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The Evolutionary History of Primate Litter Size

Jack Hansen McBride
Western Washington University, jmcbride12695@gmail.com

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The Evolutionary History of Primate Litter Size

By

Jack Hansen McBride

Accepted in Partial Completion of the Requirements for the Degree
Master of Arts

ADVISORY COMMITTEE

Dr. Tesla Monson, Chair

Dr. Hilary Schwandt

Dr. Todd Koetje

GRADUATE SCHOOL

Dr. David L. Patrick, Dean
Master’s Thesis

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Jack Hansen McBride

May 23rd, 2024
The Evolutionary History of Primate Litter Size

A Thesis
Presented to
The Faculty of
Western Washington University

In Partial Fulfillment
Of the Requirements for the Degree
Master of Arts

by
Jack Hansen McBride
May 2024
Abstract

Litter size plays an important role in the life history strategies of all mammalian taxa. It is one of the most important factors determining whether an organism is deemed to have a ‘slow’ or ‘fast’ life history strategy. Investigating how the evolution of litter size has influenced human evolution, extant primate biodiversity, and how it relates to other life history traits is crucial to understanding ourselves and our closest relatives. This thesis summarizes a two-pronged investigation into the evolution of litter size: 1) I performed a meta-analysis using 955 taxa within the magnorder Boreoeutheria, and 2) I performed a geometric morphometric pilot study on twinning and singleton-bearing American monkey (Platyrrhini) crania. My findings include negative correlations between litter size and gestation length, age at maturation, and maximum potential lifespan. Phylogenetic signal tests support that selection has played a role in the distribution of mean litter size across this magnorder, as well as in the distribution of other reproductive characters including gestation length and percentage of brain growth accomplished prenatally. Additionally, I present reconstructions of life history and body size for various ancestral organisms within this magnorder. My results support that twinning was common in boreoeutherian evolution and may have been the ancestral primate condition. To better evaluate these results, I compare the reconstructed values for the ancestral boreoeutherian to recent morphology-informed research on fossil mammals including *Vincelestes neuguenianus* (Theria). Finally, I present the results of the pilot study, discuss how these relate to my current hypothesis regarding litter size and morphological covariation, and discuss how I intend to expand this dataset.
Acknowledgements

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I was only able to obtain the skeletal materials used in the geometric morphometric analyses due to the support of The Leakey Foundation, who funded the time I spent collecting sample scans at the Smithsonian National Museum of Natural History. I would like to thank the museum staff, especially Darrin Lunde and Dr. Melissa Hawkins, for making my data collection possible, and my experience working in the museum a pleasurable one.

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Introduction

Litter size is an important life history trait for all animals which give live birth. Placental mammals give birth to live offspring in sets called litters. The number of offspring produced in a typical litter is an organism’s litter size. We can also compute a mean litter size, which provides another measure of typical litter size. The reproductive output of a given organism can be estimated by dividing their reproductive lifespan by their interbirth interval and multiplying the quotient by litter size. An example: \( \text{reproductive output} = (10\text{-year reproductive lifespan} / 2\text{-year interbirth interval}) \times \text{litter size of } 3 = 15 \text{ lifetime reproductive output, measured in number of offspring.} \)

Life history frameworks seek to explain variation in life history traits across extant and extinct biodiversity (Stearns, 1992). The applications of Life History Theory (LHT) include understanding extant biodiversity, the evolutionary histories of given lineages, predicting the life histories of extinct organisms, as well as identifying trends in how organisms respond to environmental change. Litter size, age at reproductive maturity, and lifespan are considered important traits within the domain of LHT (Funston et al., 2022; Jones, 2011).

Within the field of biological anthropology, life history approaches have been used to investigate questions in the evolution of primate behavior (e.g., nesting in Kappeler, 1998), human reproduction and reproductive morphology (e.g., endocranial volume, dental morphology, and prenatal growth in Monson et al., 2022), among other topics. Despite this background, many questions remain as to the evolutionary trajectories of extant primate life histories and morphological characters.
Figure 1. Typical ‘Slow’ versus ‘Fast’ Life History characteristics.

Twinning in primates, both as a species-level trait (e.g., callitrichid monkeys and some strepsirrhines) and as an element of intraspecific reproductive variation (e.g., human or chimpanzee twinning), is particularly interesting. Recent research has found that the American monkey *Callithrix kuhlii* consistently produces offspring which are chimeric in all somatic tissue (Ross et al., 2007a) and variation in twinning rate between human populations is relatively poorly understood (Blickstein and Keith, 2005). A deeper understanding of the evolutionary processes which produced extant life histories and litter size character states will generate insight and new routes of inquiry into questions related to twinning in humans and our primate relatives.
This project was designed around several major questions: 1) What is the ancestral state of litter size for primates? 2) What role has litter size played in the origins of extant primate diversity? 3) How does litter size variation in primates compare to other mammalian orders? 4) What other traits covary alongside litter size among primates and other mammalian orders?, and 5) What is the craniodental morphology of twinning primates?

I investigated these questions using two main methodologies. I used a series of phylogenetic reconstructive and comparative methods, including ancestral state reconstruction, to investigate the evolution of litter size and related characters across Boreoeutheria. The second is a pilot study on twinning and non-twinning primate cranial morphology. I collected three-dimensional (3D) data using both a handheld and a stationary 3D scanner and applied a series of geometric morphometric analytical methods.

In Chapter One, I will review the current understanding of litter size in LHT. I contextualize the role litter size has played in life history frameworks such as r/K selection theory (MacArthur, 1967). I give special attention to theories of slow and fast life history evolution, as that continuum is used by current researchers (e.g., Stearns, 1992) to explain broad selective paradigms.

Chapter Two reviews twinning in humans and our primate relatives, with special attention paid to the biology of twinning in humans and other primates as well as populational variation in twinning rates. This chapter also reviews the primate taxa which twin more often than they produce singletons, found exclusively in Platyrhini and Strepsirrhini. This chapter includes discussion of relevant research focused on non-primate mammals.

I describe my methods in detail in Chapter Three. This includes description of the life history trait dataset that I collated (N=955 mammalian taxa). I also detail the phylogenetic
methods I used to investigate relationships between litter size and other life history traits across boreoeutherian mammals, and to estimate ancestral mammalian characteristics.

Chapter Three also describes the geometric morphometric analytical process that I undertook for this project and used as a pilot investigation into skeletal variation in twinning taxa. I collected a sample of (N=60) Platyrhini crania from the Smithsonian Natural Museum of Natural History, landmarked these crania, and used these landmarks to compare the cranial morphology of two platyrrhine monkey species (*Saguinus oedipus* and *Sapajus apella*). I designed these methods around craniofacial sex dimorphism in these taxa.

I then present the results of these analyses in Chapter Four. In Chapter Five, I discuss the implications of these findings. The closing chapter, Chapter Six, summarizes this work.

I will present a brief review of current research in LHT, focused on litter size and related reproductive traits, in the following chapter. Litter size has been studied across many taxa, including those focused on in this thesis. This review will contextualize the proceeding chapters and provide the framework within which this research was conducted. LHT is an area which features substantial interdisciplinary collaboration. This review will begin to ground this project as intersectional with biological anthropology, medical anthropology, public health studies, mammalogy, ecology, and evolutionary biology. Chapters Two and Three are particularly important to frame the findings of the phylogenetic reconstructive methods, including which of my findings are novel and which support prior assertions within the literature.
Chapter 1 – Review of Litter Size in Life History Theory

Life History Theory

Life history theory is a framework which seeks to analyze biodiversity using the lens of distinct organismal life history strategies. These strategies involve the sequence of events which drive an organism’s birth, growth and development phases, adulthood and reproduction, and senescence (Flatt and Heyland, 2011). Life history traits are pieces which make up these overall strategies. These pieces include things like length of gestation, adult body mass, whether an organism hibernates or enters torpor, and litter size.

Litter Size and r/K Selection

Early life history theorists quickly recognized that offspring number is a defining trait of an organism’s life history strategy. In mammals, litter size; in birds, clutch size, etc. In r/K selection theory MacArthur (1967) posited that unstable, unpredictable environments favor, and thus select for, organisms which produce many offspring (r-selected organisms) as the environment will dictate the loss of some or many of these offspring, regardless of investment. On the other end of the spectrum, stable environments were predicted to favor organisms which produce fewer offspring, where they can invest resources in said offspring and the likelihood is that these investments will pay off (K-selected organisms).

The number of offspring produced at a given birth is a substantial determinant of how much investment each individual offspring can receive in terms of the finite energies and resources available to the parents and, in some species, other community members. Over the course of evolution this pattern leads to differences in other reproductive traits, behaviors, and
even morphology. In an avian example, Thys et al. (2019) identified intraspecific behavioral covariation with offspring number. *Parus major* females with smaller clutches demonstrated a greater intensity of nest-defense behaviors than those with larger clutches (Thys et al., 2019).

A strict application of the *r/K* continuum is no longer common in part because it does not apply neatly in all cases (e.g., Stearns and Koella, 1986). It has since been replaced by a less binary framework describing life history trade-offs. The litter size trade-off involves trading the advantages of producing many offspring for the capacity to use more prenatal and postnatal resources on each individual offspring. These trade-offs function alongside selection to place organisms along a slow (similar but not identical to *K*-selected) to fast (*r*-selected) life history continuum (Stearns, 1992). Life history traits classically associated with slow life histories include small numbers of offspring (small litters), large neonatal and adult body mass, increased length of adolescence and maturation periods, among others.

There remain many questions regarding the origins of organismal life histories which are made up of an assemblage of slow and fast life history traits, as extant biodiversity does not consist of organisms which can be easily placed onto this continuum relative to one another. An organism may exhibit some features which would typically be associated with slow life histories and others with fast life histories.

Callitrichids demonstrate high levels of allomaternal care compared to closely related taxa (Burkart and van Schaik, 2010; Heldstab et al., 2019). This is typically associated with slow life history organisms. Callitrichids also produce a greater number of offspring than these other platyrhines (Ernest, 2003) which is typically associated with fast life history organisms. Classification is further complicated by intraspecific variation in life history traits and in attempts to apply these concepts to extinct organisms (discussed in Capdevila and Salguero-
Fossil morphology can be compared to the morphology of living organisms to make assertions about the soft tissues and behavioral ecology of the extinct organism. These assertions must be phylogenetically informed and consider patterns of covariation which may diverge even within a single genus or species.

Litter size is paramount to any framework positing life history variation in these terms. Low fecundity phenotypes, organisms which produce relatively few offspring, are present throughout mammals (Ernest, 2003). What ecological circumstances initially favored these phenotypic expressions?

*Litter Size and Resource Availability*

Itô and Iwasa (1981) review the limitations of $r/K$ selection, citing the existence of low fecundity phenotypes in stressful environments coupled with presumably low interspecific competition (mountain streams, deep sea, and the Antarctic). These researchers propose that the ability of an organism’s young to procure food may be a more generalizable ecological pressure which can explain the appearance of low fecundity phenotypes in a greater number of environments (Itô and Iwasa, 1981).

Every proposed life history theory posits a different set of environmental and genetic restrictions on the appearance of low fecundity. Funston et al. (2022) propose that ‘slower’ life history characters may have begun arising in early placental mammals as part of rapidly increasing body size. Early placental mammals radiated into a variety of niches never before filled by mammals, and body size increases were crucial to this diversification (Bertrand et al., 2022; Funston et al., 2022). This indicates a selective environment favoring low fecundity genotypes, yet the factors which drove certain lineages towards slower life histories and others
towards faster life histories remain unclear. How did phenotypic plasticity shape these trajectories and play a role in clade divergence?

The relationship between the appearance of low fecundity phenotypes and a selective pressure on the ability of young to procure food is supported by theories in early primate evolution, including the angiosperm hypothesis. Through analysis of a Paleocene plesiadapiform, *Carpolestes simpsoni*, Bloch and Boyer (2002) infer that euprimates likely arose from a common ancestor which was adapted to terminal branch feeding, not visual predation nor specialized leaping. Their phylogenetic analysis supports that Carpolestidae and the earliest euprimates are closely related. *C. simpsoni* features opposable halluces with long fingers and nails but lacks orbital convergence or leaping-adapted tarsal bones (Bloch and Boyer, 2002). Primates may have arisen, deriving relatively lower fecundity genotypes and phenotypes, from organisms in just such ecological circumstances (Itô and Iwasa, 1981).

Rasmussen (1990) discusses similar ecological circumstances inhabited by an arboreal marsupial. He specifically discusses terminal branch feeding in the marsupial *Caluromys* genus. This taxon is relatively encephalized, produces small litters compared to other didelphids, is arboreally agile, and has large eyes (Rasmussen, 1990). All these traits make it more primate-like than other didelphid genera. He concludes that the initial divergence of primates from closely related non-primate mammals likely involved exploitation of fruits accessible via terminal branch feeding (Rasmussen, 1990). Derived feeding behaviors, feeding postures, and morphological traits allowing for the exploitation of fruit and other resources available at branch termini would appear to have been selected for alongside the production of relatively fewer offspring, as crucial aspects of the divergence of primates from other mammals.
In the case of *Alopex lagopus*, a canid species, researchers have concluded that their extremely variable litter size may be an evolutionary response to immediate resource levels combined with resource predictability. The existence of different genotypes which both retain some degree of litter size plasticity was determined by geographically correlated differences in placental scar number. The absolute largest litter sizes are observed in unstable areas with low predictability, but all populations show variation (Tannerfeldt and Angerbjörn, 1998).

Litter size and resource availability covary in the primate lineage, and the shift towards slower life histories, including the production of fewer offspring, may have been a response to increased food access by and for offspring. These locomotive and feeding adaptations may have allowed for the increased investment necessary for this strategic trade-off (producing smaller litters) to ‘pay off.’ The grasping and use of a variety of fruit and other branch-borne resources by scandentians, especially arboreal ones (e.g., Sargis, 2001), leaves questions as to the role resource availability and litter size played in the divergence of the primate lineage from its closest relatives.

*Litter Size and Infant Mortality*

Mortality rate is one of the best predictors of life history variables (Promislow and Harvey, 1990; Stearns and Koella, 1986). These variables include traits that I examined for this thesis, such as litter size, neonatal weight, and interbirth interval, among others. In a large mammalian sample, litter size was found to increase with higher mortality rates (adult and juvenile), and neonatal weight was found to decrease with higher mortality rates (Promislow and Harvey, 1990). Juvenile mortality had an impact greater than adult mortality across every trait studied (Promislow and Harvey, 1990).
Other studies that focus on single or few lineages have found that litter size is not associated with a decrease in maternal survival nor a decrease in subsequent litter size (Risch et al., 2007). For example, in a sample of 999 *Spermophilus richardsonii* squirrels, survival increased alongside larger litter size (Risch et al., 2007). Boutin et al. (1988) also found that in 99 *Ondatra zibethicus* muskrat litters survival was not significantly correlated with litter size and offspring survival was grouped. Litters tended to all die or survive at a higher rate than the average young survival rate in the population (Boutin et al., 1988).

These findings demonstrate how complicated it is to place various litter size states along an imagined linear continuum of life history ‘speed.’ Life history traits coevolve in evolutionary lineages, and taxa have arrived at their current litter size state only through a process of contemporaneous changes with other life history, morphological, and behavioral traits.

Litter size can also vary interspecifically, as is seen in *Acomys*, a rodent genus which produces mean litters of 2-3 offspring (Frynta et al., 2011). Additionally, both within and between species, litter size covaried alongside maternal body weight and presence of immature females in the family group (Frynta et al., 2011). This was interpreted as litter size increasing with resourced maternal condition and hospitable social environment in these captive populations. This supports that increased offspring survival may prompt phenotypically plastic larger litter size, bucking the mortality trends identified across larger swathes of mammalian taxa.

This genus, *Acomys*, features a relatively longer gestation period and smaller litter (hallmark ‘slow’ life history traits) compared to other genera in the family Muridae (Frynta et al., 2011). These features make it more ‘primate-like’ in terms of reproductive life history, yet these trends of covariation do not offer clear insight into the ecological circumstances which would
result in deriving even smaller litters as occurred in the primate lineage. A better understanding of the evolutionary history of litter size allows these trends to be evaluated more effectively. Theories explaining the response of litter size to mortality risk must be refined based on both extant and extinct morphology and life histories (e.g., in Bertrand et al., 2022).

