EVOLUTIONARY ECOLOGY OF MAMMALIAN PLACENTAL INVASIVENESS

by

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ABSTRACT

Eutherian mammals differ markedly in placental form and function. In species with invasive placentation the fetal epithelium of the placenta is bathed in flowing maternal blood, giving the fetus an opportunity to extract resources directly from the mother and to secrete substances which modify maternal blood chemistry. In species with non-invasive placentation the fetal tissues are separated from maternal blood by a barrier of maternal cell layers which limits the ability of the fetus to control resource transfer during gestation. My thesis explores the role played by placental invasiveness in the evolution of eutherian brain size, life history and reproductive isolation. I find that the relationships between brain size, body size and lifespan, and the rate at which hybrid inviability evolves, differ strikingly between species with invasive versus non-invasive placentation. I propose a number of physiological mechanisms that may account for such differences among eutherian mammals.

Keywords: Eutheria; Placenta; Reproductive physiology; Evolution; Brain size; Life history

Subject headings: Mammals – reproduction; Mammals – evolution; Reproduction – physiology; Placenta

DEDICATION

I dedicate this thesis to my partner, lover, intellectual sparring-partner and best friend, Sonia Memetea. Without her unfailing support and encouragement, far beyond the call of duty or reason, it would never have been possible.

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CHAPTER 1: INTRODUCTION

The development of a permanent chorioallantoic placenta during gestation is one of the characteristics that distinguishes eutherian mammals from other vertebrate groups (Nowak 1999). The placenta is an organ of fetal origin that bears the fetal genotype (Mossman 1987). It varies markedly in morphology and physiology within and between most eutherian orders (Enders and Carter 2004; Mossman 1987; Ramsey 1982). A vast amount of research effort has been devoted to documenting this placental diversity (Mossman 1987; Appendix 1) and, more recently, to tracing its evolutionary ancestry (Carter et al. 2004; Wildman et al. 2006). Nevertheless, the functional role of the placenta in the evolution of mammalian life history, behaviour and ecology remains obscure. While the possession of a chorioallantoic placenta is the most prominent physiological distinction between eutherian mammals and other vertebrate groups, hypotheses regarding the evolution of characters unique or exaggerated in the eutherians – including large brain and body size, long gestation and long lifespan (Eisenberg 1981) – have been understood in terms largely divorced from details of reproductive anatomy (for example, Bielby et al. 2007; Boyce 1988; Charnov 1991; Charnov and Morgan Ernest 2006; Eisenberg 1981). This thesis is an approach toward unravelling the relationships between placentation and eutherian evolution. I focus on five outstanding problems in the evolutionary biology of mammals: the role of physiology in life history evolution, the evolution

of brain size, the role of parent-offspring conflict in the evolution of maternal-fetal interactions, and the nature of reproductive incompatibilities resulting in the divergence and speciation of mammalian taxa.

The role of physiology in life history evolution

Life history theory is based upon the notion that individual animals must divide their finite resources between a variety of physiological functions at a variety of different times. The quantities of resources devoted to growth, reproduction and maintenance are often negatively correlated with each other, and are thus seen as reflecting strategic economic trade-offs between functions whose common currency is energetic expenditure. Life history theory analyzes these strategic trade offs not only as individual choices but also as the products of natural selection optimizing fitness over each individual's entire course of life, and this body of theory has been successful in predicting a variety of relationships and associations between economic trade-offs, behaviour and demography (Charnov 1993; Roff 1992; Stearns 1992).

