of 0.6 for the database comprising 92 different subjects. Different from the dataset analyzed in Chapter 4, the dataset used here combines growth information with maternal GCs information and it is half the size (n=45). Specifically, it was missing the prenatal maternal GCs information of subjects of age 2-3 and 5-7years old (Figure 3). Therefore, to control for local data lacking in the 2-3 and 5-7years time windows I used a more parsimonious span value of alpha = 1 to predict and plot LOESS values.

Recently, Berghänel et al. (2016) measured the effect of prenatal maternal GCs on offspring growth and size-for-age in the same study population of wild Assamese macaques here investigated. However, in that study subjects' age spanned from 0 to 18 months and therefore the conclusions on the potential acceleration of growth were limited to the infancy period. This study aims at replicating what was previously investigated with the integration of new data on juveniles and adults to test for long-term effects of prenatal maternal GCs on growth and is not going to be a stand-alone paper. Although the remote-measurement method and the statistical approach were different, the results were consistent: as observed in immatures (Berghänel et al., 2016), also juveniles and adults showed accelerated growth and higher body-size index. Overall, the results of the linear and the non-linear approaches converged. They showed that prenatal maternal stress measured from fecal maternal glucocorticoids was associated with the recalibration of offspring growth. Specifically, the higher pseudo-velocity of forearm growth and increased body size index predicted for infants, juveniles, and adult offspring with increased maternal GCs during early but not late gestation suggest a timing effect moderating the potential adaptive acceleration of offspring life-history pace.

Results will be discussed in the general discussion (Chapter 6) where all the response variables from different chapters will be examined together and inspected for possible adaptive trade-offs.

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Appendix 1

Appendix 1 - Table 2 Effect of age-squared in interaction with prenatal and postnatal maternal GCs, and sex on body size index. P-values are obtained with likelihood ratio test (drop1 function). The response body size index is the $\log_n$-transformed measure of the forearm cubic length. The response and prenatal and postnatal predictors are logn-transformed to meet model requirements.

Predictor	df	LRT	P-value
Early-preGC*Age	1	0.279	0.597
Early-preGC*Age^2	1	0.098	0.754
Late-preGC*Age	1	0.638	0.425
Late-preGC*Age^2	1	0.436	0.509
PostGC*Age	1	0.003	0.955
PostGC*Age^2	1	0.013	0.909
Sex*Age	1	0.281	0.596
Sex*Age^2	1	0.930	0.335

Chapter 6 - General discussion

The aim of this thesis is to advance our understanding of the evolutionary origins of developmental plasticity by testing predictions of the iPAR hypothesis on a wild long-lived animal. Specifically, I contributed to the ongoing debate on the nature of responses to adversities during sensitive periods of offspring's ontogeny and relying on strategic trade-offs between body growth (Chapter 4-5) and quality-related attributes (Chapter 2-3).

With a cross-sectional approach, I combined long-term data on maternal glucocorticoid levels estimated from fecal glucocorticoid metabolites with (i) GC levels of infant, juvenile and adult offspring, (ii) information on their gut microbiome richness and composition, (ii) and biometric measure (i.e., forearm length).

To assess the internal consistency of the iPAR hypothesis, I tested three main predictions associated with the programming of adaptive responses and related trade-offs:

a) Adaptive responses persist into adulthood and depends on the timing of the exposure
b) Prenatal maternal GC levels negatively affect quality-related attributes
c) Prenatal maternal GC levels accelerate offspring pace of life

In my first study, I observed that maternal GC levels during gestation are associated with long-term hyperactivity of the offspring HPA axis (while controlling for postnatal effect) measured at three different age categories, but only when the maternal exposure increased during early gestation. Across several models, early gestation significantly predicted increased mean, max, and range values of offspring GCs equally in infants, juveniles, and adult offspring (Chapter 2). Knowing that GC levels are higher in prenatally challenged offspring, I proceeded with the second study and investigated the effect of maternal GCs during gestation and postnatal phase on offspring gut microbial diversity and composition (while controlling for offspring GC levels). In the second study (Chapter 3), I found that higher maternal GC levels during early gestation were associated with a microbial signature characterized by reduced bacterial richness, increased relative abundance of several pro-inflammatory organisms, and reduced abundance of many short-chain fatty acids (SCFAs) producers with a general lower capability of the bacterial community of harvesting energy during the rich season. Quite puzzling, offspring GCs significantly predicted *Firmicutes* to *Bacteroidota* ratio and the results indicate a better energy-harvesting capability in offspring with elevated GC levels.