**Litter Size and Body Size**

Slow life histories and singleton litters have arisen multiple times in mammals, including within *Homo sapiens, Loxodonta africana*, and *Balaenoptera musculus* (Ernest, 2003), and in an array of ecological contexts. This pattern of high investment into a single offspring has been associated with increased brain size (DeSilva and Lesnik, 2008) and prolonged maturation (Aiello and Wells, 2002) in the human lineage. In contrast, in Chiroptera and Dermoptera, the production of single offspring has been associated with aerial locomotor requirements (Fokidis and Risch, 2008; Garbino et al., 2021). Broadly, increased litter size has been negatively correlated with body size dimorphism in a sample of non-primate mammals (Carranza, 1996) and positively correlated with reduced body size in a sample of primates (Harvey and Clutton-Brock, 1985). These findings suggest that larger litters are generally produced by smaller-bodied, less sexually dimorphic mammals.

**Litter Size and Placentation**

The relationship between placentation and litter size variation is currently under study. Placentation types are generally described as varying along a continuum of less invasive to more invasive, with ‘invasive’ referring to the amount of direct contact with the maternal body. Fetal tissues are in direct contact with the maternal blood supply in more invasive placentation.
Multiple tissue layers separate fetal tissues from maternal blood in the most noninvasive placentation, while fewer layers of tissues may separate the fetus from maternal blood in intermediately invasive placentation. Fetal tissues are bathed in maternal blood during gestation in the most invasive types (Capellini et al., 2011). Small bodied species with invasive placenta tend to have relatively large litter sizes and small brains, while large bodied species with invasive placenta tend to have relatively small litter sizes and large brains (Elliot, 2007). This finding is interesting in that invasiveness appears to make a life history strategy more extreme, but this can be by making it ‘slower’ or ‘faster,’ depending on context. The context here is body size, which may be a correlate with distinct prenatal resource investment strategies (Elliot, 2007). Variability in placental invasiveness may be one method in which clade-specific litter size variation occurs – altering the relationship of litter size with other life history traits, as well as with environmental factors.

**Litter Size and Genetics**

The genetics underlying both intra- and interspecific litter size variation are still poorly understood. Most of the work in this area has focused on domestic animals related to food production and agriculture (Argente, 2016; SanCristobal-Gaudy et al., 2001). For example, a study on pigs found differential expression of 189 genes between pregnant pigs of high and low prolificacy (Argente, 2016).

In primates, Callitrichid-specific nonsynonymous substitutions have been identified in *GDF9* (Harris et al., 2014). *GDF9* also exhibits a greater number of polymorphisms in human populations with higher rates of dizygotic twinning (Harris et al., 2014). Further research into the
genetic underpinnings of litter size variation is necessary to identify specific genes associated with litter size variation and identify the impact of their various polymorphisms.

Litter Size and Environmental Change

Another area which requires additional investigation is the relationship between litter size variation and environmental change. Recent research has investigated the nature of mammalian life history responses to climate change-related environmental impacts. This has produced a picture of variable life history responses, even to similar climate-related changes and within one genus.

In one species of the genus *Marmota (M. marmota)*, the reduction of winter snowfall and cold temperature severity was shown to have a negative relationship with litter size and population growth, perhaps in response to increased metabolic costs during hibernation due to reduced snow insulation (Tafani et al., 2013). In another species (*M. flaviventris*), reduced winter snowfall and low temperature severity was found to be associated with increased population growth and litter size, likely due to the earlier onset of spring and longer period of resource availability (Ozgul et al., 2010).

McLean et al.'s (2019) findings support Ozgul et al.'s (2010) earlier work, where litter size of *Peromyscus maniculatus* was correlated positively with length of the frost-free period. However, McLean et al. (2019) found that populations of *P. maniculatus* generally reduced over the long-term in these changing environments, despite potential positive impacts of warmer temperatures and earlier spring arrivals. McLean et al. (2019) theorized this might be due to replacement by more generalist taxa such as *P. leucopus*. 
Noting that trends in life history variation are idiosyncratic even within genera is an important consideration for life history theorists and requires the assemblage of large comparative datasets including individual-level trait data as well as environmental data over extended periods of time. This is particularly important in the context of phenological mismatch, where changes in seasonality and the climate can produce mismatches between organisms and the resources they depend upon. This is because the impacts of global climate change have not been geographically or seasonally uniform, nor are they advancing in uniform manner (Butchart et al., 2010; Hitz and Smith, 2004).

In the United Kingdom *Rana temporaria* frog larvae are exposed to a greater degree of predation from *Triturus* newts because the newts are entering ponds earlier due to temperature increases, yet this has not had an equal impact on the phenology of frog reproduction (Visser and Gienapp, 2019). McLean and Guralnick (2021) identify photoperiod (duration of periods of light and dark) and temperature as the strongest prompters of phenological shifts in reproduction (McLean and Guralnick, 2021). Thus, phenological mismatches might be expected to occur regularly with organisms which interact with conspecifics which are terrestrial versus aquatic or semi-aquatic, or have different activity patterns (diurnal, nocturnal, crepuscular, etc.).

There also appear to be phylogenetic trends in covariation of litter size with moisture and temperature levels in North American mammals. In this area, litter size is maximum when primary productivity is highest (Weller et al., 2024). These results support the existence of a diverse range of litter size optimization strategies in mammalian life histories, which are best understood in a phylogenetic framework. This research illustrates the importance of broad comparative studies across mammals to identify clade-specific trends of variation and feature species and individual-level trait data.
These data can be used in conjunction with bioenergetic models which predict habitat expansion and reduction based on species ecological requirements (temperature, etc.) and various climate scenarios. Humphries et al. (2002) model this on the little brown bat (*Myotis lucifungus*) to test whether their model correctly predicts population extent under current climate conditions. This manner of model has also predicted that northern environments will be equally impacted by the appearance of new species, previously restricted by biogeography from inhabiting these latitudes, as they are by the extinction of old species (Humphries et al., 2004). A specific prediction is that the Canadian arctic will experience an increase in hibernator taxa (Humphries et al., 2004).

This type of modeling can only offer insight into the futures of specific species and populations if provided with sufficient understanding of organismal socioecology and morphology. This can only be obtained by studies into living populations, including potentially experimental manipulations of litter size variation (e.g., manipulations of litter size in *Tamiasciurus hudsonicus*, Humphries and Boutin, 2000), as well as phylogenetically broad investigations into life history trait evolution and variation, and clade-specific studies focusing on the connection between morphology and life history traits. The latter two methodologies are those I utilize to get a better understanding of the evolutionary history of litter size.

Investigations comparing human and nonhuman primate morphology, behavior, and life histories to other taxa are one method with which our species can understand itself and examine how our evolutionary context has shaped our brains and bodies. This is essential given the unprecedentedly rapid changes humans can create in both our immediate and global environments.
I compare humans to our primate relatives and our more distant mammal relatives so that I can generate more information about how the tendency towards producing single offspring and investing many resources, including from individuals other than the mother, has shaped human evolution and continues to impact our reproductive and social lives. A generalizable framework of how litter size interfaces with other life history traits can be used to investigate the evolutionary past and provide a deeper understanding of extant life histories. It can also be used in connection with climactic models to predict how global climate change will impact the idiosyncratic life history strategies present within mammals.

In the following chapter I will review twinning in primates, focusing on *Homo sapiens*, twinning American monkeys, and twinning strepsirrhines. This chapter will further contextualize my phylogenetic reconstruction findings, as well as provide necessary background to the geometric morphometric pilot study which I performed. I have focused my investigation on litter size in primates and related mammal groups so that I can investigate this history in the human lineage, while contextualizing said history within a broader mammalian evolutionary framework.
Chapter 2 – Primate Life History and Litter Size

_Homo sapiens_ is a prime example of a species with a life history made up of both prototypically ‘slow’ and ‘fast’ traits. Humans generally produce singletons with large brain masses at birth (Ernest, 2003; DeSilva and Lesnik, 2008) yet have a reduced interbirth interval compared to our closest relatives: _Pan, Pongo_, and _Gorilla_ (Thompson, 2013). This allows for humans to produce more offspring during their reproductive lifespans, despite investing heavily in each offspring. This makes _H. sapiens_ a difficult species to place into generalizable life history frameworks. Yet, understanding the origins of our species, and the evolutionary background which creates everyone today, is necessary given the unrivaled technological abilities and planetary impact of which our species is capable.

Modern humans are in many ways a typical primate in terms of litter size state. Within primates, most species give birth to singleton litters, with occasional twins or larger litter sizes (Ernest, 2003; Leutenegger, 1979; Tacutu et al., 2018). Bearing singletons is the norm but, as discussed, is not a universal trait for primates. There is individual variation within species and across individual lifespans, and certain primate taxa rarely produce singletons. However, twinning in humans has been associated with adverse birth outcomes, often exacerbated by environmental factors (Blickstein and Keith, 2005; Isaacson et al., 2021).

Allomaternal Care, Brain Size, and Litter Size

Isler and van Schaik (2012a) analyzed a compiled dataset of 445 mammal taxa and found that allomaternal care had a positive relationship to overall brain size in most mammals (as well as when considering placental mammals as a whole). Yet in primates, allomaternal care had a
positive relationship with reproductive output, while some aspects of allomaternal care negatively correlated with brain size (Isler and van Schaik, 2012a). *Homo sapiens* showed a mixed pattern, demonstrating behavioral and life history similarities to the positive relationships exhibited by both the primates and non-primates (e.g., large amounts of maternal and offspring provisioning and a large brain size, like the trend in Carnivora; large amounts of infant carrying and protection and a high reproductive rate, like the trend in Primates).

A similar study, using 478 species, found that male parent care (paternal care) in mammals is correlated with the evolution of larger brains, and other forms of allomaternal care correlate with increased reproductive rate (Heldstab et al., 2019). This may help illuminate the distinct trends identified by Isler and van Schaik (2012a), yet humans remain an intriguing case in showing advanced levels of both types of care alongside large brains and high reproductive output.

Identifying when this specific confluence of life history traits emerged, with the most advanced cooperative breeding behaviors accompanying both relatively large brains and a high reproductive output, may be key to answering questions about the initial dispersal of our lineage into Eurasia, the extinction of all non-modern human species, and identifying what aspects best differentiate our species from the extinct members of our lineage.

*Twinning in Primates*

Our primate relatives are often characterized as being more on the ‘slow’ side of the life-history continuum, yet researchers have observed population-level variation in twinning rates in many primates, including humans (Blickstein and Keith, 2005). In *Macaca*, the most widespread genus other than *Homo*, provisioning was associated with a significantly higher rate of twinning
over two 28-year periods: 0.137% twinning frequency when provisioned, 0.027% non-provisioned frequency (Sugiyama et al., 2011). Despite this intraspecific variation in plural birth rate, most primate taxa produce singletons most of the time (Ernest, 2003).

In general, primates that typically produce singletons demonstrate less variation in litter size. This is supported by the relatively low prevalence of multi-offspring births in singleton-bearing taxa such as humans and other catarrhine primates (Blickstein and Keith, 2005; Geissmann, 1990). This intuitively makes sense, as a shift of one offspring up to twins would be a 100% increase in litter size, a 50% or greater relative increase compared to taxa which produce mean litters greater than one. This also makes intrauterine position effects a very uncommon factor in singleton taxa life histories, compared to a routine factor in taxa with larger litters.

The callitrichids are a family of American primates made up of taxa which generally give birth to twins. Several species have even been observed to produce litters of three or more at a higher rate than singletons (McCoy et al. 2019; Ross et al. 2007b; Rutherford et al. 2021). Twins and larger litter sizes are reported as common in some strepsirrhine species including *Galago crassicaudatus*, *Galago demidovii*, and *Microcebus murinus* (Ernest, 2003; Nash, 1983).

Singletons have been documented as more common than litter sizes of three or more in most galagos and some of the twinning callitrichids (Masters et al., 1988; McCoy et al., 2019; Nash, 1983). Some (e.g., Leutenegger, 1979) argue that litter size in callitrichids is a derived trait, resulting from an evolutionary shift towards smaller adult body size. Contrastingly, Leutenegger asserts that twinning strepsirrhines have retained both small body size and larger litters (Leutenegger, 1973). Thus, he posits that the ancestral state of litter size of primates was twinning, while the ancestral state of Platyrrhini was singleton-bearing (Leutenegger, 1973, 1979). Testing this position was one motivation of this thesis. I encountered findings which
suggest that larger-bodied galagos have multiple births more frequently than smaller-bodied
galagos (Nash 1983), which would seem to contradict the above reasoning (associating phyletic
nanism with phyletic twinning).

Dermoptera (colugos, also known as flying lemurs) and Scandentia (tree shrews) are
closely related to Primates. These taxa display litter size diversity similar to that seen within
primates. All colugos have been reported as primarily having singleton litters (Byrnes et al.,
2011; Ernest, 2003; Tacutu et al., 2018). Tree shrews are documented to have larger litter sizes,
with twins being the most common (Ernest, 2003; Tacutu et al., 2018; Xiao et al., 2017).

Both groups of mammals have been used to refine primate phylogenies and in attempts to
reconstruct the characteristics of the earliest primates and plesiadapiformes, the extinct group of
mammals believed to be the closest relatives to primates (Bloch and Boyer, 2002; Sargis, 2004).
Dermoptera, often considered to be our closer relative of the two, have singletons. However,
their locomotion pattern (gliding) has been proposed as an explanation for their singleton litters,
comparable to the singleton litters present in all but one family of Chiroptera (bats; Byrnes et al.,
2011; Carranza, 1996; Garbino et al., 2021).

Being the product of a singleton birth, the most common primate trait, has been
associated with positive outcomes, such as higher reproductive output at adulthood. This is true
even for some of the callitrichid monkey species mentioned above, well known for their
production of twins (McCoy et al., 2019). Additionally, callitrichid monkey individuals born into
isosexual litters (litters of all one sex) were underrepresented as parents once they became adults
(McCoy et al., 2019; Rutherford et al., 2021). Not all litters are necessarily equivalent, even if
they are the same size.
Morphological characters which produce, or permit, this intraspecific variation provide an interesting comparison to human reproductive traits, especially given that humans show variation regarding twinning frequency both geographically, temporally, and within populations (Blickstein and Keith, 2005; Isaacson et al., 2021; Razzaque et al., 1990). Some of this variation is directly attributable to fertility treatments, a behavioral adaptation of our species which does not have any reflection in skeletal morphology (Blickstein and Keith, 2005; Darwin, 1996). Nonetheless, understanding the consequences and origins of producing multiple offspring in our order would help refine the implementation of fertility treatments, and allow for more detailed information regarding outcome and treatment options given individual and environmental context.

Biology of Multifetal Births

Plural births can result from one fertilized egg, or zygote, or multiple (Hall, 2003). Twins generally are monozygotic (arising from one zygote, also known as identical twins) or dizygotic (arising from two zygotes, non-identical or fraternal twins). Triplets can be monozygotic, dizygotic, or trizygotic. Collectively, plural births can be referred to as either monozygotic or polyzygotic (arising from more than one zygote).

Numbered terminology (e.g., dizygotic, trizygotic) can be useful in cases where, for example, a triplet trio is made up of two identical and one non-identical offspring (Allen, 1988). Both kinds of twinning occur in humans and non-human primates (Blickstein and Keith, 2005; Ely et al., 2006; Ross et al., 2007a). An important note, and one that has led to the likely ongoing underestimation of monozygotic twinning rates, is the presence of discordance in monozygotic
twins. For example, up to 10% of monozygotic twins show ‘mirror-image’ differences: e.g., right versus left-handedness (Blickstein and Keith, 2005; Hall, 2003).

A review found that testosterone transfer from male to female fetuses is greater when said fetuses are adjacent in the uterus, and these effects have been demonstrated in mice and pigs to include permanently altered hormone levels, behavioral changes, among other shifts (Ryan and Vandenbergh, 2002). These position effects must surely be a factor in the twinning callitrichids, and it is unknown how these effects may interact with the routine chimerism present in these taxa (Martin, 2012; Ross et al., 2007a). Further research into generations of callitrichid litters is necessary to begin to address these questions.

_Twilling and Chimerism in American Monkeys_

Chimerism, or the presence of multiple genotypic expressions within a single individual, generally presents on a small scale in humans (often called microchimerism). It is commonly found within maternal parents who have given birth and have genetic material from their offspring present in their bodies postpartum, called fetomaternal microchimerism (Bianchi et al., 1996). Microchimerism is also encountered in both monozygotic and polyzygotic twins (Johnson et al., 2020; Yunis et al., 2007). Other forms of chimerism include artificial chimerism resulting from tissue and bodily fluid donations, or tetragametic chimerism wherein two separate fertilized eggs fuse to form a crossed-cell line individual (Baxter et al., 2019; Hall, 2003; Tardif and Ross, 2019; Yunis et al., 2007).