While mammalian life history may be understood as a long-term game of evolutionary economics, a major question in life history theory is to explain how evolutionary trade-offs between investment in various functions at various times is ultimately the product of physiological structures and mechanisms (Ricklefs and Wikelski 2002). The role of physiological mechanisms in life history evolution has often been discussed under the rubric of "constraint", in the trivial sense that the activity or growth of an individual may be limited by the availability of an energetic surplus over and above maintenance requirements (i.e., Gittleman and

Thompson 1988). The last one or two decades, however, have seen the rising popularity of a coevolutionary view of life history evolution in which physiological structures set bounds on the range of possible life histories, but are also subject to change due to natural selection on life history itself (Ricklefs 1991; Ricklefs and Wikelski 2002). As such, research in evolutionary physiology has increasingly involved the description of functional physiological interactions among various components of life history traits, and the tracing of the transformations of these physiological interactions over evolutionary time (Wikelsi and Ricklefs 2001; Zera and Harshman 2001).

The eutherian placenta is a physiological structure of significance to life history evolution since one of its primary roles is to mediate the transfer of maternal resources – including nutrients, structural materials and oxygen – to provision the growth and development of offspring (Mossman 1987; Père 2003). In this thesis, I consider the importance to life history evolution of placental invasiveness, an important character related to the number of maternal cell layers separating fetal from maternal blood. In general I refer to placentation in which fetal tissues are bathed in maternal blood as "invasive" and placentation in which fetal tissues are separated from maternal blood by cell layers of maternal origin as "non-invasive" (Figure 1), which is a coarse version of Grosser's tripartite classification of placental types (Grosser 1909; King 1992). The nature of this interhemal barrier is expected to be important with respect to life history evolution since it is the locus of resource exchange between mother and offspring. Chapters 2 and 3 of this thesis assess the role of placental variability

on mammalian life history evolution by identifying life history trade-offs which differ between species grouped as placentally-invasive versus non-invasive. I attempt to provide specific physiological hypotheses for the observed results, with a special emphasis on differences in the evolution of brain size in the two groups of mammals.

Evolution of brain-body allometry in mammals

Chapter 2 of this thesis uses an allometric approach (Harvey and Pagel 1988) to examine the relationships between brain size and placental invasiveness. Body parts or life history parameters are said to scale *isometrically* when their magnitudes are a fixed proportion of total body size; for example, the total volume of blood in a mammal's body scales isometrically with body mass, being around 7% of body mass in species of any size. Body parts or life history parameters are said to scale *allometrically* when their magnitudes scale disproportionately with body size; for example, as a function of increasing body size, bone mass increases more rapidly than total body mass, such that the skeleton of a shrew accounts for just 5 per cent of its total body mass while that of an elephant accounts for nearly 20 per cent (Lindstedt 1987). The scaling of brain size (BR) to body size (BO) is modelled by the allometric equation, $BR = a \cdot BO^b$, where a is the intercept and b is the gradient of the linear relationship between log(BR) and log(BO) (Harvey and Pagel 1988). The exponent b of the brain-body allometry of mammals is considered to be 0.75 (Martin and Harvey 1985). However, marked departures from this value within large taxonomic groups such as mammalian orders (Martin and Harvey 1985; Pagel and Harvey

1988), lead us to view the notion of a single brain-body allometric slope for all mammals with suspicion, while the cause and significance of such variation remains uncertain.

In Chapter 2 I use traditional and comparative statistical methods to show that the slope of mammalian brain-body allometry is substantially and significantly steeper in species with invasive placentation than in species with non-invasive placentation. This pattern holds across mammals as a whole, across age classes, and within mammalian clades that exhibit diversity in placental form. The steeper allometric slope is associated with relatively more rapid prenatal brain growth and precocious neurological development at birth, in species with invasive placentation. Studies of comparative placentation indicate a simple mechanism for these differences: in species with invasive placentation, fatty acids essential for mammalian brain development can be extracted by the fetus from the maternal bloodstream, but in species with non-invasive placentation they must be synthesized by the fetus itself. These results suggest that mammalian brain-body scaling cannot be described adequately by a single allometric equation, casting doubt over the utility of general models of allometry applicable uniformly to all taxa (i.e., Blum 1977; West et al. 1997; White and Seymour 2005). Instead, these results urge us to evaluate the physiological basis of mammalian scaling, especially the underlying mechanisms of resource acquisition exhibited by structures during their development.