In the third and fourth studies, I first explored different statistical approaches to investigate forearm growth in relation to life-history milestones, observed that quadratic-plateau models and local

polynomial regressions were equally valid methods to describe the non-linear relationship between forearm length and age, tested local polynomial regression performance in describing pseudo-velocity curves according to different span values, and compared pseudo-velocity curves with a different dataset built using a different remote-measurement approach (Chapter 4). Thus, I applied the validated method to test the effect of prenatal maternal GCs on offspring growth, and observed long-term increased body-size index and accelerated pseudo-velocity curves of forearm growth in prenatally challenged offspring (Chapter 5). Consistently with the second and the third study, maternal GC levels during early gestation were a better predictor of phenotypic recalibration and lead to accelerated growth and longer forearm length in infants, juveniles, and adult offspring.

In this final chapter, I frame my findings on the nature of prenatal maternal effects. First, I discuss the mere persistence of all the phenotypical traits into adulthood (short-term vs long-term effects), and then I interpret their nature (adaptive vs non-adaptive), discuss trade-offs resulting from iPAR, consider the limitations of my studies and provide an outlook of potential future directions.

1 Adaptive developmental plasticity: time is of the essence

Across the studies presented in this thesis, it emerged one central element shaping phenotypic outcomes of elevated maternal GCs: the timing of exposure. Overall, increased prenatal maternal GC levels during the first half of gestation (early-preGC) led to stronger and more stable effects translating into more pronounced and persisting phenotypic variation when compared to effects of late prenatal maternal GC levels (late-preGC), and postnatal maternal GC levels (postGC).

In this section, I discuss my findings in light of the observed centrality of the timing of adversity exposure within the debate on the persistence of maternal effects and the adaptive nature of developmental plasticity.

1.1 Long-term programming of the offspring phenotype

The adaptive calibration model (ACM) predicts short-term physiological changes (e.g., HPA axis functionality) in response to early-life adverse conditions in long-lived species (Del Giudice et al., 2011): it posits that adaptive plasticity concerns immediate physiological functions and translates into temporary adaptations. Particularly informative on the persistence of maternal effects and against predictions of the ACM are the results from my first study. While investigating prenatal maternal effects on offspring's HPA axis activity I observed a long-term increase of offspring GC levels in all age classes. Such long-term hyperactivation of the HPA axis is in contrast with what observed in wild chimpanzees (Girard-Buttoz et al., 2021). In a recent empiric test on the persistence of the effects of early-life adversity in wild juvenile chimpanzees, maternal loss induced alterations in diurnal cortisol slopes only when the insult occurred within 2 years from the measurement — after that, it was not visible anymore (Girard-Buttoz et al., 2021). Like wild immature chimpanzees, also female baboons facing maternal loss alone did not show higher GC levels in adulthood (Rosenbaum et al., 2020), while an embedded and persisting effect was observed with the increasing number of adversities experienced (i.e., cumulative adversity) (Rosenbaum et al., 2020; Tung et al., 2016). I argue that the HPA axis is very susceptible to the programming of GCs and can induce long-term effects if such a trigger is applied during sensitive stages of offspring's ontogeny (McGowan and Matthews, 2018; V.G. Moisiadis and Matthews, 2014a; Reynolds, 2013). A modest variation of maternal GC levels could induce long-term alterations of the HPA axis functionality due to the higher sensitivity of early gestation. It is possible that, for a long-term effect to occur (like the one triggered by moderate variation of early-preGC), cumulative adversities must hit the same individual during his postnatal

development (Rosenbaum et al., 2020; Tung et al., 2016), otherwise the effect could fade with time. Supporting this, the juvenile chimpanzees were orphaned after they were 4 years old (Girard-Buttoz et al., 2021), and thus they probably reached an age at which the effects were weak, and much stronger adversity would have been required for a long-term effect to occur (McGowan and Matthews, 2018; Moisiadis and Matthews, 2014b).

Crucially, the timing of the exposure was also associated with the persistence of other physiological traits into adulthood, and early-preGC was a better predictor when compared with late-preGC and postGC. Elevated early-preGC were associated also with: (i) a persisting bacterial community with reduced diversity, higher relative abundance of potentially pro-inflammatory organisms, and lower SCFA-producers; (ii) a lower energy-harvesting capability during the rich season and lower metabolic-resource diversification; (iii) accelerated body growth and increased body-size index. The variation in all these traits persisted into adulthood with strong indications against short-term effects, and in favor of persisting phenotypic variations in response to maternal GCs during early gestation.