Twinning is not consistent across primates, nor is chimerism, which varies widely between taxa. Callitrichids (marmosets and tamarins) are the only primates known to regularly
produce dizygotic twins where offspring exhibit chimerism in sperm (Ross et al., 2007a) and an array of hematopoietic tissues: bone marrow, blood, the spleen, the thymus (Baxter et al., 2019).

The prevalence and profundity of chimerism is much greater in the twinning American monkeys (callitrichids) than in humans. Peripheral blood chimerism (a form of microchimerism) has been observed in 8% of human twin pairs and 21% of human triplets (Johnson et al., 2020; van Dijk et al., 1996), whereas Ross et al. (2007a) encountered 72.2% of callitrichid twin pairs with chimeric tissues, including chimerism in brain, muscle, and various organ tissues, in addition to 57.1% of sperm samples (Ross et al., 2007a). The much greater prevalence of chimeric transfer in this primate clade raises interesting questions as to the life history trajectory of these monkeys. The twinning of this clade, as well as that of other twinning taxa (e.g., some strepsirrhine primates), can be compared to variation between humans, the extant apes, and extinct hominoid lineages.

The regularity with which callitrichids produce twins (Leutenegger, 1979; Tardif and Ross, 2019) combined with the different reproductive physiology and outcomes associated with twinning in these taxa compared to human twinning, all serve to demonstrate how selective pressure has produced distinct life history traits amongst relatively close relatives.

Many callitrichids primarily produce twins (Ernest, 2003; Leutenegger, 1973, 1979), and most individuals are chimeric twins in at least several species (Ross et al., 2007a, 2007b). This may help explain why it is so common in these taxa. Chimeric twinning may help offset some of the adverse outcomes associated with twinning (Isaacson et al., 2021; Ross et al., 2007a). However, adverse outcomes associated with plural birth still seem to appear in some callitrichid life histories (McCoy et al., 2019; Ross et al., 2007b; Rutherford et al., 2021).
Worley et al. (2014) report the whole genome sequence of *Callithrix jacchus* (a twinning callitrichid monkey). They describe the presence of distinct forms of genes such as *WFIKKN1* which are thought to correspond to changes in immune-system function which circumvent potentially deleterious responses to chimerism (Worley et al., 2014). Whole genomes of multiple callitrichid taxa may help illuminate questions as to the timing of these adaptations, and whether individual species show specific genotypic expressions of this life history strategy.

Dunbar (1995) theorizes that twinning, and other adaptations which increase reproductive output, like the absence of lactational amenorrhea, could only have become selectively advantageous with allomaternal care from male parents already present. Thus, he posits that monogamous male mating strategies predate twinning in this lineage (Dunbar, 1995). Increases in allomaternal care do not always result in increases in mean litter size (e.g., *H. sapiens*), so identifying clade-specific factors such as chimerism allows us to consider why these trends differ. Research on variation between callitrichid species and populations, and phylogenetic reconstructive analyses such as the ancestral state reconstructions which I performed, help to clarify these evolutionary and adaptive questions.

*Evolutionary History of Litter Size*

Examining the relationship between reduction in litter size and morphological changes in our lineage and making comparisons to other lineages where litter size has been under selection, can provide us with information regarding the evolution of human reproduction. Comparative morphological analyses of primate taxa which display a range of litter sizes may reveal characters which illuminate the ways in which reproductive energetics change over time. Research has also provided evidence of skeletal variation occurring within our species at time
scales as short as one millennium (Dzupa et al., 2021). These analyses can also reveal proximate features which may be more evolutionarily responsive to these changes, and thus can be used to trace phylogenetic relationships (Brasil et al., 2020; Lewton, 2012). This motivates the geometric morphometric pilot study portion of this investigation, which examines craniodental morphology of primate taxa.

Reproductive traits, including litter size and reproductive energetics, can also be used to assess the evolutionary history of hominids. Energetics has been proposed as a contributing factor to Neanderthal extinction, perhaps deriving from cold adaptations that ceased being advantageous in the changing Upper Paleolithic (Ocobock et al., 2021; Ponce de León et al., 2008). Other theories of Neanderthal extinction include cultural practices (Conard, 2011), diet and dietary practice differences (Henry, 2017; Hockett, 2012), interbreeding with anatomically human populations that had larger population sizes (Stringer and Crété, 2022), and growth rate (Ponce de León et al., 2008). Morphological characteristics which indicate which hominids may have been well-suited to the delivery of a single, large offspring when compared to anatomically modern humans may help illuminate theories of Neanderthal extinction, hominid variation, the relative success of different primate taxa over time, and the origins of human-like traits.

Biocultural evolution is a key consideration for questions related to Neanderthal extinction and about what confluence of environmental and phylogenetic circumstances created the anatomically modern human life history strategy. Dunsworth and Eccleston (2015) discuss how, based on absolute brain and body masses from the fossil record, childbirth may not have been truly modern until relatively recently, within the past 500,000 years (Dunsworth and Eccleston, 2015). This is also supported by prenatal growth rates reconstructed from fossil dental and cranial anatomy (Monson et al., 2022).
*H. sapiens*’ short interbirth intervals and large, energetically expensive infants are often used to define us as a species. The delivery of the absolute largest infant sizes (including use of technological interventions such as caesarean section, postpartum care, etc.) may have become evolutionarily tolerable only recently in the history of our species with advances in medical technologies. Prior research supports that some hominids, like Neanderthals, may have lived in smaller communities than modern humans, with females migrating to new groups (Skov et al., 2022). This is similar to what has been observed in the behavior pattern of modern populations of *Pan troglodytes*, with some groups showing an order of magnitude greater mitochondrial diversity than Y-chromosome diversity (Skov et al., 2022).

Isler and van Schaik (2012a, 2012b) suggest that an advanced level of cooperative care is crucial to making the modern human life history strategy tolerable and scalable. A lack of any one of these biocultural factors may have inhibited Neanderthals in a) developing the same pattern as seen in anatomically modern humans, or b) avoiding negative impacts of such high reproductive output and large offspring, especially if faced with competition from relatively advantaged modern human populations.

Within hominids, reproduction of one large offspring has been theorized to be part of what ignited the trend toward sociality and slow life history. This is epitomized in anatomically modern humans (Aiello and Key, 2002). This is especially interesting when looking at sociality and intelligence in other primate lineages. Callitrichids outperform their most closely related taxa (Cebidae: capuchins and squirrel monkeys) in socially oriented cognitive tasks (Burkart and Van Schaik, 2010). Different reproductive characters (twins versus singleton litters) may nonetheless be convergent and lead to similar outcomes (enhanced sociality based on the need for parental cooperation) even within primates.
Litter size is thus a crucial concept when applied to modern humans. Many traits which were considered unique to humans have more recently been observed in other extant primate species, such as rotational birth, the process of an infant rotating to allow for the widest dimensions of its body to pass through the various parts of the birth canal (Trevathan, 2015). The implications of a better understanding of modularity within our reproductive morphology extend to existing phylogenetic debates, human and animal wellbeing, and ecological management (Clune et al., 2013; Lewton, 2012).

**Litter Size and Neonatal Environment**

Litter size and twinning plays an important but poorly understood role in determining neonatal environment. The developing fetus is exposed to different environmental conditions based on maternal diet and, crucially here, whether other fetuses are developing alongside it. The impact of neonatal environment has been investigated in rodents, a taxon that is regularly studied in laboratory settings due to the combination of their relatively close relationship to primates and their quick reproduction.

These studies have found that intrauterine position, including the number and sex of fetuses in neighboring positions, impacts development and life history throughout an organism's life. These impacts can include changes to physiology, morphology, and behavior. For example, female mice whose fetal position was bounded by male siblings were more likely to initiate fights with male mice during experimental manipulations on the impacts of testosterone on female mice (Gandelman et al., 1977; Ryan and Vandenbergh, 2002). In both morphological and behavioral assessments, female mice with male siblings on either side while in-utero showed the most ‘male-like’ variation (Gandelman et al., 1977).
Neonatal environment, including number and sex of offspring, has been found to influence age at menarche in humans (Almond and Currie, 2011; Ryan and Vandenberghe, 2002; Yermachenko and Dvornyk, 2014). Age at menarche is also influenced by birth and postnatal factors such as prematurity and birth weight (Yermachenko and Dvornyk, 2014). Prematurity and birth weight are likewise influenced by twinning (Blickstein and Keith, 2005; Isaacson et al., 2021). In Isaacson et al.’s (2021) study, the median weight of singletons was 750 grams higher than first-born twins and 790 grams higher than that of second-born twins. Twinning can have concomitant and potentially compounding effects across the body and throughout the lifespan, though the long-term impacts of the changes in birthing weight and period are relatively poorly understood (Blickstein and Keith, 2005; Isaacson et al., 2021). It is clear that litter size can be implicated in a broad web of phenotypic effects.

**Twinning and Primate Body Size**

Montgomery and Mundy (2013) investigated trends of covariation for neonatal and adult body mass in a sample of 101 species including 11 callitrichid taxa and 5 cheirogaleid taxa. These two families include the smallest primates and feature twinning species (Ernest, 2003; Montgomery and Mundy, 2013). Their findings support that the smallest taxa in both groups exhibit phyletic nanism, that in each case that it is a derived rather than an ancestral state (Montgomery and Mundy, 2013).

However, these findings indicate that, uniquely among primates, American monkey body size reduction is associated with reduced prenatal growth rate rather than reduced length of growth phases (shorter gestation lengths and younger maturation). They discuss similarities between the fossil hominid *Homo floresiensis* and small-bodied callitrichids. Both lineages
exhibit reduced body mass, lack megadonty, and show similar levels of moderately reduced levels of encephalization (Burger et al., 2019; DeSilva and Lesnik, 2008; Montgomery and Mundy, 2013; Sacher and Staffeldt, 1974). The slow prenatal growth rate in callitrichids has also been implicated in the evolutionary loss of the third molars in this lineage (Monson et al., 2019).

Based on findings supporting the advanced social-cognitive capabilities of both small-bodied callitrichids (Burkart and van Schaik, 2010) and *H. floresiensis* (Jungers and Baab, 2009) investigations into how this complexity was maintained despite reductions in both absolute and relative brain size are necessary. This is another aspect informing my pilot study into craniofacial morphology in primates. The assembling of a large dataset of extant and fossil primate crania can be used to assess patterns of morphological covariation with reproductive life history traits.

*Human Twinning*

In humans, twinning has been associated with many factors, with many pregnancies experiencing adverse outcomes. There is evidence of cultural recognition of twinning dating back to at least approximately 31,000 cal BP. Researchers described double-burial of monozygotic infants with evidence that the grave was reopened to add the second sibling upon its passing, 50 days after the first twin (Teschler-Nicola et al., 2020). The presence of a single grave containing one infant at the same locale (Gravettian site, Krems-Wachtberg, Austria), and the evidence for reopening, illustrate that twinning has likely been recognized, and traditions altered in consideration of twinning, for at least this long in human biocultural history (Teschler-Nicola et al., 2020).

Research has identified trends associated with twinning across large samples. The mothers of twins are older and have had more prior pregnancies (Isaacson et al., 2021). Twins
are more likely to be delivered via Cesarean section (Isaacson et al., 2021), to be born preterm (less than 37-week gestations), and have smaller birth weights (Blickstein and Keith, 2005; Isaacson et al., 2021). Twins are also more likely to be stillborn. Studies have found that the second twin to be born was more likely to be stillborn than the first (Isaacson et al., 2021). The study confirms many expectations of twin births but contextualizes the results in the fact that the study site (Botswana) has a higher frequency of adverse outcomes for all births than high-income countries (Isaacson et al., 2021).

Anatomically modern humans show a range of twinning frequencies (Blickstein and Keith, 2005), and the prevalence of technological fertility interventions has only increased over the past decades (Blickstein and Keith, 2005; Hall, 2003). Identifying advantages of twinning in other species may allow us to refine these technologies and our reproductive care practices. Additionally, analyzing when and how other primate lineages have experienced shifts in litter size state may provide insight into potential significance of human variation in twinning rate.

Beyond these goals, this understanding will enhance understanding of human wellbeing, sociality, and myriad other life history characteristics given the close link between reproduction, fitness, and lifelong health and wellness (Darwin, 1996; Ellison, 2001; Paul, 2010). The diversity of plural birth present in primates alone (twinning, singletons, chimeric or microchimeric, etc.) presents an opportunity to refine our understanding of litter size variation, how this trait has shaped primate diversity today, and how this trait relates to the interfacing of given primate species and their environments.
Major Aims of the Study

This study has several major aims that advance our understanding of twinning and primate litter size using a variety of methods:

**AIM 1:** Analyze the phenotypic relationships between litter size and other life history traits (e.g., adult body mass) over evolutionary time.

**AIM 2:** Estimate the ancestral condition of litter size in Primates and Boreoeutheria.

**AIM 3:** Assess the phenotypic relationship of craniofacial morphology to twinning in Primates.

In the following chapter I will discuss the samples I gathered to address these aims. I will also describe the methods I used to investigate litter size variation over boreoeutherian evolutionary history, as well as craniofacial and litter size covariation in two primate taxa. I designed these methods to investigate the above aims, generated by this review into litter size and primate evolution.
Chapter 3 – Methods

I designed this thesis to analyze the statistical relationship between litter size and other life history traits, reconstruct the litter size state of various ancestral mammals, and investigate the impacts of twinning on craniofacial morphology in primates. A key goal for this project is to assess litter size using a greater number of species and broader range of taxa than prior investigations. I also began collecting a 3D scan dataset and designing a methodology which would allow a large portion of this life history dataset to be assessed alongside skeletal morphological traits.

I collated data on a large sample of eutherian mammals and used phylogenetic statistical analyses to test questions related to litter size. I then 3D scanned N=60 American monkeys to complete a preliminary analysis of craniofacial morphology in twinning and non-twinning primates.

Litter Size and Life History Analyses

I collated data on life history for N=955 species of extant eutherian mammals. The breakdown of species and the references for the data are presented in Table 1. The majority (over 90%) of extant families were represented for every clade within Boreoeutheria. This includes representatives of all extant families within Primates, Dermoptera, and Scandentia.

I selected the placental mammal magnorder Boreoeutheria because it includes Primates, my primary focal group, as well as the rest of the Euarchontoglires (Clades Dermoptera, Scandentia, Rodentia, and Lagomorpha) and all Laurasiatheria (Clades Artiodactyla, Chiroptera, Perissodactyla, Carnivora, Pholidota, Erinaceidae, Soricidae, Talpidae, Solenodontidae). I
elected to focus on Boreoeutheria so that my analyses include multiple litter size states in addition to several shifts in litter size state across these various clades.

**Table 1.** Taxa represented in dataset of life history characters & their distribution throughout magnorder Boreoeutheria.

<table>
<thead>
<tr>
<th>Clade</th>
<th>Families (n)</th>
<th>Extant Families in Clade (Percent represented: n / EFC)</th>
<th>Species (n)</th>
<th>References*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artiodactyla*</td>
<td>10</td>
<td>10 (100%)</td>
<td>147</td>
<td>1, 2, 4, 5, &amp; 6</td>
</tr>
<tr>
<td>Carnivora</td>
<td>15</td>
<td>15 (100%)</td>
<td>176</td>
<td>1, 5, &amp; 6</td>
</tr>
<tr>
<td>Cetacea</td>
<td>11</td>
<td>14 (78.6%)</td>
<td>42</td>
<td>1, 5, 6, &amp; 8</td>
</tr>
<tr>
<td>Chiroptera</td>
<td>11</td>
<td>18 (61.1%)</td>
<td>88</td>
<td>1, 5, &amp; 6</td>
</tr>
<tr>
<td>Dermoptera</td>
<td>1</td>
<td>1 (100%)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Erinaceomorpha</td>
<td>1</td>
<td>1 (100%)</td>
<td>9</td>
<td>1 &amp; 6</td>
</tr>
<tr>
<td>Lagomorpha</td>
<td>2</td>
<td>2 (100%)</td>
<td>19</td>
<td>1 &amp; 6</td>
</tr>
<tr>
<td>Perissodactyla</td>
<td>3</td>
<td>3 (100%)</td>
<td>11</td>
<td>1 &amp; 6</td>
</tr>
<tr>
<td>Pholidota</td>
<td>1</td>
<td>1 (100%)</td>
<td>2</td>
<td>1 &amp; 6</td>
</tr>
<tr>
<td>Primates</td>
<td>16</td>
<td>16 (100%)</td>
<td>158</td>
<td>1 - 7</td>
</tr>
<tr>
<td>Rodentia</td>
<td>30</td>
<td>30 (100%)</td>
<td>264</td>
<td>1, 5, &amp; 6</td>
</tr>
<tr>
<td>Scandentia</td>
<td>2</td>
<td>2 (100%)</td>
<td>7</td>
<td>1, 2, &amp; 6</td>
</tr>
<tr>
<td>Soricomorpha</td>
<td>3</td>
<td>3 (100%)</td>
<td>31</td>
<td>1 &amp; 6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>106</strong></td>
<td><strong>116 (91.4%)</strong></td>
<td><strong>955</strong></td>
<td><strong>1-8</strong></td>
</tr>
</tbody>
</table>


Larger litters are typical in Rodentia, Erinaceomorpha, Lagomorpha, and Soricomorpha. Smaller litters typical in Chiroptera, Artiodactyla, Primates, Dermoptera, Pholidota, and  

33
Perissodactyla. Scandentia and Carnivora are intermediate in terms of mean litter size. Most of these groups feature subsidiary clades which do not follow the overarching trend, e.g., twinning in primates like *Saguinus oedipus*, singletons borne by the rodent *Plagiodontia aedium* (Ernest, 2003). This sample features multiple examples of both positive and negative shifts in litter size which may then be compared during analysis.