Placentation, brain size and life history

Evolutionary explanations for the diversity of brain size among mammalian species have long been a major area of research and controversy (Gayon 2000). While social and ecological hypotheses predominate (Allman et al. 1993a; Allman et al. 1993b; Barton 1996; Dunbar 2003a; Dunbar 2003b; Fish and Lockwood 2003; Gittleman 1986; Jones and MacLarnon 2004; Lindenfors 2005; Mann et al. 1988; Sawaguchi and Kudo 1990; Shultz and Dunbar 2006), a complementary body of work rooted in evolutionary physiology has based its hypotheses on the fact that brain tissue is metabolically expensive and in a sense competes for energy with other structures and functions during growth and development (Aiello 1997; Aiello and Wheeler 1995; Hladik et al. 1999). Due to the fact that brain tissue requires a great investment in its life-long maintenance and especially its initial development during pregnancy and childhood (i.e. Laughlin, van Steveninch et al. 1998), brain size seems a likely candidate for involvement in life history trade-offs over resource usage.

As discussed above, understanding the relationship between physiology and life history outcomes is a major aim of contemporary evolutionary biology. I approach the issue by drawing upon evolutionary economic models of brain size evolution. The growth of neural tissue is an unusual form of investment in that the "value of neural capital" increases throughout the life span of an individual as new knowledge, skills and information are acquired (Kaplan et al. 2003a). Evolutionary economics models predict the existence of synergistic evolutionary processes relating brain size, longevity and parental investment. High levels of

encephalization provide a context in which longevity is favoured by natural selection, since the overall return on neural investment accumulates over the full lifespan of the animal and since the size of these returns increases with age. Conversely, since knowledge, skills and the ability to learn may promote the survival of an animal over a long period of time, natural selection should favour large brains in long-lived animals. In both cases, selection will favour a shift in the timing of initial investment in brain growth to ever earlier ages, freeing up time for an extended period of learning and experimentation in subadult mammals (Kaplan et al. 2003a; Kaplan et al. 2003b; Kaplan and Robson 2002). These considerations lead to the prediction that mammals are better able to reap the cognitive rewards of slow life histories when they are equipped with a mechanism for the rapid prenatal transfer of large quantities of nutritive and structural resources. Similarly, invasive placentation should be selectively favoured in species with extensive brain growth and longevity, and correlations between life history "slowness" and encephalization should be stronger in species with invasive placentation than in species with non-invasive placentation. These hypotheses are described and tested in Chapter 3, and the results are discussed within the general context of mammalian life history evolution.

Parent-offspring conflict

Conflict over the level of resource transfer during early life is expected to arise from asymmetries between the inclusive fitnesses of parents and offspring. The role of the placenta in such conflict has been suggested by a number of

authors (Crespi and Semeniuk 2004; Haig 1993; Wells 2003; Zeh and Zeh 2000). All else being equal, a mother (or more specifically, a maternal autosomal gene) is equally related (by one half) to all of her offspring, and her optimal strategy should be to divide resources equitably between all of her young. Each offspring, on the other hand, is more related to itself (with a coefficient of relatedness of one) than to its full siblings (with which it shares one half of its genes) or half siblings (with which it shares one quarter of its genes). Genes expressed in the fetus or placenta acting to increase the proportion of maternal resources received by some focal offspring at the expense of its siblings or future siblings will thus be favoured by natural selection. To the contrary, maternally-expressed genes which equalize the distribution of maternal resources between offspring should be favoured by natural selection acting on the mother (Godfray 1995; Lessells and Parker 1999; MacNair and Parker 1979; Trivers 1974). These ideas have been used to interpret a number of unusual characteristics of human placentation and pregnancy including the extremely high titers of placentally-secreted hormones and the invasion of maternal spiral arteries by tissues of fetal origin (Haig 1993).