1.2 Prenatal maternal GCs induce adaptive responses

Biological embedding occurs when adversity alters developmental processes with stable and long-term alterations of physiological states (Biological embedding model: BEM; Hertzman 1999, 2012; Power and Hertzman 1997). Like the iPAR hypothesis, both the ACM and the BEM explain phenotypic variation in physiological functions produced by early adverse conditions but the ACM conceptualize them as short-term adaptive alterations (Del Giudice et al., 2011), and the BEM predicts long-term embedded biological patterns (Hertzman, 2012; Power and Hertzman, 1997). Moreover, BEM can be understood as a developmental constrain model since the long-term alterations are not considered adaptive (Hertzman, 2012).

Although I found an overall indication of long-term prenatal maternal effects which could suggest potential support for the developmental constraints hypothesis and the BEM, I argue that such effects observed in wild Assamese macaques are not the mere product of constraints embedded into physiological systems but are the outputs of programmed adaptive responses to maternal clues. However, the observed long-term effects are not against the ACM if socio-ecological conditions do not vary between the later life phases, when the offspring are phenotyped, but considering the high environmental unpredictability such scenario is unlikely and difficult to test.

First, as shown in Chapter 2 overall prenatal maternal GC levels predicted reproductive performance, and mothers with elevated GC levels during gestation showed a higher annual rate of surviving offspring. Interestingly, these findings are supported by the results of the fourth study (Chapter 5): early-preGC increased body-size index and pseudo-velocity of forearm growth. Although accelerated growth may directly increase the risk of immature starvation and therefore mortality risk when energy intake is not sufficient (e.g., early adversity), a higher growth rate increases offspring survival and accelerates sexual maturation with potentially higher fitness benefits (Dmitriew, 2011). Theoretically, developmental constraints could prompt an acceleration of the pace of life to ensure *immediate* survival (Lu et al., 2019). While prenatal maternal GCs triggered accelerated growth and increased annual rate of surviving offspring, the acceleration in growth was not limited to the short-term, but persisted with age and translated into larger adults and longer forearm length indicating a programmed recalibration of growth and an accelerated pace of life that would give potential benefits not only immediately (e.g., ensuring immediate survival), but also on the long run (e.g., increased fitness) (Dmitriew, 2011; Spencer et al., 2022).

Second, elevated early-preGC were associated with hyperactivity of the HPA axis indicating higher allostatic load, and with an overall gut bacterial community more similar to what observed in captive/sick individuals (e.g., reduced diversity, higher abundance of potentially pro-inflammatory bacteria, lower SCFA-producers, lower *Firmicutes* to *Bacteroidota* ratio and thus energy-harvesting capability). All these elements point at detrimental health states and higher "wear and tear" associated with early-preGC. However, I also observed that elevated offspring GC levels (also increased by early-preGC) were associated with higher *Firmicutes* to *Bacteroidota* ratio (*Firmicutes*/*Bacteroidota*) and this effect increased with age (Chapter 3).

The investigations on gut microbiome effect on metabolic pathways are recent and we are still exploring all the implications linked with variation in the relative abundance of specific organisms (Bernstein, 2017; Carabotti et al., 2015; Chen et al., 2019; Devillard et al., 2007; Fujimura et al., 2010; Le Chatelier et al., 2013). Especially the analysis on the *Firmicutes*/*Bacteroidota* (ratio) informs on the ability of the bacterial community, and therefore of the holobiont, to ferment and metabolize carbohydrates and lipids (Jones et al., 2019; Lopez-Legarrea et al., 2014; Sawaswong et al., 2021; Stojanov et al., 2020). The main tendency is to associate unbalance in the typical *Firmicutes*/*Bacteroidota* with dysbiotic states irrespective of the direction of such effect: elevated *Firmicutes*/*Bacteroidota* is associated with pathological metabolic outcomes inducing obesity, while a decreased *Firmicutes*/*Bacteroidota* is associated with a complex of inflammatory reactions in the intestine (Stojanov et al., 2020). Although there is no consensus on interpreting higher

Firmicutes/*Bacteroidota* as a hallmark of obesity and more caution is suggested in causal associations with pathological metabolic outcomes (Magne et al., 2020), most of the studies concord on the higher capacity of *Firmicutes* to ferment and metabolize carbohydrates and lipids (Clayton et al., 2018; Nagpal et al., 2018; Newman et al., 2021; Sawaswong et al., 2021; Stojanov et al., 2020). Puzzling, I observed an opposite effect direction of maternal and offspring GC levels on *Firmicutes*/*Bacteroidota* depending on the timing of the exposure: elevated early- and late-preGC decreased *Firmicutes*/*Bacteroidota*, while both the postGC and the offspring GC levels increased it. Thus, different hypothetical scenarios can be pictured to interpret the results.