I collected data on litter size as well as on multiple other life history and size traits. These traits include gestation period in days, maximum longevity in years, age at female and male maturity in years, birth and adult weights measured in grams, litters per year, age at weaning in days, interbirth interval in days, and neonatal and adult brain mass in grams. See all data references in Supplemental Table S5.

I began collecting these data with a search for online repositories of life history characters using a series of key terms (Table 2). These sets of terms always included ‘data,’ ‘dataset,’ or ‘database.’ These were accompanied by a life history term. I began the search with ‘litter size’ and proceeded based on other desired characteristics. If data appeared incorrect, I checked the source material and submitted corrections to the dataset managers. In the case of one source (Crile and Quiring, 1940), there is a clear example of species misidentification where two *Aotus* specimens are reported to weigh over 8 and over 9 kilograms respectively (larger than many howler monkeys). This dataset has found its way into many compiled datasets and publications, and in general goes unnoticed because it produces average values which accord to primate norms (e.g., for overall assessments of relative brain size, etc.). Nonetheless, it should be excluded from datasets due to the impossibility of knowing to which primate species these specimens truly belong.
Table 2. Data collection search terms used to find life history traits present in collated database.

<table>
<thead>
<tr>
<th>Life history term used:</th>
<th>Databases encountered:</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Litter Size’</td>
<td>AnAge (Tacutu et al., 2018); Animal Diversity Web* (Fredrickson, 2009; Shefferly, 2004a, 2004b); Ecological Archives E084-093 (Ernest, 2003).</td>
</tr>
<tr>
<td>‘Placentation’</td>
<td>Cappelini et al. (2011) database.</td>
</tr>
</tbody>
</table>

*Used to verify or supplement other databases, confirm missing or disputed variables.

I also calculated encephalization quotients (EQs). Encephalization is the ratio of actual brain size to expected brain size based upon body size and phylogenetic relationships. It is commonly used as a proxy for intelligence in primate studies (e.g., Boddy et al., 2012; Rightmire, 2004). I computed an encephalization quotient (EQ) via the following formula, described by Jerison (1985): $EQ = \frac{brain\ mass}{(0.12 \times body\ mass^{(2/3)})}$. EQ is used to produce a comparison between real versus expected brain mass, the latter estimated by this denominator. This formula is in regular use, but there is ongoing evaluation of its validity in comparative analyses (e.g., Peñaherrera et al., 2017).

Based on this ongoing assessment and on Bertrand et al.’s (2022) methodology, I generated additional quotients for analysis: 1) an EQ using their formula (EQ2), and 2) my own phylogenetic EQ (PEQ) created from the dataset assembled here, using the slope and intercept values from a PGLS regression of logarithmically transformed brain and body masses (2022). PEQ was created using log$_{10}$ transformed brain and body masses.

$$EQ2 = \frac{brain\ mass}{(0.21 \times body\ mass^{(0.64)})}$$

$$PEQ = \frac{brain\ mass}{(0.49 \times body\ mass^{(0.55)})}$$
I also used published formulas to calculate prenatal growth rate (PGR, Montogomery and Mundy, 2013), brain-body ratio (BBR, Sacher and Staffeldt, 1974), percentage of brain growth accomplished before birth (PGN, Eisert et al., 2014), and percentage of brain growth accomplished post-birth (PGV, Eisert et al., 2014):

\[
PGR = \frac{\text{neonatal mass (birth weight)}}{\text{gestation length (days)}}
\]

\[
BBR = \frac{\text{brain mass}}{\text{adult mass}}
\]

\[
PGN = \frac{\text{neonatal brain mass}}{\text{adult brain mass}}
\]

\[
PGV = \frac{(\text{adult brain mass} - \text{neonatal brain mass})}{\text{adult brain mass}}
\]

All body and brain size data as well as the larger longevity data (maximum lifespan, age at male and female maturity) were size-corrected (\(\log_{10}\)) prior to analyses.

Analytical Methods – Statistical Analysis

These analyses were performed using RStudio (Posit Team, 2022), on base R version 4.2.2 (R Core Team, 2022). I generated a series of summary statistics for the dataset using the describeBy function in psych (Revelle, 2023). This function reports summary statistics (sample size, mean, standard deviation, median, minimum and maximum values, range, skew, kurtosis, and standard error) by grouping variables Order (in this case, Order or clade), Family, and Genus.

I then used the read.tree function in ape (Paradis et al., 2022). This function reads a file containing one or more trees in parenthetic format, in this case a NEXUS file. I used the smaller tree, containing 4,125 species, from the supplemental data of Faurby and Svenning’s (2015) paper. I used this phylogeny to inform the analyses which require relatedness values.
I calculated the statistical relationship between litter size and every other life history trait collected within a phylogenetic framework using Phylogenetic Generalized Least Squared analyses (PGLS). PGLS analyses are used to estimate the association between traits or sets of traits while correcting for phylogenetic relatedness between taxa.

PGLS comparisons were run between litter size and birth weight, adult weight, brain mass, neonatal brain mass, gestation length, EQ, proportion of brain growth accomplished prenatally, prenatal growth rate, age at female and male maturity, litters per year, interbirth interval, maximum longevity, encephalization index, and brain-body ratio. PGLS comparisons were also run between prenatal growth rate and log$_{10}$ brain mass, proportion of brain growth accomplished prenatally and prenatal growth rate, and prenatal growth rate and brain mass. An additional PGLS calculation comparing brain mass and adult body weight was done to produce a phylogenetically informed encephalization quotient (PEQ) as outlined in Bertrand et al.’s (2022) work on the evolution of mammalian encephalization (see PEQ and EQ2 above). All PGLS analyses were calculated using the pgls function in caper (Orme et al., 2018).

I calculated the phylogenetic signal for each life history trait using the function phylosig in phytools (Revell, 2012) for both Pagel’s Lambda ($\lambda$) and Blomberg’s $K$ (Diniz-Filho et al. 2012). Phylogenetic signal tests are used to quantify the tendency of related biological species to resemble each other more than any other species. They produce a value which signifies how closely a given trait adheres to this tendency within a given phylogeny, comparing the character states for the trait present within the phylogeny and analyzing how closely the trait variation corresponds to phylogenetic relatedness.

Pagel’s lambda and Blomberg’s $K$ analyses each test for phylogenetic signal yet use different frameworks (Pagel 1999; Blomberg et al. 2003). Pagel’s Lambda is a tree
transformation which stretches tip branches relative to internal branches, making the tree more like a complete polytomy (Walsh et al. 1999). Lambda produces values between zero and one. When an estimated lambda equals zero, the traits are presumed to have no phylogenetic signal, indicating the trait evolved independently of phylogenetic relatedness or might be under strong natural selection. A Lambda of one corresponds to a Brownian motion model of evolution. Any other value suggests an intermediate model (Molina-Venegas and Rodríguez 2017).

$K$ variance differs. Brownian motion still produces an expected value of one, but $K$ values range from greater to less than one. A $K$ value under one is interpreted as taxa resembling each other less than expected, while a $K$ of more than one is interpreted to signify that they are more closely related than Brownian motion would predict (Blomberg et al. 2003; Diniz-Filho et al. 2012).

I created bivariate plot visualizations of trends in this data using the qplot function of **ggplot2** (Wickham, 2016). I then performed a principal component analysis (PCA) on litter size, litters per year, age at male and female maturity (both years), maximum longevity (years), logarithmically transformed adult weight and brain mass (both grams), and gestation length (days) using the prcomp function in the **stats** package built-in to R (R Core Team, 2022).

Principal component analyses (PCAs) compute variables called principal components (PCs) which are linear combinations of the input variables. The first PC has the largest possible variance. Therefore, the first PC (or PC1) will explain the largest part of the variance in the data. The second component, or PC2, is computed under a constraint of being orthogonal to the first component and to explain the largest possible variance. This proceeds for the following PCs. Principal component analyses seek to summarize important information about data by
simplifying multiple interrelated variables into new variables, the PCs, which more closely align to the data’s variance, sometimes called inertia.

I made plots of PC1 and PC2 as well as of PC2 and PC3 using qplot (Wickham, 2016). I then used the fviz_pca_var function in the \textit{factoextra} package (Kassambara and Mundt, 2020) to graph the impact of every life history character on PCs 1 and 2. I also visualized correlation between these select life history variables by creating a correlation matrix with the cor function in \textit{stats} (R Core Team, 2022) and the ggcorrplot function of the package \textit{ggcorrplot} (Kassambara, 2023).

\textit{Analytical Methods – Ancestral State Reconstruction}

Ancestral state reconstructions (ASRs) are also sometimes called ‘Character Mapping.’ They are a method of using measured characteristics of individuals, populations, or taxa alongside a representation of these individual’s or taxon’s relatedness to statistically infer the characteristics which would have been present within their common ancestors. The reconstruction of evolutionary relationships among taxa is one way we can inform our understandings of evolutionary history (e.g., Garbino et al., 2021; Kappeler, 1998).

I elected to reconstruct ancestral states for the following life history characters: ‘Litter Size’ (n=955), ‘Gestation’ length in days (n=797), ‘BBR’ (Brain-body Ratio, n=666), PGR (Prenatal Growth Rate, n=658), log\textsubscript{10}PGR (n=658), ‘ML’ (Maximum Longevity in years, n=729), AW (Adult Weight in grams, n=944), ‘EQ’ (Encephalization Quotient, n=666), ‘EQ2’ (n=666), and log\textsubscript{10}‘B’ (Brain mass in grams, n=667). These traits are often used in life history analyses, and they cover mass variables as well as reproductive patterns.
I estimated ancestral values for these life history characters using contMap in *phytools* (Revell, 2012). This function maps continuous variables using phylogenetic relatedness. I quantified the estimated values at internal nodes using fastAnc in *phytools* (Revell, 2012). This generates a maximum-likelihood ancestral state for any continuous trait.

**Table 3.** Life history character sample sizes & internal nodes in ancestral state reconstructions.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Abbreviation</th>
<th>Sample Size (n)</th>
<th>Internal Nodes (ASR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litter Size</td>
<td>LS</td>
<td>955</td>
<td>954</td>
</tr>
<tr>
<td>Gestation Length (days)</td>
<td>G</td>
<td>797</td>
<td>796</td>
</tr>
<tr>
<td>Maximum Longevity (years)</td>
<td>ML</td>
<td>729</td>
<td>728</td>
</tr>
<tr>
<td>Male Maturity (years)</td>
<td>MM</td>
<td>447</td>
<td>NA</td>
</tr>
<tr>
<td>Female Maturity (years)</td>
<td>FM</td>
<td>653</td>
<td>NA</td>
</tr>
<tr>
<td>Birth weight (grams)</td>
<td>BW</td>
<td>691</td>
<td>NA</td>
</tr>
<tr>
<td>Adult weight (grams)</td>
<td>AW</td>
<td>944</td>
<td>943</td>
</tr>
<tr>
<td>Litters per year</td>
<td>LY</td>
<td>648</td>
<td>NA</td>
</tr>
<tr>
<td>Interbirth interval (days)</td>
<td>IB</td>
<td>492</td>
<td>NA</td>
</tr>
<tr>
<td>Neonatal brain mass (grams)</td>
<td>NBrain / NB</td>
<td>110</td>
<td>NA</td>
</tr>
<tr>
<td>Adult brain mass (grams)</td>
<td>Brain / B</td>
<td>668</td>
<td>NA</td>
</tr>
<tr>
<td>Encephalization quotient</td>
<td>EQ</td>
<td>666</td>
<td>665</td>
</tr>
<tr>
<td>Brain-body Ratio</td>
<td>BBR</td>
<td>666</td>
<td>665</td>
</tr>
<tr>
<td>Proportion of brain growth accomplished prenatally</td>
<td>PGN</td>
<td>107</td>
<td>NA</td>
</tr>
<tr>
<td>Prenatal growth rate</td>
<td>PGR</td>
<td>658</td>
<td>657</td>
</tr>
</tbody>
</table>

NA: Not used for ancestral state reconstruction. ASR: Ancestral State Reconstruction

Litter size was mapped as a continuous trait onto a tree with 955 tips and 954 internal nodes. All traits were mapped onto trees according to available sample size (Table 3).
ancestral state reconstructions with and without node labels can all be viewed in Supplemental Table S6. Each node number can be associated with the accompanying values reconstructed using fastAnc in Supplemental Table S6.5.

**Craniofacial Morphological Analyses**

A key goal of this thesis was to conduct a preliminary analysis investigating craniofacial morphology in twinning and non-twinning primates.

**Materials**

I performed this geometric morphometric pilot analysis using N=60 Platyrrhini cranial scans. This sample was made up of n=30 *Saguinus oedipus* and n=30 *Sapajus apella* crania (sex balanced samples: n=15 males, n=15 females).

*Saguinus oedipus* is a twinning platyrrhine monkey, and *Sapajus apella* is a closely related singleton-bearing platyrrhine monkey. *Saguinus oedipus* is a callitrichid monkey (family: Callitrichidae). This family is known for taxa with relatively small body sizes, which produce twins, which perform well on socio-cognitive evaluations, and which feature cooperative polyandrous mating strategies (Buckner et al., 2015; Burkart and van Schaik, 2010; Sussman, 2003). *Sapajus apella* is a cebid monkey (family: Cebidae). This family is known for taxa with relatively high levels of encephalization, tool use, and high cognitive performance (Byrne et al., 2022)

These primates are both only found in Central and South America and are primarily arboreal (Byrne et al., 2022). The last common ancestor of all extant callitrichids may have lived around 14 million years ago (Buckner et al., 2015), and cebid and callitrichid monkey lineages
may have diverged some 20 million years ago (Byrne et al., 2022). I selected both a cebid and callitrichid species, as these clades have historically been linked and even considered a singular family (e.g., Garbino and Martins-Junior, 2018), and the position of Aotidae is more poorly resolved (Byrne et al., 2022; Perez et al., 2013).

**Data Collection Methods**

I scanned all crania at the Smithsonian National Museum of Natural History (NMNH). These scans were obtained from the NMNH with the assistance of Dr. Darrin Lunde and Dr. Melissa Hawkins and with funding from The Leakey Foundation. The *S. apella* scans were taken via Artec Space Spider (property of the WWU Anthropology Department) and the *S. oedipus* scans were taken with the NMNH Artec Micro, after I received instruction in its use from Dr. Hawkins.

The scans were processed and exported using Artec Studio Professional (version 16). With these scans exported into Wavefront (.obj) files, I uploaded them for landmarking into 3D Slicer (Kikinis et al., 2014). I landmarked (Figure 2) the scans using the control points module. My 25-landmark protocol (Table 4) was modified from that described by Jung and von Cramon-Taubadel (2022). I aligned scans using the fiducial registration wizard module, allowing for easier reproduction of landmark placement using a base landmark template for each species.