Such evolutionary conflicts of interest may play out in a variety of ways, ranging from one participant "winning" the conflict, to both participants engaging in a never-ending "tug of war" in which each adaptation that arises in one party selects for a counteradaptation in the other party. I use data on prenatal and postnatal growth rates to assess the evidence for parent-offspring conflict over resource allocation during pregnancy. I consider the control of nutrient transfer

during pregnancy to differ between species with invasive versus non-invasive placentation. In the former group, fetuses can directly modify maternal blood chemistry by extracting resources and secreting hormones and other substances (Haig 1993), while in the latter group the ability of fetuses to directly modify maternal blood chemistry is likely limited. After birth this disparity should disappear, as mothers find themselves in a much stronger position to exert control over resource transfer to offspring by modifying the frequency and duration of suckling. Conflict over the allocation of resources under invasive placentation will thus be evidenced by high prenatal and perhaps low postnatal growth rates compared to the rates found in species with non-invasive placentation. My findings are broadly consistent with this hypothesis; I find that the correlation between prenatal brain growth rate and postnatal body growth rate differs in sign between species grouped as placentally-invasive versus non-invasive. A discussion and interpretation of these findings is presented in Chapter 3.

Speciation

Accounting for the strikingly different rates and patterns of the evolution of reproductive isolation between animal lineages has long been a central issue in evolutionary biology. Recent theoretical and empirical work has emphasized the role of barriers to gene flow as a primary cause of population divergence and speciation in nature (Coyne and Allen Orr 2004). In Chapter 4 I consider the possibility that differences in placental structure translate into differences in the strength of reproductive incompatibility between divergent mammalian

populations. Using mitochondrial DNA sequences I show that the maximum genetic distance at which interspecific mammalian pregnancies yield viable neonates is significantly greater in clades with invasive placentation than in species with non-invasive placentation. Moreover, sister species with invasive placentation exhibit higher allopatry in their geographic ranges, suggesting that formerly separated populations in mammals with this placental type fuse more readily on recontact. These differences are apparently driven by the stronger downregulation of maternal immune responses under invasive placentation, under which fetal antigens directly contact the maternal bloodstream. These results suggest that, in addition to its interactions with brain size and life history, placental invasiveness mediates a major component of reproductive isolation in mammals.

The following three chapters of my thesis explore the role of placentation in these outstanding problems in the evolution of eutherian mammals. I end with a discussion of the principal results and suggestions for future directions of research.

CHAPTER 2: DIVERGENT BRAIN-BODY ALLOMETRY AND HETEROCHRONY BETWEEN EUTHERIAN MAMMALS WITH INVASIVE VERSUS NON-INVASIVE PLACENTATION

Introduction

Body size allometry, in which the dimensions of body parts and the values of life history variables scale consistently with body size across species, has been the subject of biological study, speculation and controversy for over a century (Gould 1966; Stearns 1980). A central notion in the study of allometry is that such patterns of scaling reflect fundamental and perhaps universal constraints on the transfer of energetic resources within living organisms, and ultimately on their development, function and evolution. Biological research has documented interspecific differences in the intercept and gradient of allometric slopes, and in deviation of each species from an observed allometric slope, with mammalian brain-body allometry being one of the best studied patterns (Gayon 2000). Such studies help to identify ecological or life history correlates of variation in the scaling of body size and it components, which have been explained in terms of trade-offs in the allocation of limited energetic resources to different body parts, functions, activities and time periods throughout an animal's life span.

Analysis of the development of energetically expensive tissues (Aiello 1997; Aiello and Wheeler 1995) is expected to yield important insights into the

origin and evolutionary diversification of animal allometry for two reasons. First, such tissues place strong energetic demands on life-history trade-offs over allocation of resources to growth versus fecundity. Second, expensive tissues also mediate strong selection on the proximate mechanisms of resource acquisition during prenatal and infant growth. In terms of its maintenance energy requirements, the brain is the most costly tissue of the mammalian body, consuming over twenty times the energy of skeletal muscle per unit mass at rest (Aiello 1997; Aiello and Wheeler 1995; Laughlin et al. 1998). The brain is also extremely costly in terms of the structural components that are required for its growth, to such an extent that brain growth may be the main rate-limiting process operating during fetal development (Martin 1996).