If lower *Firmicutes*/*Bacteroidota* is associated with a complex of inflammatory reactions in the intestine and therefore indicate detrimental health states, elevated maternal GC levels during gestation may translate into detrimental health, while PostGC and OffspringGC would theoretically reduce such negative effects. I argue that this scenario is quite unlikely since elevated prenatal maternal GC levels induce hyperactivation of the HPA axis and thus the programmed negative effects would be canceled out or buffered by a hypothetical "beneficial" effect of PostGC and OffspringGC. Yet, the lack of correlation between Early/LatepreGC and PostGC dampens such hypothesis and the beneficial effects of higher maternal GC levels during lactation and higher offspring GC levels would go against the paradigms of the developmental origins of disease theory (Barker, 2007; Barker et al., 1993; Cottrell, 2009; Reynolds, 2013).

Alternatively, if higher *Firmicutes*/*Bacteroidota* is an indication of higher risks for pathological metabolic outcomes, elevated maternal GC levels during gestation would reduce the risks, while increasing postGC and offspring GC levels would lead to an increase in such risks. In this alternative scenario, only the effect of postGC and offspringGC is coherent with potentially detrimental effects of early adversity on health, and a reduced risk for pathological metabolic outcomes associated with prenatal maternal adversity would go against the same paradigm (Barker, 2007; Hertzman, 2012; McGowan and Matthews, 2018; Reynolds, 2013; Sheriff and Love, 2013; Wadhwa et al., 2009).

Although possible, I struggle to find how prenatal maternal adversity could improve health and that would surely be a rare exception considering the large body of literature against it (Amato et al., 2016; Barker et al., 1993; Conti et al., 2012; Cottrell, 2009; Fujimura et al., 2010; Goulet, 2015; Hanson and Gluckman, 2014; Hartman et al., 2019; Lindström, 1999; Malani et al., 2022; V.G. Moisiadis and Matthews, 2014b; Wells et al., 2016). My interpretations of the *Firmicutes*/*Bacteroidota* results are more cautious: I argue that the *Firmicutes*/*Bacteroidota* is a marker too broad to be used as a hallmark of the health states but it may provide some precious indications on potentially energetic adaptive strategies to buffer higher "wear and tear". A bacterial community with higher

Firmicutes/*Bacteroidota* is associated with enhanced energy-harvesting capability and I observed offspring GC levels positively associated with *Firmicutes*/*Bacteroidota* with such relation intensifying with age. If prenatally challenged individuals need to sustain a general hyperactivation of the HPA axis, a gut microbial community with the improved capability of energy harvesting would be essential to provide the higher costs necessary to maintain fully operative the higher-demanding physiological system. From an evolutionary perspective, an holobiont coping better with long-term "wear and tear" could have potential fitness advantages. Thus, it is possible that prenatally stressed holobionts could exhibit energy-related adaptations that would allow them to endure higher GC levels and energetic demands, and avoid the collapse of the physiological systems.

The role of the gut microbiome in mediating potential energetic constraints and adaptive strategies associated with early adversity is still largely unexplored. Although very stimulating, the latter hypothesis needs to be supported by direct tests on metabolic markers. Until then, the most parsimonious interpretation is that elevated prenatal maternal GC during gestation might be associated with variation in the relative abundance of different genera of the *Firmicutes* and *Bacteroidota* compared to postGC and offspringGC leading to marked differences in the results at the phylum level. However, it remains unclear whether such differences translate into alterations of typical metabolic pathways and whether potential alterations are beneficial or detrimental.