This landmarking protocol captures both size and shape information for each cranium within the sample.
<table>
<thead>
<tr>
<th>Landmark</th>
<th>Abbreviated Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glabella</td>
<td>Most anterior midline point on the frontal bone</td>
</tr>
<tr>
<td>Nasion</td>
<td>Intersection of the nasofrontal suture and the midsagittal plane</td>
</tr>
<tr>
<td>Nasomaxillare</td>
<td>The most inferior point on the nasomaxillary suture</td>
</tr>
<tr>
<td>Alare</td>
<td>Lateral point on the nasal aperture, perpendicular to the nasal height</td>
</tr>
<tr>
<td>Subspinale</td>
<td>Midline point where the inferior nasal spine becomes the maxilla</td>
</tr>
<tr>
<td>Prosthion</td>
<td>Anterior midline point on maxillary alveolar process between incisors</td>
</tr>
<tr>
<td>Orbitale, superior</td>
<td>Superior midpoint of the orbital margin</td>
</tr>
<tr>
<td>Zygooritale</td>
<td>Where zygomaticomaxillary suture intersects inferior orbital margin</td>
</tr>
<tr>
<td>Zygomaxillare</td>
<td>The most inferior, anterior point on the zygomaticomaxillary suture</td>
</tr>
<tr>
<td>Porion</td>
<td>The most superior point on the margin of the external auditory meatus</td>
</tr>
<tr>
<td>Mastoidale</td>
<td>The most inferior, lateral, posterior point on the mastoid process</td>
</tr>
<tr>
<td>Inion</td>
<td>Midline point where superior nuchal lines merge in external occipital</td>
</tr>
<tr>
<td>Lambda</td>
<td>The midline point where the sagittal and lambdoid sutures intersect</td>
</tr>
<tr>
<td>Bregma</td>
<td>The point where the coronal and sagittal sutures intersect</td>
</tr>
<tr>
<td>M1-M2</td>
<td>Lateral point on the alveolus between M1 and M2</td>
</tr>
<tr>
<td>Jugale</td>
<td>Point in depth of the notch between the processes of the zygomatic</td>
</tr>
<tr>
<td>C1/P3</td>
<td>Most lateral point on the alveolus between the permanent canine and P3</td>
</tr>
<tr>
<td>Incisivon</td>
<td>The most posterior inferior point on the incisive fossa</td>
</tr>
<tr>
<td>Palatomaxillare</td>
<td>Midline point of intersection of the palatine and the maxillary bones</td>
</tr>
<tr>
<td>Basion</td>
<td>Where anterior margin of foramen magnum intersects midsagittal plane</td>
</tr>
<tr>
<td>Opisthion</td>
<td>Where post. margin of foramen magnum intersects midsagittal plane</td>
</tr>
<tr>
<td>Foramen magnum</td>
<td>Lateral point on the margin of the foramen magnum</td>
</tr>
<tr>
<td>Occipital condyle</td>
<td>Most inferior point on the occipital condyle</td>
</tr>
<tr>
<td>Sphenosquamosal</td>
<td>Intersection of the infratemporal crest and sphenosquamosal suture</td>
</tr>
<tr>
<td>Temporal fossa</td>
<td>The most posterior, inferior point on the temporal fossa</td>
</tr>
</tbody>
</table>

*For full descriptions of landmark placement please see Appendix A.

I tested one hypothesis about craniofacial variation within Euarchontoglires:

**H1)** There is reduced cranial size dimorphism in Euarchontoglires with litters greater than one. Justification and Expectations: Body size dimorphism has previously been negatively correlated with litter size amongst non-primate mammals (Carranza, 1996). I expect to find
reduced dimorphism in euarchontoglires with larger litter sizes because cranial size is tightly correlated with body size.

**H1 - null** There is no cranial size dimorphism between these species.

This dataset was selected to perform an initial pilot study, using two closely related taxa with different litter size states, which would offer support for or begin to refute this hypothesis, at least for the subsection which are the primates. Additionally, this pilot study will allow me to refine this methodology and use it on an expanded dataset in future research.

**Analytical Methods – Geometric Morphometric Analyses**

I used SlicerMorph’s Generalized Procrustes Alignment (GPA) module to export the eigenvalues, eigenvectors, principal component (PC) scores, mean shape, and combined output which contains new Procrustes aligned coordinates, centroid sizes, and Procrustes distances into excel file format to be further analyzed in R and RStudio (Rolfe et al., 2021).

I first analyzed this data using the t.test function in R’s basic *stats* package (R Core Team, 2022). This function performs a Welch Two Sample t-test on vectors of data. I then plotted the data using ggplot from the *ggplot2* package (Wickham, 2016) and *sm.density.compare* from the *sm* package (Bowman and Azzalini, 2024), and finally I used the Hedges_g function in the *TestDimorph* package (Abulnoor et al., 2023). The Hedges_g function quantifies the size of sex difference in quantifiable traits. Hedges’ g is a measure of effect size (Hedges, 1981).

I focused my analyses on centroid size. The centroid is a measure of size, calculated as the square root of the sum of squared distances of all the landmarks of an object from their center of gravity (Klingenberg, 2016). I chose centroid as the basis upon which I will begin to support
or refute my initial hypothesis regarding craniofacial sexual dimorphism’s relationship with litter size in primates.

In addition, I analyzed Procrustes distance using these methods. Procrustes distance is similar but distinct from centroid size. It is the square root of the sum of squared distances between corresponding landmarks. It is the differences which cannot be removed by scaling, translation, or rotation and is therefore useful as a measure of shape as opposed to size difference (Klingenberg, 2016; Slice, 2007). I thus focus on centroid as a measure of size variation, and Procrustes distance as a tool to gain insight into potential shape variation (though it is not entirely free from the influence of size).

All data necessary to reconstruct these results and perform additional analyses are available in supplemental files uploaded alongside this thesis. For any questions, corrections, or miscellaneous correspondence please contact the author.
Chapter 4 – Results

Litter Size and Life History Analyses

I investigated litter size and life history variation in 955 boreoeutherian mammals across 13 orders (Table 5). I used summary statistics, phylogenetic signal tests, phylogenetic generalized least squares (PGLS), and ancestral state reconstructions (ASRs) to characterize this variation. I then visualized trends in the data using bivariate plots and a principal component analysis (PCA) of selected life history characters including litter size.

Table 5. Mean values for select life history traits in boreoeutherian clades

<table>
<thead>
<tr>
<th>Clade</th>
<th>n (species)</th>
<th>Avg.* litter size</th>
<th>Avg. gestation length (days)</th>
<th>Avg. adult weight (grams)</th>
<th>Avg. brain mass (grams)</th>
<th>Avg. EQ**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artiodactyla</td>
<td>147</td>
<td>1.3</td>
<td>213.7</td>
<td>129279</td>
<td>204.3</td>
<td>0.93</td>
</tr>
<tr>
<td>Carnivora</td>
<td>176</td>
<td>2.7</td>
<td>110.8</td>
<td>68851</td>
<td>137.2</td>
<td>1.05</td>
</tr>
<tr>
<td>Cetacea</td>
<td>42</td>
<td>1</td>
<td>368.3</td>
<td>12830899</td>
<td>2261.1</td>
<td>2.49</td>
</tr>
<tr>
<td>Chiroptera</td>
<td>88</td>
<td>1.1</td>
<td>112.3</td>
<td>117</td>
<td>1.7</td>
<td>0.61</td>
</tr>
<tr>
<td>Dermoptera</td>
<td>1</td>
<td>1</td>
<td>105</td>
<td>1300</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Erinaceomorpha</td>
<td>9</td>
<td>3.8</td>
<td>36.2</td>
<td>566</td>
<td>3.2</td>
<td>0.36</td>
</tr>
<tr>
<td>Lagomorpha</td>
<td>19</td>
<td>3.5</td>
<td>34.3</td>
<td>1635</td>
<td>9.3</td>
<td>0.59</td>
</tr>
<tr>
<td>Perissodactyla</td>
<td>11</td>
<td>1</td>
<td>406.7</td>
<td>861480</td>
<td>562.4</td>
<td>0.64</td>
</tr>
<tr>
<td>Pholidota</td>
<td>2</td>
<td>1</td>
<td>67</td>
<td>7682</td>
<td>17.6</td>
<td>0.83</td>
</tr>
<tr>
<td>Primates</td>
<td>158</td>
<td>1.2</td>
<td>163</td>
<td>6839</td>
<td>72.3</td>
<td>1.71</td>
</tr>
<tr>
<td>Rodentia</td>
<td>264</td>
<td>3.7</td>
<td>44.7</td>
<td>1235</td>
<td>5.7</td>
<td>0.64</td>
</tr>
<tr>
<td>Scandentia</td>
<td>7</td>
<td>2</td>
<td>49</td>
<td>175</td>
<td>3.4</td>
<td>0.97</td>
</tr>
<tr>
<td>Soricomorpha</td>
<td>31</td>
<td>4.8</td>
<td>25.8</td>
<td>54</td>
<td>0.4</td>
<td>0.46</td>
</tr>
</tbody>
</table>

*Avg.: Average.
**EQ: Encephalization Quotient.

There is a clear association between variables associated with slow versus fast life history strategies in my data. Across Boreoeutheria, longer gestation lengths are found in taxa with smaller litters. This also extends to other classically slow and fast traits: longer adolescent
periods are associated with smaller litters, and higher degrees of encephalization are observed in taxa with smaller litters (Figure 3).

Average litter size ranges from 1 to 9.9 across this dataset. The clades with the smallest mean litter sizes (≤1.5) are Artiodactyla, Cetacea, Chiroptera, Dermoptera, Perissodactyla, Pholidota, and Primates (Figure 3). The clades with the largest mean litter sizes (≥3.5) are Lagomorpha, Erinaceomorpha, Rodentia, and Soricomorpha. Carnivora has a mean litter size of 2.66 and Scandentia has a mean litter size of 1.957 offspring.

Average gestation length ranges from 15 to 517 days in this sample. The order with the longest mean gestation length is Perissodactyla, at 406.727 days. The clade with the shortest mean gestation length is Soricomorpha, at 25.8 days.

Adult weight (grams) reaches its maximum extremes in Cetacea. Within these taxa, brain mass (grams) is also the absolute highest, though, relatively, the largest bodied taxa are less encephalized. Interspecific analyses scaled by body size indicate that body mass increases much faster than brain mass in cetacean, e.g., *Balaenoptera musculus* (blue whale) body mass is 680 times larger than *Tursiops truncatus* (bottlenose dolphin), but its brain is only about 2.3 times larger than *T. truncatus*.

Full results are summarized in supplementary materials (Tables S1-S6.5). Please see supplemental materials for all additional results not represented in the main text. This includes descriptive data categorized by order, genus, and family (Table S1), full phylogenetic signal results (Table S2), full phylogenetic generalized least square (PGLS) results (Table S3), and the collated dataset (Table S4, fully sourced in Table S5).
Figure 3. Mean Litter Size (LS) and Encephalization Quotient (EQ). Note that EQ tends to be lower in lineages where average litter size is larger.

Phylogenetic Signal and Phylogenetic Generalized Least Squares (PGLS)

A key goal for this study was to assess the phylogenetic signal of key life history traits. Almost all traits measured have statistically significant phylogenetic signal using both Pagel’s lambda and Blomberg’s $K$ tests (Table 6). A Pagel’s lambda ($\lambda$) value of one corresponds to phylogenetic conservation in line with a Brownian motion model of evolution, while values closer to zero indicate that traits are less phylogenetically conserved than expected.

A Blomberg’s $K$ value closer to one indicates strong phylogenetic signal, while a result less than one can be interpreted as traits differing between closely related taxa, potentially due to
strong selection. In contrast, a $K$ value greater than 1 is interpreted as taxa resembling each other more closely than expected for a given trait. Blomberg’s $K$ is useful due to this difference in bound compared to Pagel’s lambda, which cannot detect if traits are more closely related than Brownian motion would predict.

**Table 6.** Phylogenetic signal results for life history traits sampled in this study

<table>
<thead>
<tr>
<th>Life history trait</th>
<th>Pagel’s lambda</th>
<th>Lambda $p$-value</th>
<th>Blomberg’s $K$</th>
<th>$K p$-value</th>
<th>Sample Size (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS</td>
<td>0.853</td>
<td>&lt;0.001</td>
<td>0.057</td>
<td>0.001</td>
<td>955</td>
</tr>
<tr>
<td>G</td>
<td>0.935</td>
<td>0</td>
<td>0.436</td>
<td>0.001</td>
<td>797</td>
</tr>
<tr>
<td>EQ</td>
<td>0.659</td>
<td>&lt;0.001</td>
<td>0.116</td>
<td>0.001</td>
<td>666</td>
</tr>
<tr>
<td>Log$_{10}$ML</td>
<td>0.946</td>
<td>&lt;0.001</td>
<td>0.324</td>
<td>0.001</td>
<td>729</td>
</tr>
<tr>
<td>Log$_{10}$Male Maturity</td>
<td>0.872</td>
<td>&lt;0.001</td>
<td>0.272</td>
<td>0.001</td>
<td>447</td>
</tr>
<tr>
<td>Log$_{10}$Female Maturity</td>
<td>0.892</td>
<td>&lt;0.001</td>
<td>0.278</td>
<td>0.001</td>
<td>653</td>
</tr>
<tr>
<td>BBR</td>
<td>0.758</td>
<td>&lt;0.001</td>
<td>0.096</td>
<td>0.001</td>
<td>666</td>
</tr>
<tr>
<td>Log$_{10}$PGR</td>
<td>0.96</td>
<td>&lt;0.001</td>
<td>0.692</td>
<td>0.001</td>
<td>658</td>
</tr>
<tr>
<td>PGN</td>
<td>0.844</td>
<td>&lt;0.001</td>
<td>0.349</td>
<td>0.001</td>
<td>107</td>
</tr>
</tbody>
</table>

LS: Litter Size; G: Gestation Length (days); PGN: Percentage of brain growth accomplished before birth; FM: Female maturation time (days); MM: Male maturation time (days); ML: Maximum longevity (years); PGR: Prenatal growth rate; BBR: Brain-body ratio; EQ: Encephalization Quotient.

My results demonstrate that certain life history traits are more phylogenetically conserved than others (those with higher lambda values and $K$ values closer to one). Log-transformed prenatal growth rate has the strongest phylogenetic signal in this sample. This is followed by gestation length as measured by $K$, or maximum longevity as measured by lambda. Litter size and the percentage of brain growth accomplished prenatally also show high phylogenetic signal values. When analyzing untransformed prenatal growth rate, lambda remained significant while $K$ was no longer significant. This is likely due to Pagel’s lambda being more effective in cases
where the data are closer to the Brownian motion expectation, corresponding to findings that $K$
underperforms other measures of phylogenetic signal in such cases (Münkemüller et al., 2012).

My findings indicate that, despite their interrelatedness, selection has not operated
identically upon each of these life history characters. Identifying which traits are more
phylogenetically conserved than others can provide insight into which traits are more constrained
and thus less likely to rapidly evolve.

I used phylogenetic generalized least square (PGLS) analyses to examine the relationship
between traits over evolutionary time. I statistically compared mean litter size values with values
of the other life history traits to assess which traits are correlated when taking phylogenetic
structure into account. I also included several other trait-to-trait comparisons in my analysis
(Table 7).

**Table 7.** Statistically significant results of the PGLS analyses comparing life history traits

<table>
<thead>
<tr>
<th>Life history traits</th>
<th>Slope ± SE</th>
<th>t</th>
<th>df</th>
<th>p-value</th>
<th>$\lambda$</th>
<th>Adjusted $R^2$</th>
<th>Sample size (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(BW) &amp; (LS)</td>
<td>-0.069 ± 0.014</td>
<td>-4.994</td>
<td>689</td>
<td>&lt; 0.001</td>
<td>0.965</td>
<td>0.034</td>
<td>691</td>
</tr>
<tr>
<td>(B) &amp; (LS)</td>
<td>-0.039 ± 0.011</td>
<td>-3.688</td>
<td>665</td>
<td>&lt; 0.001</td>
<td>0.973</td>
<td>0.019</td>
<td>667</td>
</tr>
<tr>
<td>(G) &amp; (LS)</td>
<td>-4.451 ± 1.264</td>
<td>-3.522</td>
<td>795</td>
<td>&lt; 0.001</td>
<td>0.931</td>
<td>0.014</td>
<td>797</td>
</tr>
<tr>
<td>(PGN) &amp; (LS)</td>
<td>-0.044 ± 0.012</td>
<td>-3.644</td>
<td>105</td>
<td>&lt; 0.001</td>
<td>0.754</td>
<td>0.104</td>
<td>107</td>
</tr>
<tr>
<td>(FM) &amp; (LS)</td>
<td>-0.059 ± 0.008</td>
<td>-7.373</td>
<td>651</td>
<td>&lt; 0.001</td>
<td>0.878</td>
<td>0.076</td>
<td>653</td>
</tr>
<tr>
<td>(MM) &amp; (LS)</td>
<td>-0.037 ± 0.01</td>
<td>-3.699</td>
<td>445</td>
<td>&lt; 0.001</td>
<td>0.853</td>
<td>0.028</td>
<td>447</td>
</tr>
<tr>
<td>(LY) &amp; (LS)</td>
<td>-0.081 ± 0.03</td>
<td>-2.686</td>
<td>646</td>
<td>&lt; 0.01</td>
<td>0.939</td>
<td>0.010</td>
<td>648</td>
</tr>
<tr>
<td>(ML) &amp; (LS)</td>
<td>-0.022 ± 0.005</td>
<td>-4.537</td>
<td>727</td>
<td>&lt; 0.001</td>
<td>0.934</td>
<td>0.026</td>
<td>729</td>
</tr>
<tr>
<td>(PGR) &amp; (B)</td>
<td>1.022 ± 0.026</td>
<td>39.189</td>
<td>515</td>
<td>&lt; 0.001</td>
<td>0.843</td>
<td>0.748</td>
<td>517</td>
</tr>
<tr>
<td>(B) &amp; (AW)</td>
<td>0.55 ± 0.011</td>
<td>50.972</td>
<td>664</td>
<td>&lt; 0.001</td>
<td>0.866</td>
<td>0.796</td>
<td>666</td>
</tr>
</tbody>
</table>

LS: Litter Size; BW: Birth Weight (grams); G: Gestation Length (days); PGN: Percentage of brain growth accomplished before birth; FM: Female maturation time (days); MM: Male maturation time (days); LY: Number of litters produced per year; ML: Maximum longevity (years); PGR: Prenatal growth rate; B: Brain mass (grams); AW: Adult weight (grams). Mass and longevity data here is transformed.