Previous studies have described various social and ecological correlates of adult mammalian encephalization (Allman et al. 1993a; Allman et al. 1993b; Barton 1996; Dunbar 2003a; Dunbar 2003b; Fish and Lockwood 2003; Gittleman 1986; Jones and MacLarnon 2004; Lindenfors 2005; Mann et al. 1988; Sawaguchi and Kudo 1990; Shultz and Dunbar 2006). Here we adopt a physiological perspective in order to explore how the growth and allometry of developing brains may also be influenced by functional constraints and tradeoffs in resource transfer and allocation during the prenatal period. The anatomical-physiological structure of most significance to the developing mammalian fetus is the placenta, which develops from fetal extra-embryonic ectoderm and varies markedly in form and function among eutherians (Mossman 1987). The placenta has also been identified as an arena in which genetic conflicts over the rate and

magnitude of resource allocation are made manifest (Crespi and Semeniuk 2004; Haig 1993). Specifically, invasive forms of placentation may be associated with enhanced fetal mobilization of maternal resources, and the fetal manipulation of maternal energy budgets by secretion of hormones and other substances into her bloodstream, resulting in improved resource acquisition by the fetus during pregnancy (Haig 1993).

Our primary hypothesis is that invasive forms of placentation are associated with accelerated prenatal brain growth, which may translate into differences in patterns of brain-body allometry. In order to test this hypothesis we group mammalian species according to their form of placentation. We refer to species with hemochorial placentas (in which fetal tissue is in direct contact with flowing maternal blood) as exhibiting invasive placentation, while species with epitheliochorial or endotheliochorial placentas (in which fetal tissue is separated from flowing maternal blood by maternal epithelia) exhibit non-invasive placentation (see Figure 1). Phylogenetic analysis indicates that the ancestral eutherian placental condition was apparently of an invasive form, and the occurrence of non-hemochorial placentation in extant taxa is the result of 9 to11 independent evolutionary transitions occurring in the Insectivora, primates, bats, rodents, Afrotheria, Xenarthra and at the root of Laurasiatheria (Elliot and Crespi 2006; Wildman et al. 2006). The taxonomic distribution of these placental types is described in Table 1.