2 Adaptive trade-offs: testing the iPAR hypothesis

The iPAR model proposes adaptive phenotypic variation in response to predicted adult' somatic states (Berghänel et al., 2016; Nettle et al., 2013; Nettle and Bateson, 2015). Adversities during ontogenetic sensitive periods will induce a soma less likely to survive at any age, organisms would use their somatic-state quality to predict reduced lifespan and thus would recalibrate their pace of life by accelerating growth and reproduction at further costs to quality-related attributes (Nettle et al., 2013). According to the iPAR hypothesis, both nutritional and social adversities can be cues that trigger adaptive recalibrations, and that makes it one of the most generalizable evolutionary models of developmental plasticity (Berghänel et al., 2016; Lu et al., 2019; Malani et al., 2022; Nettle et al., 2013). Lower quality of adult somatic states, reduced lifespan, accelerated growth, and sexual maturation are all indications of adaptations to maximize fitness by living faster under the expectation of dying younger (Lu et al., 2019; Malani et al., 2022; Nettle et al., 2013; Nettle and Bateson, 2015).

Recently, rare empiric investigations on the effect of prenatal maternal food availability and maternal GCs on long-lived organisms established that prenatal maternal GC effects can result from the iPAR and related trade-offs (Berghänel et al., 2016, 2015). These investigations were conducted in the same population studied here and showed adaptive trade-offs (i.e., ↑ growth and ↓ immune system) in immature subjects (Berghänel et al., 2016, 2015). Here, I aim at extending previous findings on timing effects and trade-offs in immature subjects with tests on the persistence of programming effects and life-history recalibrations in adulthood and potential long-term trade-offs shaping adaptive developmental plasticity under the assumptions of the iPAR model.

It has been proposed that the crucial test for an iPAR is the demonstration of a fitness cross-over: showing individuals that experienced early adversity and accelerated reproduction with higher fitness (estimated from longevity or lifetime reproductive success) than challenged offspring that did not accelerate reproduction will conclusively test the iPAR (Malani et al., 2022; Weibel et al., 2020). Since longevity data and information on lifetime reproductive success were not yet available, I focused my investigation on the adaptive trade-offs related to the iPAR hypothesis.

2.1 Trade-offs

To test predictions of the iPAR hypothesis, I investigated the correlations between naturally caused inter-individual variation in prenatal and postnatal maternal GC levels and (i) offspring quality-related attributes, and (ii) speed of growth. In this seasonal species, there was too little variation in age at first reproduction to test how it related to early adversity. Specifically, I used information on the HPA axis activity and the gut microbiome richness and composition as measures of offspring's physiological and health states, and measures of forearm length as a proxy for body growth.

2.1.1 Quality-related attributes

Defining health states is not a simple task since the multidimensionality of the attributes involved — the broader the approach, the more useful the answer (Hyland et al., 2014). Moreover, the health states can be understood better through the investigation of the "unit", by including informative elements of both the host and the hosted community as a whole. Investigations on the gut microbiota community have been proved to contribute precious information on the host's health states (Gacesa et al., 2022), and represent a good option for non-invasive studies of wild protected populations (Amato et al., 2016; Björk et al., 2019). Chronically elevated stress levels can repeatedly activate compensatory physiological mechanisms and result in higher allostatic load: the "wear and tear" of

the body (Maestripieri and Hoffman, 2011). Allostatic load can alter the processes of aging, impair health states and translate into reduced longevity (Maestripieri and Hoffman, 2011).

The results from the analyses on the gut microbial composition associated with prenatal maternal GC levels, and its moderation effect of age are particularly informative and support the higher "wear and tear" indicated by the HPA axis hyperactivity. Broadly, I observed that prenatal maternal GC levels (especially during early gestation) altered the gut bacterial composition typically detected during specific periods of the offspring's ontogeny. Although my approach was cross-sectional, the offspring of all ages showed dysbiotic states characterized not only by a reduced richness (e.g., reduction of the relative abundance in 15% of the genera) but also by variation in the relative abundance of specific organisms whose age-related pattern of variation is known.