There is a significant relationship between all life history and body size traits assessed in this study. The highest correlations are between brain mass and adult body mass, and between brain mass and prenatal growth rate. These positive correlations indicate that animals with faster rates of prenatal growth achieve larger adult brain and body masses within this sample. Litter size is negatively correlated with all other nominally slow life history traits (Table 7 and Figure 4).

**Figure 4.** Correlation Plot of Life History characters. LS: Litter Size; Log*AW: Adult Weight (grams); G: Gestation Length (days); LogML: Maximum Longevity (years); LogBrain: Brain Mass (grams); LogMM: Male Maturity (years); LogFM: Female Maturity (years); LY: Litters per Year.

*Logarithmically transformed (base 10).
This correlation plot illustrates the general life history trends across this dataset. Increasing litter size is negatively correlated with virtually every other life history trait, all of which are associated with slow life history strategies. The exception is litters per year, where there is a weak positive association.

There is considerable variability in litter size, body size, and gestation length across clades (Figure 5). Rodentia features taxa which demonstrate the full range of litter size state and substantial variation in gestation length. Carnivora also shows a wide spread of both traits. Both these clades are cosmopolitan and fill a wide range of ecological niches. Carnivora also shows high levels of variation in terms of body size.

Overall, litter size appears to be limited by gestation length - taxa with small litter size can have a wide range of gestation lengths. However, large litters are only produced by taxa with short gestation lengths.

![Figure 5](image.jpg)

**Figure 5.** Bivariate Plot of log$_{10}$-transformed gestation length (days) and log$_{10}$-transformed litter size.
Principal Component Analyses

I performed a principal component analysis (PCA) on litter size (LS), gestation length in days (G), as well as logarithmically transformed adult weight in grams (LogAW) brain mass in grams (LogBrain), litters produced per year (LY), logarithmically transformed male and female maturity in years (LogMM and LogFM), and logarithmically transformed maximum longevity in years (LogML). PCA allows for visualization of trait variation in multivariate space. The PCA results, including proportion of variance, are reported in Table 8.

Table 8. Principal component analysis of select life history characters

<table>
<thead>
<tr>
<th>Principle component</th>
<th>Standard deviation</th>
<th>Proportion of Variance</th>
<th>Cumulative Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC1</td>
<td>2.4356</td>
<td>0.7415</td>
<td>0.7415</td>
</tr>
<tr>
<td>PC2</td>
<td>0.8719</td>
<td>0.0950</td>
<td>0.8366</td>
</tr>
<tr>
<td>PC3</td>
<td>0.7954</td>
<td>0.0791</td>
<td>0.9157</td>
</tr>
<tr>
<td>PC4</td>
<td>0.5207</td>
<td>0.0339</td>
<td>0.9495</td>
</tr>
<tr>
<td>PC5</td>
<td>0.4765</td>
<td>0.0284</td>
<td>0.9779</td>
</tr>
<tr>
<td>PC6</td>
<td>0.3483</td>
<td>0.0152</td>
<td>0.9931</td>
</tr>
<tr>
<td>PC7</td>
<td>0.1785</td>
<td>0.0040</td>
<td>0.9971</td>
</tr>
<tr>
<td>PC8</td>
<td>0.1532</td>
<td>0.0029</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

Here (Figures 6 and 7) we can see that clades group along these principle component categories. The leftward wing of figure 6 represents slower life history organisms: lower values of PC1 and higher PC2 values are associated with slow life history organisms, with long lifespans, large body size, long gestation lengths, and small litters. This side of the figure is dominated by cetaceans and a smaller number of primates and perissodactyls. The furthest leftward cetacean is *Balaena mysticetus* (lifespan of up to 211 years) and primate is *Homo sapiens* (lifespan of up to 122.5 years). Not the largest of their clade, but the longest-lived in each case. In the other direction we are seeing fast life history organisms, with higher values of
PC1 but similarly high values of PC2. This side of the figure is dominated by rodents with some soricomorphs. The most impactful variable in PC1 is maximum longevity, followed by adult mass in grams.

**Figure 6.** Bivariate Plot of PC1 and PC2. PC1 reflects maximum longevity and adult mass in this sample and captures 74.2% of the total variation, and PC2 captures 9.5% of the variation. *Balaena mysticetus* and *Homo sapiens* stand apart, with the most negative scores within their respective lineages. Only litter size, litters per year, and gestation length are not log transformed.

In the center of this figure (6) we can see organisms with relatively less extreme life history strategies (neither very ‘fast’ nor very ‘slow’). These taxa are moderate in adult body mass and longevity. Here we observe many artiodactyls, carnivorans, and some chiropterans.

Here (Figure 7) clades also group together, and PC2 reflects a distinct combination of variables. Low values of PC2 indicate that an organism has a longer lifespan compared to other similarly sized boreoeutherian mammals. Most primates and cetaceans are centrally located, alongside many carnivorans and some artiodactyls. Chiropterans and some rodents are the most independently clustered in this plot. The most leftward primates are *Microcebus murinus* and
*Cebus capucinus* which both have relatively long lifespans for their body size. These are the closest in value to the independently clustered chiropterans which uniformly feature longer lifespans than their body size would predict.

**Figure 7.** Bivariate Plot of PC2 and PC3. PC2 reflects the ratio of body size to longevity (logarithmically transformed adult mass in grams divided by logarithmically transformed maximum longevity) and captures 9.5% of the variation. PC3 captures 7.9% of the variation. The lowest value of PC2 is found in *Rhinolophus hipposideros*.

This principal component analysis reaffirms associations identified within my PGLS analyses. Litter size and litters per year have an inverse effect on the variable explaining the greatest amount of variance in the dataset compared to both female and male maturity, maximum longevity, gestation length, and increasing brain and body mass (Figure 8). For principal component two, gestation length and the mass variables have an inverse impact on the next most explanatory variable computed in the PCA, compared to all the other life history characters analyzed here. Longevity and longevity relative to body mass are key traits in this analysis.
Figure 8. PCA Biplot, PC1 (Dim1) and PC2 (Dim2), Impact of Variables and Data Organized by Clade. Log*AW: adult mass in grams; LogBrain: adult brain mass in grams; LS: litter size; LY: litters per year; LogML: maximum longevity in years; LogMM: male maturity in years; LogFM: female maturity in years; G: gestation length in days.
* Logarithmically transformed (base 10).

Ancestral State Reconstructions

My model predicts that twinning, or approximately twinning, is the ancestral condition for Primates, Boreoeutheria, Laurasiatheria, Euarchontoglires, Cetartiodactyla, Carnivora, and remained the ancestral condition for the last common ancestor of Haplorhini, within the primate clade.

These reconstructions predict that the ancestral boreoeutherian produced litters which averaged 2.165 offspring, had a gestation length of 124.152 days, a brain mass of 8.371 grams (using log10 values), an adult body mass of 945.045 grams (using log10 values), a prenatal growth rate of 0.677 (using log10 values), a brain-body ratio of 0.014 (via reconstruction) or 0.009 (via reconstructed brain and body mass values), and a maximum longevity of 19.203 years. Using the
predicted values of brain and body size in these $\log_{10}$ ancestral state reconstructions, the ancestral boreoeutherian had an EQ of 0.724 and an EQ2 of 0.497 (compared to 0.835 and 0.582, predicted by their respective reconstructions).

Table 9. Reconstructed LH characters of selected last common ancestors

<table>
<thead>
<tr>
<th>LCA (Last Common Ancestor)</th>
<th>Estimated Litter Size</th>
<th>Estimated Gestation Length (days)</th>
<th>Estimated EQ</th>
<th>Estimated EQ2</th>
<th>Estimated Maximum Longevity</th>
<th>Estimated Brain Mass (grams)</th>
<th>Estimated Adult Weight (grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carnivora - ≈66ma$^1$</td>
<td>2.32</td>
<td>114.87</td>
<td>0.97</td>
<td>0.70</td>
<td>23.21</td>
<td>33.63</td>
<td>5660.10</td>
</tr>
<tr>
<td>Chiroptera - ≈57.9ma$^2$</td>
<td>1.39</td>
<td>140.66</td>
<td>0.65</td>
<td>0.44</td>
<td>17.90</td>
<td>1.92</td>
<td>133.18</td>
</tr>
<tr>
<td>Cetartiodactyla - ≈81ma$^3$</td>
<td>1.69</td>
<td>214.70</td>
<td>0.99</td>
<td>0.74</td>
<td>29.32</td>
<td>69.01</td>
<td>21606.37</td>
</tr>
<tr>
<td>Rodentia - ≈74ma$^4$</td>
<td>2.83</td>
<td>74.59</td>
<td>0.71</td>
<td>0.48</td>
<td>14.01</td>
<td>4.55</td>
<td>423.95</td>
</tr>
<tr>
<td>Primates - ≈77ma$^5$</td>
<td>1.73</td>
<td>127.73</td>
<td>1.14</td>
<td>0.79</td>
<td>22.88</td>
<td>10.23</td>
<td>765.07</td>
</tr>
<tr>
<td>Boreoeutheria - ≈107ma$^1$</td>
<td>2.17</td>
<td>124.15</td>
<td>0.84</td>
<td>0.58</td>
<td>19.20</td>
<td>8.37</td>
<td>945.05</td>
</tr>
</tbody>
</table>

$^1$Zhou et al., 2012. $^2$Jones et al., 2005. $^3$Zhou et al., 2011. $^4$Kay and Hoekstra, 2008. $^5$Steiper and Young, 2006.

These reconstructions predict that the ancestral primate produced litters which averaged 1.732, had a gestation length of 127.729 days, a brain mass of 10.227 grams (using $\log_{10}$ values), an adult body mass of 765.066 grams (using $\log_{10}$ values), a prenatal growth rate of 0.499 (using $\log_{10}$ values), a brain-body ratio of 0.017 (via reconstruction) or 0.013 (via reconstructed brain and body mass values), and a maximum longevity of 22.88 years. Using the predicted values of brain and body size in these $\log_{10}$ ancestral state reconstructions, the ancestral primate had an EQ of 1.019 and an EQ2 of 0.695 (compared to 1.144 and 0.79, predicted by their respective reconstructions).
Table 10. Internal primate nodes

<table>
<thead>
<tr>
<th>LCA</th>
<th>Litter Size</th>
<th>Gestation Length (days)</th>
<th>EQ</th>
<th>EQ2</th>
<th>Brain-Body Ratio (BBR)</th>
<th>Maximum Longevity (years)</th>
<th>Brain Mass (grams)</th>
<th>Adult Weight (grams)</th>
<th>BBR (using ASR values)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primates - ≈77-74ma</td>
<td>1.73</td>
<td>127.73</td>
<td>1.14</td>
<td>0.79</td>
<td>0.017</td>
<td>22.88</td>
<td>10.227</td>
<td>765.066</td>
<td>0.013</td>
</tr>
<tr>
<td>Strepsirrhini - ≈57ma</td>
<td>1.43</td>
<td>137.23</td>
<td>1.23</td>
<td>0.85</td>
<td>0.016</td>
<td>24.47</td>
<td>12.319</td>
<td>850.862</td>
<td>0.014</td>
</tr>
<tr>
<td>Haplorhini - ≈46ma</td>
<td>1.53</td>
<td>139.03</td>
<td>1.29</td>
<td>0.89</td>
<td>0.018</td>
<td>34.94</td>
<td>12.042</td>
<td>815.806</td>
<td>0.015</td>
</tr>
<tr>
<td>Platyrrhini - ≈43ma</td>
<td>1.11</td>
<td>174.65</td>
<td>2.02</td>
<td>1.42</td>
<td>0.019</td>
<td>32.86</td>
<td>38.890</td>
<td>2177.768</td>
<td>0.018</td>
</tr>
<tr>
<td>Catarrhini - ≈38ma</td>
<td>1.05</td>
<td>189.78</td>
<td>1.94</td>
<td>1.42</td>
<td>0.012</td>
<td>39.01</td>
<td>91.186</td>
<td>8398.512</td>
<td>0.011</td>
</tr>
</tbody>
</table>

*Using the values produced via the adult weight and brain mass ancestral state reconstructions.
\(^1\)Steiper and Young, 2006. \(^2\)Pozzi et al., 2014.

Both Cetartiodactyla and Chiroptera are predicted to have a last common ancestor which produced smaller litters than those of Primates. Nonetheless, the ancestral primate is predicted to have had the greatest values for most measures of encephalization (EQ, EQ2) and the prototypical slow life history trait, maximum longevity (ML). The ancestral primate is predicted to have been longer lived than other smaller-bodied ancestral organisms Rodentia and Chiroptera, and closer in predicted longevity to the larger-bodied ancestral cetartiodactyl and carnivoran. Both the ancestral chiropteran and primate have relatively increased longevity.

Litter size variation here is also not clearly organized along superorder lines even within this selected subset. We see the largest predicted ancestral litter size in Rodentia, the second largest in Carnivora, and the third largest in Primates. Litter size has convergently increased and decreased in multiple lineages within Boreoeutheria. This includes the eventual trajectories
which lead to very small-bodied singleton taxa like many chiropterans and very large-bodied singleton taxa like many cetaceans. Within both Chiroptera and Primates, some lineages (e.g., Vespertilionidae and Callitrichidae respectively) have derived larger litters after diverging from a singleton bearing last common ancestor.

Primates is more similar in many life history measures to Cetacea than to its closer relative, Rodentia. Convergent evolution of cetaceans and primates in terms of life history strategy is a major trend apparent within these data. Another area of similarity is between extant chiropterans and my predictions for the earliest primates, both with high longevity relative to body size.

Figure 9. Ancestral state reconstruction of litter size in Primates (n=158). Note that the ASR reconstructs a lower litter size when estimated in primates alone, without a phylogenetic comparative framework.
Above we can see a primate-specific ancestral state reconstruction (Figure 9). Most taxa are represented in red, indicating they produce singletons. The blue and green colors indicate larger average litter size. Larger litters are found only in Strepsirrhini (e.g., *Cheirogaleus major*) and Callitrichidae, a family of Platyrhini (e.g., *Saguinus oedipus*). I also note the derived nature of *Callimico goeldii*, which is singleton-bearing with respect to the last common ancestor of the callitrichids. My findings also support that twinning in strepsirrhines is a derived trait and provide several examples of convergent evolution with the other strepsirrhine and anthropoid twin-bearers.

Within Boreoeutheria, there is more variability of litter size in Rodentia and Carnivora. Litter size is less variable in Cetartiodactyla, Primatomorpha, and Chiroptera. Almost all taxa in these clades give birth to singletons. Soricomorpha, Erinaceomorpha, Lagomorpha, and Rodentia tend toward larger litters.