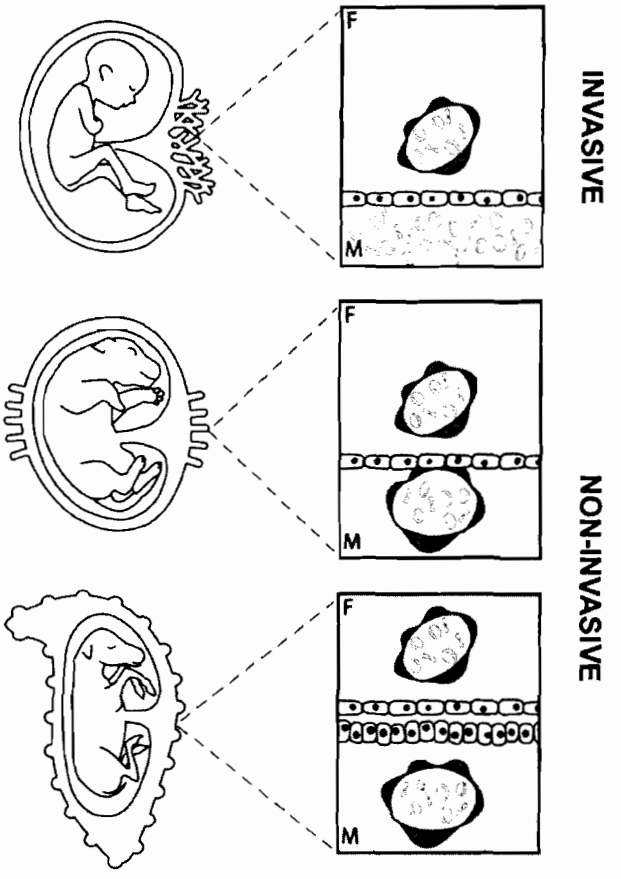


Figure 1. Invasive and non-invasive forms of placentation in eutherian mammals. Left: schematic representations of invasive and non-invasive forms of placentation (top: hemochorial placentation in human beings; middle: endotheliochorial placentation in canines; bottom: epitheliochorial placentation in horses; the placenta is of fetal origin, and maternal tissues - which surround the fetal-placental unit - are not drawn for the sake of clarity). Right: arrangement of maternal (M) and fetal (F) tissue layers corresponding to the invasive and non-invasive forms illustrated; open nucleated cells represent epithelial layers, dark grey nucleated cells represent the endothelial wall of blood vessels, light gray areas represent connective tissue and open dumbell-shaped cells represent hemocytes. Only in invasive placentation is the placenta in direct contact with maternal blood. See text and Table 1 for further details.

Table 1. Distribution of invasive (hemochorial) and non-invasive (epitheliochorial or endotheliochorial) placentation in eutherian mammals analysed in this study

Clade	Placentation	Number of families and species included in this study
Afrosoricidae	Hemochorial	1 family, 11 species
Artiodactyla	Epitheliochorial	8 families, 87 species
Carnivora	Endotheliochorial	11 families, 38 species
Cetacea	Epitheliochorial	7 families, 25 species
Chiroptera	Hemochorial	6 families, 95 species
Chiroptera	Endotheliochorial	8 families, 41 species
Hyracoidea	Hemochorial	1 family, 1 species
Lipotyphla	Hemochorial	3 families, 18 species
Lipotyphla (Talpidae)	Epitheliochorial	1 family, 6 species
Lagomorpha	Hemochorial	1 family, 4 species
Macroscelidea	Hemochorial	1 family, 2 species
Perissodactyla	Epitheliochorial	3 families, 5 species
Primates (Haplorrhini)	Hemochorial	9 families, 53 species
Primates (Strepsirrhini)	Epitheliochorial	7 families, 18 species
Proboscidea	Endotheliochorial	1 family, 2 species
Rodentia	Hemochorial	13 families, 43 species
Rodentia (Heteromyidae)	Endotheliochorial	1 family, 21 species
Scandentia	Endotheliochorial	1 family, 1 species
Xenarthra (Dasypodidae)	Hemochorial	1 family, 1 species
Xenarthra (Bradypodidae)	Endotheliochorial	1 family, 1 species

We use classical and phylogenetic statistical methods to test for differences in the brain-body allometric slope exhibited by species with invasive vs. non-invasive placentation, across mammals as a whole and within focal clades. We further test for differences in prenatal and postnatal brain and body growth rates between these two groups, and analyze data on relative precocity at birth, our prediction being that invasive hemochorial placentation is associated with relatively advanced neurosensory development at birth. Finally, we describe a physiological mechanism that may account for our findings, compare it with alternative hypotheses, and discuss our results in the context of existing evolutionary theories of encephalization.

Methods

Data on brain mass and body mass at birth and adulthood, gestation length, age and mass at weaning, and age at first reproduction for 471 mammals of known placental type were gathered from the literature (Burton 2006; Carey and Judge 2000; Fish and Lockwood 2003; Hayssen et al. 1993; Jones and MacLarnon 2004; Lindenfors 2002; Marino 2006; Morgan Ernest 2003; Pérez-Barbería and Gordon 2005; Symonds 2001; White and Seymour 2003). In order to boost the size of the Chiropteran dataset, placentation was assumed to be uniform within each genus of bat (a pattern found in anatomical studies of all bat and non-bat mammalian genera to date); however, inclusion or exclusion of this Chiropteran data did not have substantive effects on the overall results presented below. Unless stated otherwise, adult body mass was based on female data. In

order to account for covariance between body size and the value of life history variables such as gestation length or weaning age, we carried out linear regression of each Log_{10}-transformed life history variable against Log_{10} body mass, and used the residual for each species in our analysis.