Among several taxa, I observed variation in the relative abundance of the genus *Bifidobacterium,* a well-established marker of dysbiotic states and detrimental health (Gacesa et al., 2022). Typically, this health-promoter organism inhibits pathogen attachment to intestinal cells, promotes protections against entero-pathologies, and is involved in folate biosynthesis (Lugli et al., 2020; Modrackova et al., 2021; Thomson et al., 2018, 2018). Importantly, the relative abundance of *Bifidobacterium* decreases with age in humans and other primates (Arboleya et al., 2016; Janiak et al., 2021; Kato et al., 2017; Odamaki et al., 2016; Oki et al., 2018; Petrullo et al., 2022). When *Bifidobacterium* was largely present (i.e., in the top 20 most abundant genera during the rich season), its relative abundance was negatively associated with prenatal maternal GC levels and age, indicating an acceleration of the typical age-related reduction of *Bifidobacterium*. Both development and aging are characterized by patterns of variation in diversity and composition of gut microbial community which mature and vary with age according to specific host-species (Bosco and Noti, 2021; Duan et al., 2019; Franceschi et al., 2018; Kato et al., 2017; Reese et al., 2021; Rodriguez and Martiny, 2020; Wilmanski et al., 2021; Yao et al., 2021). The reduction of *Bifidobacterium* relative abundance, an increase in several pro-inflammatory organisms, and a reduction of many SCFAs producers (anti-inflammatory) are all clues of detrimental health and have been observed also in aging wild Assamese macaques (Sadoughi et al., 2022). Moreover, such detrimental effects intensified with age indicating that older subjects were affected more negatively by prenatal maternal GCs than younger ones and faced higher "wear and tear". I argue that prenatally challenged subjects could therefore face long-term dysbiotic states which can intensify in older subjects benefitting less from the health-promoting and anti-inflammatory organisms and paying higher immune-related costs associated with the increase of the pro-inflammatory ones.

The analyses on bacterial composition showed a significant effect of maternal GC levels only during the rich season. The most parsimonious explanation for this is that a lower number of infants sampled during the rich season and resulting data imbalance may have driven the results. In my studies, the rich season always preceded the lean season chronologically. Therefore, the infants were younger during the rich season and produced a smaller amount of fecal matter with consequent difficulties in the collection of fecal samples. However, in juveniles and adults, many bacterial organisms were present during the rich season and were basically absent or changed in the relative abundance during the lean season: first among all, the well-established health-promoting *Bifidobacterium.* Clearly, it is not possible to establish prenatal maternal effects on such an important keystone organism if it is absent during the lean season. Although not marked, such seasonal differences in the bacterial composition may be one important driver of differences in the results.

At the genus level, most of the between-season difference in the bacterial composition was associated with variation of the genus *Treponema,* an organism very common in primate species (McKenna et al., 2008) and whose functionality in the gut is still unexplored (Angelakis et al., 2019). This genus includes several non-pathogenic organisms which are progressively disappearing in humans under the ecological pressure of industrialization so that a higher relative abundance of *Treponema* is now a distinctive trait of non-human primate holobionts (Manara et al., 2019). Unfortunately, the gap in the literature did not allow me to speculate on the potential functionality and health-related effects of this keystone organism for non-human primates, and future studies will have to answer such stimulating questions. However, the relative abundance of *Treponema* was not affected by any predictor related to prenatal or postnatal maternal GCs, offspring GCs and age, but it strongly varied with season. This suggests that *Treponema* may be affected more by external factors like diet supporting findings on its progressive reduction in humans (Manara et al., 2019; McKenna et al., 2008). While less marked, I observed seasonal differences in the relative abundance of other organisms which informed the potential health condition of the subjects during the rich and the lean season. Importantly, the samples collected during the lean season — the most energetically-challenging period of the year in the study population (Heesen et al., 2013; Touitou et al., 2021) — showed a lower abundance of important good-health indicators like *Bifidobacterium, Faecalibacterium, Roseburia* and *Blautia* (Arboleya et al., 2016; Clayton et al., 2018; Fujimura et al., 2010; Gacesa et al., 2022; Liu et al., 2021; Wilmanski et al., 2021). Quite puzzling, samples from the lean season showed also a higher abundance of members belonging to the *Christensenellaceae* and the *Rikenellaceae* family, both associated with increased health and longevity in humans (Tavella et al., 2021; Waters and Ley, 2019). Interestingly, the reason why both these families have been counted as good-health indicators in humans is because they contrast fat accumulation (Beaumont et al., 2016;

Reinders et al., 2017; Tavella et al., 2021; Waters and Ley, 2019). *Christensenellaceae* and *Rikenellaceae* correlate with each other, have been consistently reported as a marker of lean phenotypes, and are negatively related to visceral fat mass (Oki et al., 2016; Tavella et al., 2021; Waters and Ley, 2019). I argue that a lower abundance of good-health indicators and a higher abundance of members of *Christensenellaceae* and *Rikenellaceae* during the lean season may indicate that all the subjects were experiencing energetic challenges due to potential lower food availability, lower food quality, or higher energetic demand during the lean season (Heesen et al., 2013; Touitou et al., 2021), and that it can translate into decreased energy-stored. It is possible that such a non-neglectable energetic challenge may temporarily obfuscate the maternal effects on the gut microbial community and drive seasonal differences in the results. Re-sampling of the same subjects during the next rich season and a more balanced dataset may unveil seasonal differences.