My reconstruction indicates that litter size diversified into the variety of states which we see in modern day boreoeutherians after the appearance and subsequent divergence of Laurasiatheria and Euarchontoglires. The last common ancestor of Euarchontoglires is predicted to have had a mean litter size of 2.23 and the last common ancestor of Laurasiatheria is predicted to have had a mean litter size of 2.11. These superorders then diverged into the many mammalian species of Boreoeutheria which now includes the largest extant mammal (Motani and Pyenson, 2024) and the two smallest extant mammals (Jürgens, 2002).
Figure 10. Ancestral state reconstruction of litter size (N=955)
Comparing Ancestral State Reconstructed Values with Fossil Data

I compared my reconstructed states for the various encephalization quotient values, brain-to-body ratios, and brain and \( \log_{10} \) brain size to those obtained via skeletal material analysis on *Vincelestes neuquenianus* (Bertrand et al. 2022). I selected *Vincelestes neuquenianus* because this taxon is temporally associated with the early cretaceous (Bertrand et al., 2022; Macrini et al., 2007). This corresponds to estimates of boreoeutherian divergence (Wu et al., 2021; Zhou et al., 2012). In Table 11, I summarize how closely these reconstructed values correspond to those generated by Bertrand et al. (2022) using materials and prior findings regarding the *Vincelestes* genus. This genus is not a boreoeutherian mammal but is used to assess how closely these values resemble the fossil record of this period.

The reconstruction of adult weight (grams) most closely corresponds to the adult weight estimated using this fossil taxon’s skeletal material. Untransformed brain mass and encephalization quotient correspond the least well, predicting much greater values than those generated using *Vincelestes neuquenianus*. The next best approximation after adult weight would appear to be the phylogenetically informed encephalization quotient. This genus, *Vincelestes*, is a relatively well-known stem therian. It is represented by six nearly complete skulls, more than a dozen mandible and mandibular fragments, and additional postcranial elements. A description of the material used to generate their values can be found in Macrini et al.’s 2007 paper.
Table 11. ASR results compared to values from Bertrand et al. (2022)

<table>
<thead>
<tr>
<th>Value</th>
<th>This study</th>
<th>Value</th>
<th>Bertrand et al. (2022); Vincelestes†</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>EQ2</td>
<td>0.582</td>
<td>EQ2</td>
<td>0.18</td>
<td>69.072</td>
</tr>
<tr>
<td>EQ2(2)*</td>
<td>0.497</td>
<td>EQ2</td>
<td>0.18</td>
<td>63.783</td>
</tr>
<tr>
<td>BBR</td>
<td>0.014</td>
<td>BBR</td>
<td>0.004</td>
<td>71.429</td>
</tr>
<tr>
<td>BBR(2)*</td>
<td>0.009</td>
<td>BBR</td>
<td>0.004</td>
<td>55.556</td>
</tr>
<tr>
<td>Brain Mass**</td>
<td>114.722 g</td>
<td>Endocranial volume</td>
<td>2.37 cm³</td>
<td>97.934</td>
</tr>
<tr>
<td>Brain Mass</td>
<td>8.371 g</td>
<td>Endocranial volume</td>
<td>2.37 cm³</td>
<td>71.688</td>
</tr>
<tr>
<td>EQ</td>
<td>0.84</td>
<td>EQ2</td>
<td>0.18</td>
<td>78.571</td>
</tr>
<tr>
<td>PEQ</td>
<td>0.511</td>
<td>EQ2</td>
<td>0.18</td>
<td>64.775</td>
</tr>
<tr>
<td>PEQ(2)*</td>
<td>0.395</td>
<td>EQ2</td>
<td>0.18</td>
<td>54.43</td>
</tr>
<tr>
<td>AW</td>
<td>945.0445 g</td>
<td>Body mass</td>
<td>674 g</td>
<td>28.68</td>
</tr>
</tbody>
</table>

*The (2) here indicates a value calculated using reconstructed brain mass and adult weight values, as opposed to the value generated via ancestral state reconstruction of said variable.

**Without transforming data.

“This study”: Ancestral boreoeutherian; “Bertrand et al. (2022); Vincelestes”: V. neuquenianus. AW: Adult weight (grams). EQ: Encephalization Quotient; PEQ: Phylogenetic Encephalization Quotient; EQ2: Encephalization Quotient 2; BBR: Brain-to-Body Ratio.

I decided to compare my values to an additional two fossil taxa, Vintana sertichi and Triconodon mordax (Table 12). These taxa are the most temporally proximate to boreoeutherian divergence estimates, after Vincelestes. They bracket this time. Vintana is a more recent mammal, estimated at 65-75 million years old. Triconodon is older than Vincelestes, estimated at 155-165 million years. (Bertrand et al., 2022). These data are sourced by Bertrand et al. (2022) from Hoffman et al. (2014) and Quiroga (1980) for Vintana and Triconodon respectively.
These additional comparisons highlight that identifying fossilized remains which are both temporally proximate to ancestral state reconstructive targets and which are thought to resemble the ancestral state of a given clade is quite problematic. The younger but very large-bodied *Vintana* does not appear to share many physical characteristics in common with my predictions pertaining to the ancestral boreoeutherian. The older *Triconodon* appears to be more similar to an ancestral form of this magnorder, though my predictions of both standard and phylogenetically informed encephalization quotients are the most distinct from those produced for this fossil taxon.

**Table 12.** ASR results compared to additional values from Bertrand et al. (2022)

<table>
<thead>
<tr>
<th>Value</th>
<th>This study</th>
<th>Value</th>
<th>Vintana†</th>
<th>% change</th>
<th>Triconodon†</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>EQ2</td>
<td>0.582</td>
<td>EQ2</td>
<td>0.31</td>
<td>46.735</td>
<td>0.16</td>
<td>72.509</td>
</tr>
<tr>
<td>EQ2(2)*</td>
<td>0.497</td>
<td>EQ2</td>
<td>0.31</td>
<td><strong>37.626</strong></td>
<td>0.16</td>
<td>67.807</td>
</tr>
<tr>
<td>BBR</td>
<td>0.014</td>
<td>BBR</td>
<td>0.003</td>
<td>78.571</td>
<td>0.006</td>
<td>57.143</td>
</tr>
<tr>
<td>BBR(2)*</td>
<td>0.009</td>
<td>BBR</td>
<td>0.003</td>
<td>66.667</td>
<td>0.006</td>
<td>33.333</td>
</tr>
<tr>
<td>Brain Mass**</td>
<td>114.722 g</td>
<td>Endocranial volume</td>
<td>18.3 cm³</td>
<td>84.048</td>
<td>0.82 cm³</td>
<td>99.285</td>
</tr>
<tr>
<td>Brain Mass</td>
<td>8.371 g</td>
<td>Endocranial volume</td>
<td>18.3 cm³</td>
<td><strong>118.612</strong></td>
<td>0.82 cm³</td>
<td>90.204</td>
</tr>
<tr>
<td>EQ</td>
<td>0.84</td>
<td>EQ2</td>
<td>0.31</td>
<td>63.095</td>
<td>0.16</td>
<td>80.952</td>
</tr>
<tr>
<td>PEQ</td>
<td>0.511</td>
<td>EQ2</td>
<td>0.31</td>
<td><strong>39.335</strong></td>
<td>0.16</td>
<td>68.689</td>
</tr>
<tr>
<td>PEQ(2)*</td>
<td>0.395</td>
<td>EQ2</td>
<td>0.31</td>
<td><strong>21.519</strong></td>
<td>0.16</td>
<td><strong>59.494</strong></td>
</tr>
<tr>
<td>AW</td>
<td>945.0445 g</td>
<td>Body mass</td>
<td>6793 g</td>
<td><strong>618.902</strong></td>
<td>143.3 g</td>
<td>84.837</td>
</tr>
</tbody>
</table>

*The (2) here indicates a value calculated using reconstructed brain and adult mass values.**

**Without transforming data.***

**Inverse difference (ancestral state value lower than that predicted using fossil specimen). “Vintana”: *Vintana sertichi* (Bertrand et al., 2022); “Triconodon”: *Triconodon mordax* (Bertrand et al., 2022). AW: Adult weight (grams). EQ: Encephalization Quotient; PEQ: Phylogenetic Encephalization Quotient; EQ2: Encephalization Quotient 2; BBR: Brain-to-Body Ratio.

*Craniofacial Morphological Analyses*
Welch Two Sample t-test Results – Sexual Dimorphism

In addition to my analysis of litter size and life history, I conducted a pilot investigation of how craniofacial morphology correlates with litter size variation. I scanned, landmarked, and analyzed morphological variation within and between two species of platyrrhine monkey, one a singleton-bearing species (Sapajus apella) and the other a twinning species (Saginus oedipus).

I assessed cranial size dimorphism across taxa using centroid size as a proxy for overall cranial size in this study. Procrustes distance is used here as a proxy for size-controlled shape variation. The only statistically significant level of sex dimorphism is in S. apella, the singleton-bearing American monkey taxon (Table 13). Neither group had a statistically significant shape difference across my 25-landmark selection, and S. oedipus did not have a statistically significant level of cranial size sex dimorphism.

**Table 13.** Welch Two Sample t-test results for *Saginus oedipus* and *Sapajus apella*

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Value</th>
<th>Groups</th>
<th>t</th>
<th>df</th>
<th>p-value</th>
<th>$\mu$ (F)</th>
<th>$\mu$ (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. oedipus</em></td>
<td>Centroid</td>
<td>M &amp; F</td>
<td>-0.425</td>
<td>26.359</td>
<td>0.675</td>
<td>84.992</td>
<td>85.344</td>
</tr>
<tr>
<td><em>S. oedipus</em></td>
<td>P. Dist.</td>
<td>M &amp; F</td>
<td>0.302</td>
<td>26.369</td>
<td>0.765</td>
<td>0.041</td>
<td>0.040</td>
</tr>
<tr>
<td><em>S. apella</em></td>
<td>Centroid</td>
<td>M &amp; F</td>
<td>-5.17</td>
<td>24.785</td>
<td>&lt;0.001</td>
<td>156.304</td>
<td>169.586</td>
</tr>
<tr>
<td><em>S. apella</em></td>
<td>P. Dist.</td>
<td>M &amp; F</td>
<td>0.805</td>
<td>23.015</td>
<td>0.429</td>
<td>0.070</td>
<td>0.65</td>
</tr>
</tbody>
</table>

P. Dist. = Procrustes Distance; M & F = Male and Female.

TestDimorph Package Results: Hedges’ g – Sexual Dimorphism

I used Hedges’ g (1981) to measure effect size (Table 14). A large effect is equal to or greater than 0.8, and a small effect is equal to or less than 0.2. A $g$ value indicates how much groups differ in terms of standard deviation (Cohen, 1988; Hedges, 1981). These results support the $t$-test findings, indicating that centroid size is much more affected by sex in *S. apella* than it is in *S. oedipus*. By assessing both $p$ and $g$ values for centroid dimorphism across sex categories,
I am more confident in using both findings to provide initial support for this life history and morphology covariation hypothesis.

**Table 14. Hedges’ \( g \) Results for effect of sex on centroid size for *S. oedipus* and *S. apella***

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Trait</th>
<th>Lower</th>
<th>( g )</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. oedipus</em></td>
<td>Centroid size</td>
<td>-0.55</td>
<td>0.15</td>
<td>0.88</td>
</tr>
<tr>
<td><em>S. apella</em></td>
<td>Centroid Size</td>
<td>1.09</td>
<td>1.84</td>
<td>2.91</td>
</tr>
</tbody>
</table>

I used ggplot2 (Wickham, 2016) to visualize the geometric morphometric variation captured within my set of landmarked platyrrhine crania. Figures 11 and 12 illustrate the difference in overall craniofacial size dimorphism as captured by centroid size when using all 25 craniofacial landmarks. The centroid is the square root of the sum of squared distances of all the landmarks of an object from their center of gravity (Klingenberg, 2016). This provides a proxy for overall cranium size in these taxa.

I use boxplots and kernel density plots to visually describe the amount of variation between these two taxa as well as to visualize dimorphic variation within taxa between males and females (n=15 males, n=15 females for each species). Value overlap, average difference between populations, and other key morphometric evaluations can be visualized with these plots.
These figures illustrate the degree of dimorphism present in both taxa selected for this pilot study. There is substantially greater overlap in craniofacial size in *S. oedipus* than in the singleton-bearing *S. apella*. These data support my hypothesis regarding the negative relationship between litter size and cranial size dimorphism.
Additional Results: Interspecific Centroid and Procrustes Distance Variation

Figure 13. Interspecific Boxplots for Centroid Size and Procrustes Distance.

Table 15. Welch Two Sample \(t\)-tests for interspecific Procrustes distance and centroid size

<table>
<thead>
<tr>
<th>Value</th>
<th>(t)</th>
<th>(df)</th>
<th>(p)-value</th>
<th>(\mu) (\textit{S. apella})</th>
<th>(\mu) (\textit{S. oedipus})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centroid</td>
<td>42.938</td>
<td>32.101</td>
<td>(&lt;0.001)</td>
<td>162.945</td>
<td>85.168</td>
</tr>
<tr>
<td>P. Dist.</td>
<td>7.275</td>
<td>45.871</td>
<td>(&lt;0.001)</td>
<td>0.068</td>
<td>0.041</td>
</tr>
</tbody>
</table>

These two primates are relatively closely related, generally grouped within the superfamily Ceboidea, though sometimes considered subfamilies within Cebidae (e.g., Silvestro et al., 2019). These findings indicate that \textit{S. apella} and \textit{S. oedipus} are more similar in craniofacial shape than they are in size, and more similar in terms of Procrustes distance than centroid size, though they significantly vary in both measures. The largest members of the \textit{S. oedipus} sample are not as large craniofacially as the smallest \textit{S. apella}. However, in shape, as measured by total Procrustes distance, there is some overlap between the populations.
Chapter 5 – Discussion

Litter Size Evolution in Boreoeutheria

Life history traits within Boreoeutheria covary along lines predicted by theories of a slow-fast life history continuum (Stearns, 1992). My work uses the largest life history and phylogenetic analysis to date to investigate the evolution of this continuum. I find that litter size is significantly negatively correlated with other life history traits: smaller litter sizes are significantly associated with longer gestation length, longer adolescences, larger brain and body mass, and longer lifespans. This supports previous work in life history theory in mammals (Andrea, 2016; Harvey and Clutton-Brock, 1985; Promislow and Harvey, 1990; Tannerfeldt and Angerbjörn, 1998).

My work also demonstrates a significant correlation between prenatal growth rate and brain mass, as well as adult body mass and brain mass. The strongest correlations capture positive associations between brain and body mass, and between prenatal growth rate and brain mass. The strongest negative correlation was between litter size and percentage of brain growth accomplished prenatally. This finding supports more recent research that focused on prenatal growth rates, brain and body size specifically in primates (Monson et al., 2022) and expands this statistical relationship across the mammalian phylogeny. Additionally, my work highlights the extreme reproductive strategy of humans, who have small litter sizes alongside a relatively small percentage of brain growth accomplished prenatally (secondarily altricial). This contrasts with most other mammals sampled here including Tarsius bancanus (Western tarsiers), Gorilla gorilla (Western gorillas), Pagophilus groenlandicus (harp seals), and Equus zebra (zebras).
which all bear singletons and accomplish over 60% of brain growth before birth (compared to humans, less than 25%).

Life history traits are highly integrated and likely coevolve, leading to a continuum where organisms tend to have slower or faster life history ‘versions’ of many traits. My findings support generalizable quantity-quality trade-offs within Boreoeutheria, such as those outlined in r/K selection (MacArthur, 1967; Walker et al., 2008) and in the slow-fast life history continuum (Stearns, 1992). These trends of life history trait covariation across Boreoeutheria resemble those previously identified in primates (Harvey and Clutton-Brock, 1985) and speak to the existence of extrinsic life history constraints which can be used to inform our understanding of evolutionary change.

Novel to this work, average litter size of two or more is predicted for the last common ancestors of numerous boreoeutherian lineages (Carnivora, Cetartiodactyla, Primates, Boreoeutheria, Euarchontoglires, Laurasiatheria, >2 in Rodentia), dated between approximately 107 to 66 million years ago. The singletons and very large litters common in these orders today appear to have not been selectively advantageous until recently in the evolutionary history of this magnorder. This aligns with fossil evidence, which provides support for multi-egg clutches in many Cretaceous vertebrates (e.g., Vila et al., 2010).

During the evolution of Boreoeutheria, lineages experienced two mass extinction events, one at the Cretaceous-Paleogene boundary and the other at the Eocene-Oligocene boundary (Wu et al., 2021). Twinning appears have been the primary condition prior to this first extinction event, which occurred around 66 million years ago (Zhou et al., 2012). Chiropterans did not radiate until after this event, around 58 million years ago. My ancestral state reconstruction supports that Chiroptera had the smallest average ancestral litter size state.
My analysis supports that the range of average litter sizes in extant mammals evolved after this second extinction event. Even considering an order which originated prior to the first extinction event, such as Rodentia, most rodent lineages are thought to have diversified after the second extinction event during the late Oligocene and Neogene (Fabre et al., 2012). Twinning may have been indicative of a ‘moderate’ life history pattern which allowed certain mammal lineages to survive these extinctions, particularly the first extinction event, and outcompete other survivors to proceed into their current forms today. If not twinning, the phenotypic plasticity to respond to environmental circumstances by increasing reproductive output via larger litters (e.g., Tannerfeldt and Angerbjörn, 1998; Weller et al., 2024) may have been a crucial trait to maintain during this evolutionary history.