Comparison of allometric slopes was accomplished by fitting univariate general linear models to the data in SPSS (SPSS 2006) using placental type as a fixed factor. Slopes were considered to be significantly different when the interaction term (placenta x independent variable) was significant at $p<0.05$. Where possible, these classical approaches were replicated under a phylogenetic model using independent contrasts as implemented in the computer program PDAP:PDTREE/Mesquite (Midford et al. 2007). Tests conducted across the entire mammalian dataset were based on a recent species-level supertree of the mammals (Bininda-Edmonds and Cardillo 2007). Ordinal phylogenies used in these tests were obtained from the literature (Grenyer and Purvis 2003; Jones et al. 2002; Purvis 1995), except in a single case, a phylogeny of Geomyidae and Heteromyidae, which was reconstructed by maximum likelihood analysis of cytochrome *b* sequences using the computer program PAUP (Swofford 2003).

Data on the degree of infant precocity for 206 mammalian species was gathered from the literature (see Appendix). Measures of precocity were based on the Log_{10}-transformed age at which five developmental milestones are reached in each species: eyes open, internal auditory meatus opens, independent quadrupedal locomotion first occurs; solid food is first ingested; and weaning is complete. Univariate general linear models using a stepwise selection

protocol were used to assess the effect and significance of placentation as a predictor of precocity independent of the confounding effects of body size, gestation length, and other relevant reproductive parameters.

Results

Results are summarized in Table 2 and discussed in detail below.

Brain-body allometry

When considering all eutherian mammals in our dataset, species with invasive (hemochorial) placentation were found to exhibit a strikingly steeper neonate brain-body allometric slope than species with non-invasive placentation (β=1.020 versus 0.724; N=117, F=49.797, $p<0.001$; Figure 2). Indeed, while brain mass increases as an allometric function of body mass in species with non-invasive placentation, it increases isometrically in species with invasive placentation. The slope of the adult brain-body mass allometry was also found to be significantly steeper for the group of species with invasive placentation, though the difference was less pronounced (β=0.861 versus 0.721; N=471, F=21.788, $p<0.001$; Figure 2), suggesting that some of the difference associated with placental invasiveness in brain mass at birth is diminished by patterns of brain growth during the juvenile period, consistent with the prenatal effects of placentation. Moreover, the relatively uniform slope within each placental type at both age points is remarkable, given the broad and diverse range of taxa that make up each group in the analysis (see Table 1). These results may be influenced by the clustering of data points within taxonomic groups of mammals,

especially the existence of a clade with invasive placentation (Rodentia) characterized by small brains and small bodies, and a clade with invasive placentation (Primates) characterized by large brains and medium-sized bodies. In order to account for such potential confounding effects, the analyses were repeated using phylogenetically independent contrasts. From a phylogenetic tree of 404 mammals, we considered 267 internal contrasts for which placental type could be inferred unambiguously by maximum likelihood. A single outlying contrast was removed from the dataset prior to analysis. Regression lines forced through the origin for nodes classified as invasive or non-invasive were consistent with the results presented in Figure 2, the slope for species with invasive placentation being significantly steeper than the slope for species with non-invasive placentation (β=0.664 versus 0.558, $p<0.001$, $F=7.045$, $N=120$ invasive and 147 non-invasive contrasts).