Finally, in infants, juveniles and adults elevated prenatal maternal GC levels were associated with HPA axis hyperactivity indicating higher "wear and tear", and detrimental effects on the host's gut microbiome tended to strengthen with age. As conceptualized in the iPAR hypothesis (Berghänel et al., 2016; Malani et al., 2022; Nettle et al., 2013), both these indications suggest a general negative effect of prenatal maternal GCs on offspring's health, with a potential reduction of the lifespan and higher fitness costs (Bonier et al., 2009; Campos et al., 2021; Lindström, 1999; Schoenle et al., 2021; Tung et al., 2016; Zipple et al., 2021b).

2.1.2 Body growth

In the last study (Chapter 5), I tested the iPAR prediction that early adversity is positively associated with accelerated growth as a proxy for life-history pace recalibration. I operated under the assumption that a faster life history trajectory is associated with a quicker maturation and faster reproduction which maximize fitness (Dmitriew, 2011). The study replicated what was previously investigated in the same study population (Berghänel et al., 2016), with the integration of new data on juveniles and adults to test for long-term effects of prenatal maternal GCs on growth trajectories.

Across several models, early-preGC confirmed a major sensitive period for growth recalibration and was consistently associated with increased body-size index and accelerated pseudo-velocity curves of the forearm length. An increase of 1SD of early-preGC was associated with a higher pseudo-velocity growth curve (+0.2cm/year at 4 years) which translated into an increase of 6.6% in the adults' forearm length. Previous findings on growth rates are highly inconsistent with some studies indicating a positive (Dantzer et al., 2013; Hauser et al., 2007; Mueller and Bale, 2006; Patin et al., 2002;

Schöpper et al., 2012) and others a negative relation (Emack et al., 2008; Hauser et al., 2006) with prenatal maternal GC levels. One explanation for such inconsistencies is that although prenatal maternal GCs are related to developmental constraints, they do not always induce predictive adaptive responses. Adaptive responses could be triggered or not depending on the timing of the stressor during ontogeny, leading to different effects on placenta morphology and fetus' ontogeny (Hanson and Gluckman, 2014; McGowan and Matthews, 2018; V.G. Moisiadis and Matthews, 2014b, 2014a; Reynolds, 2013). However, my results are consistent with the trade-off reported by Berghänel et al. (2016): increased growth rate and body size at 16-18 months of age combined with eye infection during a two-month outbreak of conjunctivitis (Berghänel et al., 2016). Similarly, I observed in the same study population accelerated growth rate combined with (i) hyperactivity of the HPA axis, and (ii) dysbiotic gut microbial states. My findings integrate previous results and inform on two different aspects concerning investment strategies associated with prenatal maternal GCs: the persistence and the breadth of such adaptive trade-offs.

Theoretically, an increase in growth rates could result either from developmental constraints or via long-term recalibration of developmental trajectories that would optimize an individual's life-history under adverse conditions (Lu et al., 2019; Spencer et al., 2022). In my investigation, prenatal maternal GC levels were associated with accelerated growth and increased body-size index in infants, juveniles, and adults indicating a long-term recalibration of the pace of life which can potentially increase fitness benefits (Dmitriew, 2011; Spencer et al., 2022). Such long-term growth recalibration was combined with a long-term alteration of the HPA axis activity and microbial dysbiotic states — I argue that consistently to what was observed in immature Assamese macaques (Berghänel et al., 2016), investment into these processes may be traded off in favor of growth under the recalibration induced by prenatal maternal GC (Belsky et al., 2015; Berghänel et al., 2016; Metcalfe and Monaghan, 2001). Thus, such adaptive trade-offs may not be only temporary strategies to ensure immediate survival (Lu et al., 2019), but rather programmed long-term strategies optimizing life-history to increase fitness under a reduced life expectancy. Without a direct investigation of lifetime reproductive success, a long-term recalibration of offspring's growth trajectory and associated trade-offs with quality-related attributes suggests only partial support to the iPAR hypothesis (Weibel et al., 2020). However, under the assumption that accelerated growth is a good proxy for an increased pace of life and faster reproduction, my results provide evidence for a potential adaptive response to prenatal maternal clues of adversity in a long-lived mammal.