**Litter Size and Life History Evolution in Primates**

My ancestral state reconstruction supports that twinning (primate last common ancestor node value = 1.732) was the ancestral state of litter size for primates. This contradicts prior research using fewer taxa and distinguishes the earliest primates from most extant taxa (Kappeler, 1998; Wu et al., 2021).

These reconstructions do not support previous theories that extant primates evolved from a LCA which uniformly produced singleton offspring (Kappeler, 1998; Wu et al., 2021). My findings do not preclude the possibility of an ancestral primate which sometimes produced singleton offspring but produced larger litters at a greater rate than seen in extant primates outside of some callitrichids and cheirogaleids.

My findings support early work by Leutenegger (1979), who theorized that twinning typified the ancestral primate. However, the findings of these ancestral state reconstructions
contradict Leutenegger’s (1979) other hypothesis that twinning is ancestral in strepsirrhines. The strepsirrhine lineage is supported to have evolved from a reduced mean litter size (1.43), with some taxa deriving a larger litter size after diverging from the other strepsirrhines (Table 10). Further research looking at the paleoenvironments of the ancestral primates may further clarify the impact of clade-specific selective pressures on primate reproductive patterns.

Maximum longevity is more important in defining life history variation within my sample than adult body mass, according to principal component analysis. Additionally, longevity relative to body mass is a character which explains the second greatest degree of life history variation in this sample. For example, chiropterans, who have long lifespans and small body size, sit at the most extreme end of PC2, alongside primate Microcebus murinus, a small-bodied long-lived species with longer lifespan than would be predicted from body size alone (DiFrancesco et al., 2023).

Rodents also display a wide spread of PC2 values. Some rodents are positioned at the opposite end of the spectrum from the chiropterans, with lifespans that are short even for their relatively small size. The most extreme example is Rattus norvegicus (the brown rat). The maximum lifespan of this species is just under four years, and its median lifespan is estimated at under a year (Hubrecht and Kirkwood, 2010), primarily due to intense predation pressure.

My ancestral state predictions for the earliest primates align them in life history with chiropterans, having long lifespans relative to their small body mass, particularly compared to other boreoeutherian mammals. My reconstructions also support small body size and long lifespans for the earliest chiropterans, smaller but longer-lived than the rodent last common ancestor. In primates, this may be related to cardiovascular adaptations linked with lifetime heartbeat number, which is conserved across primates and higher than in many rodents and
domestic mammals (DiFrancesco et al., 2023). It would be intriguing to further investigate what adaptations might be linked to chiropteran longevity, especially if it is indeed an ancestrally conserved trait.

This similarity between Chiroptera and Primates cannot be due to relatedness nor to other common explanations like locomotion exclusively. The close relationship of Primates to Dermoptera is an intriguing aspect, but there is little evidence that the earliest primates were gliding (Cachel, 2015). Further investigation into the adaptations in these lineages which allowed them to increase longevity without increasing body size proportionally may have wide-ranging implications.

Comparing Results to Fossil Anatomy

A focus of recent scholarship has been whether relatively large brains were an early adaptation of all or most mammals (Bininda-Emonds et al., 2007; Bertrand et al., 2022; Masahito et al., 2018; Smith, 2022). Bertrand et al. (2022) discuss the evolutionary trajectories taken by early placental mammals, arguing that relative brain size decreased with the development of large bodies in many mammalian taxa. They also argue that surviving mammals are all more encephalized than early mammals (Bertrand et al. 2022). This may create a bias where all extant taxa differ from extinct taxa not due to relatedness. This pattern can be problematic for traditional phylogenetic reconstructive methodologies such as those used here, which rely on statistical inference using extant states and measures of relatedness alone.

In consideration of this bias, I compared the reconstructed states for various characters including my selected encephalization quotients, brain-to-body ratio, and brain and log_{10} brain size to Vincelestes neuquenianus and other fossil taxa analyzed in Bertrand et al.’s (2022) paper
(Tables 11 and 12). Using only extant taxa consistently overestimated the encephalization of the earliest ancestor taxa based on the values generated from cranial and postcranial fossils. One caveat is that *Vincelestes nequenianus* is not a direct ancestor to any extant mammals (Bertrand et al., 2022). A second caveat is that this organism may have slightly predated the divergence of boreoeutherians (Macrini et al., 2007; Zhou et al., 2012). This taxon may have had a proportionally smaller brain compared to those of its contemporaries which were the ancestors to extant organisms, within the boreoeutherian lineage.

This is unlikely to entirely explain the overestimation. The reconstructed body mass estimate is much closer to the estimates generated for *V. nequenianus* (see Table S15, Bertrand et al., 2022) than any of the encephalization-related reconstructions (Tables 11 and 12). PEQs of >=0.5 are not predicted in Bertrand et al.’s reconstructions until the middle Eocene with organisms like *Metacheiromys marshi* (2022).

Using the encephalization quotient calculation derived by Bertrand et al. (2022) made the reconstructions align more closely. Even more effective was creating my own phylogenetically informed measure of encephalization (PEQ) and using this on the reconstructed brain and body mass values from my ancestral state reconstructions. Phylogenetically informed measures may be able to control for some biasing, even more effectively than using the simpler measure, brain to body ratio (Tables 11 and 12).

When reviewing how my reconstructions compared to the other taxa associated with the Cretaceous and late Jurassic (fossils *Vintana sertichi* and *Triconodon mordax*) it became evident that using fossils will always carry the caveat that each individual specimen represents a species which was adapted to its environment in entirely different ways than the operational taxonomic unity (OTU). As such, the results further support the need to phylogenetically inform
encephalization quotients and reiterate the importance of justifying construction findings using multiple lines of evidence. It is improbable that fossils will be able to validate all reconstructive findings, increasingly so the further back in time the reconstruction reaches. It is also possible that rates of EQ evolution have changed over the last 60 million years. Identifying life history and morphological trends in extant taxa and in younger fossil lineages is essential to improving our understanding of evolution through deeper time.

Within this dataset, only blue and bowhead whales (*Balaenoptera musculus* and *Balaena mysticetus*) have an EQ below that which was predicted using the endocranial volume and skeletal proportions of *V. neuquenianus*. These cetacean EQ values are certain to be derived based on their massively increased body size (Sears and Perrin, 2009) relative to all terrestrial mammals and most aquatic mammals. This makes encephalization quotient formulas informed using relatedness essential for ancestral character analyses of encephalization and any traits related to relative and absolute brain size. Additional investigation into best methods to account for clade differences in life history variation will refine these techniques and allow them to be applied to more questions in evolutionary history. Informed methodologies are crucial in clades and environments where the fossil record is relatively poor due to preservation conditions, such as the generally small-bodied and arboreal platyrrhines (Kinzey, 1997).

*Primate Craniofacial Morphology and Litter Size*

The twinning ceboide *Saguinus oedipus* is characterized by reduced cranial size dimorphism compared to the singleton-bearing ceboide *Sapajus apella*. In ceboide, cranial size reduces alongside body size dimorphism, following the mammalian trend of body size dimorphism reducing alongside increasing litter size (Carranza, 1996). My findings here support
my initial hypothesis, although the null hypothesis cannot be refuted until the morphological dataset expands to include additional primate and non-primate mammal taxa.

Twinning euarchontoglires, as represented by *Saguinus oedipus*, have larger litters, reduced craniofacial sex dimorphism, and reduced (absent) third molars compared to the singleton-bearing euarchontoglide, *Sapajus apella*. This may be related to the evolution of cooperative breeding in these taxa (e.g., Isler and van Schaik, 2012a, 2012b). Future studies into other closely related taxa will expand on these initial results. Future comparisons will have to include twinning and singleton-bearing lemuriformes, as well as taxa from both Scandentia and Dermoptera, to provide singleton- and litter-bearing outgroups.

My geometric morphometric findings support and expand on the trends identified during the life history analyses and literature review. Identifying that cranial size is correlated with body size in this taxon is important considering recent research which found sexually dimorphic growth patterns even in lemurs which are non-dimorphic in body size (Thomas et al., 2022). Thus, a lack of adult body size dimorphism cannot be assumed to represent a lack of all dimorphism. Future research comparing lemur and callitrichid craniofacial morphology and growth patterns will be useful in identifying how relatively non-dimorphic taxa may still differ, both in how these non-dimorphic body sizes are achieved and whether this lack of dimorphism is standard across the regions of the body.

Adding *Callimico goeldii* (the only singleton-bearing callitrichid) to this dataset is a high priority alongside the addition of more twinning primates outside of Callitrichidae. Traits which my life history analyses have shown to be correlated include litter size, gestation length, and prenatal growth rates. Identifying variation in reproductive characters and craniofacial morphology between closely related taxa may be applied to theories regarding reproductive
differences between extant and extinct taxa, including human and Neanderthal populations (Ponce de León et al., 2008).

Montgomery and Mundy (2013) have used craniodental anatomy to compare the evolutionary history of small-bodied hominids like *Homo floresiensis, H. luzonensis* and callitrichid primates. These lineages have reduced body sizes which are not accompanied by evidence of megadonty, or relatively larger teeth. Relative brain size is also reduced compared to larger bodied relatives in both *H. floresiensis* and some twinning callitrichids (Montgomery and Mundy, 2013). This evidence has been used to support theories which propose that these hominid lineages are examples of primates undergoing phyletic nanism (Montgomery and Mundy, 2013; Zanolli et al., 2022). The data I have assembled here can supplement future study into theories in human and nonhuman primate evolution using morphological correlates with reproductive traits.
Chapter 6 – Concluding Remarks

In this thesis, I performed a two-pronged investigation into the evolution of litter size. I conducted a meta-analysis of life history variation using 955 taxa within the magnorder Boreoeutheria, and I performed a geometric morphometric pilot study on twinning and singleton-bearing American monkey crania. I found significantly negative relationships between litter size and gestation length, age at maturation, maximum potential lifespan, and other traits which are typically associated with having a slow life history strategy or being $K$-selected.

Phylogenetic signal tests support that the distribution of litter size across this magnorder results from population-level adaptation. This is also supported for the distribution of other reproductive characters including gestation length and percentage of brain growth accomplished prenatally as compared to after birth. Additionally, I reconstructed various ancestral states within this magnorder, including twinning for primates and relatively long lifespans for body size in primates and chiropterans. In comparing these reconstructed values for the ancestral boreoeutherian to recent morphology-informed research on fossil mammals, I confirmed the importance and validity of using phylogenetically informed encephalization quotients and carefully justifying a reconstructive finding.

Finally, I present the results of a craniofacial morphology pilot study. It supported the following hypothesis generated in my literature review: that twinning primate species are less dimorphic in cranial size. I discussed possibilities for future research using 3D scans of mammalian craniofacial morphology including questions in human evolution which these methods and data might be applied to.
Litter size plays an important role in the life history strategy of all mammalian taxa. It seems unlikely that litter size reached the extremes seen in extant boreoeutherians until relatively recently, after both the Cretaceous-Paleogene and Eocene transition mass extinction events. My results present the possibility that twinning may have been an important factor defining the life history strategy which allowed our ancestors and those of other extant lineages to survive these events. This may have restricted size dimorphism, at least in those ancestral mammals which resembled modern day euarchontoglires, if my hypothesis regarding the connection between litter size and skeletal variation holds true. Future investigations into these questions may offer additional insight into how litter size, and twinning in particular, has shaped extant morphological and life history diversity.
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https://doi.org/10.1080/106351599260184


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https://doi.org/10.48738/2022.iss2.130


https://doi.org/10.1007/s10329-010-0220-8


Appendix A – Landmarking Protocol

<table>
<thead>
<tr>
<th>Landmark</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glabella</td>
<td>The midline point on the frontal bone, aligned with superior rim of the orbits.</td>
</tr>
<tr>
<td>Nasion</td>
<td>The point of intersection of the nasofrontal suture and the midsagittal plane.</td>
</tr>
<tr>
<td>Nasomaxillare (lat)</td>
<td>The most inferior point on the nasomaxillary suture.</td>
</tr>
<tr>
<td>Alare (lat)</td>
<td>The most lateral point on the nasal aperture taken perpendicular to the nasal height.</td>
</tr>
<tr>
<td>Subspinale</td>
<td>The midline point at the inferior tip of the bony septum between the upper central incisors.</td>
</tr>
<tr>
<td>Prosthion</td>
<td>Anterior midline point on maxillary alveolar process between incisors.</td>
</tr>
<tr>
<td>Alveolare*</td>
<td>The most anterior midline point on the maxillary alveolar process between the two central incisors.</td>
</tr>
<tr>
<td>Orbitale, superior (lat)*</td>
<td>The most superior midpoint of the orbital margin, aligned with the orbitale parallel to the sagittal plane.</td>
</tr>
<tr>
<td>Ectochoonion (lat)*</td>
<td>The most lateral point on the orbital margin.</td>
</tr>
<tr>
<td>Zygooritale (lat)</td>
<td>The point where the zygomaticomaxillary suture intersects with the inferior orbital margin.</td>
</tr>
<tr>
<td>Zygomaxillare (lat)</td>
<td>The most inferior, anterior point on the zygomaticomaxillary suture.</td>
</tr>
<tr>
<td>Porion (lat)</td>
<td>The most superior point on the margin of the external auditory meatus.</td>
</tr>
<tr>
<td>Mastoidale (lat)</td>
<td>The most inferior, lateral, posterior point on the mastoid process.</td>
</tr>
<tr>
<td>Inion</td>
<td>The midline point where the superior nuchal lines merge in the external occipital.</td>
</tr>
<tr>
<td>Lambda</td>
<td>The midline point where the sagittal and lambdoid sutures intersect.</td>
</tr>
<tr>
<td>Bregma</td>
<td>The point where the coronal and sagittal sutures intersect.</td>
</tr>
<tr>
<td>M1-M2 (lat)</td>
<td>The most lateral point on the alveolus between M¹ and M².</td>
</tr>
<tr>
<td>Frontomalare temporale (lat)*</td>
<td>The most lateral point on the zygomaticofrontal suture.</td>
</tr>
<tr>
<td>Jugale (lat)</td>
<td>The point in the depth of the notch between the temporal and frontal process of the zygomatic bone.</td>
</tr>
<tr>
<td>Ectochoonion, medial*</td>
<td>The point aligned perpendicularly to the sagittal plane with the ectochoonion on the orbital margin.</td>
</tr>
<tr>
<td>C1/P3 (lat)</td>
<td>The most lateral point on the alveolus between the permanent canine and P³.</td>
</tr>
<tr>
<td>Incisivon</td>
<td>The most posterior inferior point on the incisive fossa.</td>
</tr>
<tr>
<td>Palatomaxillare</td>
<td>The midline point of intersection of the palatine and the maxillary bones.</td>
</tr>
<tr>
<td>Sphenobasion*</td>
<td>The midline point on the sphenooccipital suture.</td>
</tr>
<tr>
<td>Landmark</td>
<td>Description</td>
</tr>
<tr>
<td>--------------------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Basion</td>
<td>The point where the anterior margin of the foramen magnum intersects the midsagittal plane.</td>
</tr>
<tr>
<td>Opisthion</td>
<td>The point where the posterior margin of the foramen magnum intersects the midsagittal plane.</td>
</tr>
<tr>
<td>Asterion*</td>
<td>The point where the lambdoid, parietomastoid, and occipitomastoid sutures meet.</td>
</tr>
<tr>
<td>Occipital condyle (lat)</td>
<td>The most inferior point on the occipital condyle.</td>
</tr>
<tr>
<td>Orbitale (lat)</td>
<td>The most inferior midpoint on the lower orbital margin.</td>
</tr>
<tr>
<td>Sphenosquamosal (lat)</td>
<td>The point of intersection of the infratemporal crest and sphenosquamosal suture.</td>
</tr>
<tr>
<td>Temporal fossa (pos, lat)</td>
<td>The most posterior, inferior point on the temporal fossa.</td>
</tr>
</tbody>
</table>

*These landmarks were excluded from results presented here as I was unable to satisfactorily standardize placement prior to analyses. This expanded set is presented here as these are the current landmarks I have under consideration for use in future analyses, once I have added additional crania.