3 Limitations and conclusions

Although this thesis informs on the adaptive nature of prenatal maternal effects, it lacks an exhaustive investigation on the fitness advantages of fast reproduction (Malani et al., 2022; Weibel et al., 2020). The lack of available long-term data on lifetime reproductive success prevented me to provide a conclusive test investigating the presence of a potential fitness cross-over and thus limited my interpretations. Further limitations are given by the cross-sectional nature of the study and by the lack of the effect of diet as one important predictor in shaping gut-microbial communities. My study provides only a few snapshots of the health status of the holobiont Assamese macaques sampled during two periods of the year (infants, juveniles, and adults sampled during rich and lean seasons) and therefore it is not clear the effect of maternal stress on gut-microbial stability.

The pronounced timing-effects demonstrated here have led to the shrinking of the sample size. For many individuals, fecal samples from either one of the two prenatal phases or from the postnatal period of lactation were not available and therefore such individuals could not be included in the analyses that investigated all three periods together. Moreover, since the onset of gestation cannot be determined in the field, it is complicated to time sampling the two gestation periods. Overall, the sample size was small and the sample size per offspring age class was very small. Therefore, all results have to be taken as preliminary and will need replication.

Importantly, it is slowly emerging the role of the gut microbiome in shaping body growth. Although currently most of the studies focus on body weight and fat accumulation (Beaumont et al., 2016; Frost et al., 2019; Jones et al., 2019), we are understanding that differences in the gut microbial composition can impact bone development and health, and are associated with fast/slow growth trajectories (Chapagain et al., 2019; Li et al., 2021, 2013; Sun et al., 2009; Villa et al., 2017; Zaiss et al., 2019). Thus, the debate on the evolutionary origin of developmental plasticity may benefit from studies investigating the role of the gut-microbial community on body growth through direct investigations on the mediation of the growth hormone metabolism.

All organisms have to maintain their physiological parameters within a range of values depending on their age, sex, and species — that allows them to function properly. Chronically elevated GC levels and allostasis can induce long-lasting shifts of such parameters away from their typical range of homeostasis and move their values to aberrant or atypical levels (Maestripieri and Hoffman, 2011; McEwen and Wingfield, 2003; Romero et al., 2009). Higher stress levels can increase the reduction of telomer length and thus accelerate the natural aging processes (Epel et al., 2004; Geronimus et al., 2010; Kotrschal et al., 2007). Very stimulating, I observed that elevated prenatal maternal GC levels

were associated with the aging-like pattern of variation in microbial abundance (e.g., decrease of *Bifidobacterium*, increased relative abundance of several pro-inflammatory bacteria and decrease of anti-inflammatory ones). A more integrative approach with longitudinal data will allow a better evaluation of the costs associated with adversities during development and their impact on the recalibration of life-history traits.

Epigenetic maternal effects are among the most pronounced environmental effects on individual health and fitness (McGowan and Matthews, 2018; Perroud et al., 2014; Wadhwa et al., 2009; Zipple et al., 2021a). With this thesis, I tested competing hypotheses on the epigenetic effects of prenatal maternal GCs and provided results in support of the iPAR hypothesis. Overall, the results provided evidence of persisting detrimental effects of not traumatic and naturally driven increase of prenatal maternal GC levels combined with an acceleration of growth. The results indicate that no matter their age, prenatally challenged offspring face higher allostatic load and "wear and tear" of physiological systems which can negatively impact morbidity and reduce lifespan (Bonier et al., 2009; Campos et al., 2021; Creutzberg et al., 2021; Lewis et al., 2000; Lindström, 1999; McGowan and Matthews, 2018; Merlot et al., 2013; Novak et al., 2013; Nunn et al., 2015; Reynolds, 2013; Zipple et al., 2021b). However, to partly compensate for a predicted reduced lifespan, the offspring accelerates their life-history pace by increasing growth at the cost of reduced investment in maintenance functions and quality-related attributes. Such life-history recalibration through adaptive trade-offs could ensure the best possible offspring production under a reduced life expectancy (Del Giudice, 2014; Nettle et al., 2013; Nettle and Bateson, 2015).

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DECLARATION

I hereby declare that I have written this thesis entitled “Prenatal maternal stress effects in wild Assamese macaques (Macaca assamensis)” independently and with no other aids or sources than quoted.

Göttingen, 29th of April 2022

Simone Anzà