Of interest is the fact that species with invasive placentation tend to have smaller adult bodies than noninvasive species in this dataset (mean log adult body mass = 2.08 versus 3.54; $F=89.473$, $p<0.001$). Possible explanations for this result are provided later in this chapter. In the meantime, it is necessary to ensure that the apparent divergence in allometry is not the result of a statistical difference in body mass between the two groups of mammals in our dataset. In order to do so we used a resampling approach, selecting species randomly but with weighted probabilities such that the same uniform distribution, mean and range of body mass was reflected in each placental category. One hundred and

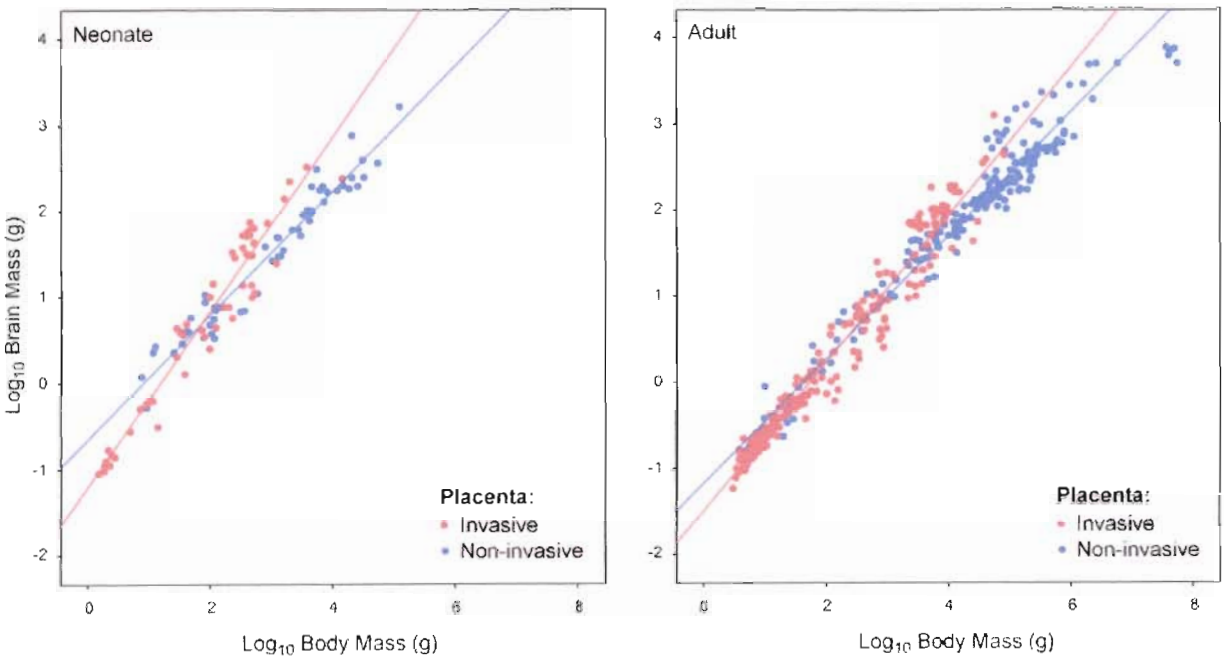


Figure 2. Brain-body allometry in adult and newborn mammals. Species with invasive (hemochorial) placentation (red) exhibit significantly steeper allometric slopes than species with non-invasive (endotheliochorial or epitheliochorial) placentation (blue).

sixty species were sampled from the original dataset (eighty invasive and eighty non-invasive) across one hundred replicates; we found no replicate in which the difference in allometric slope between the two groups was not significant ($F=18.298$, $p<0.001$). For this reason the observed pattern appears to be robust with respect to differences in mean and range of body mass between the two groups. An alternative possibility is that the data in Figure 2 is best described by a single curvilinear relationship in which the apparent distinction between the allometry of species with invasive versus non-invasive placentation is the result of the former species having a lower average body mass. While both models fit the data very well, a quadratic curve explained less variance in log brain mass than the two-line model depicted in Figure 2 ($R^2=0.974$ versus 0.985).

An alternative way of testing the robustness of this result is to conduct the same test independently for each order of mammals exhibiting diversity in placental type. Four such orders were available within our dataset. In all of them, the allometric exponent of species with invasive placentation is steeper than that of species with non-invasive placentation, and this difference in slope is statistically significant in three of the four cases (Figure 3).

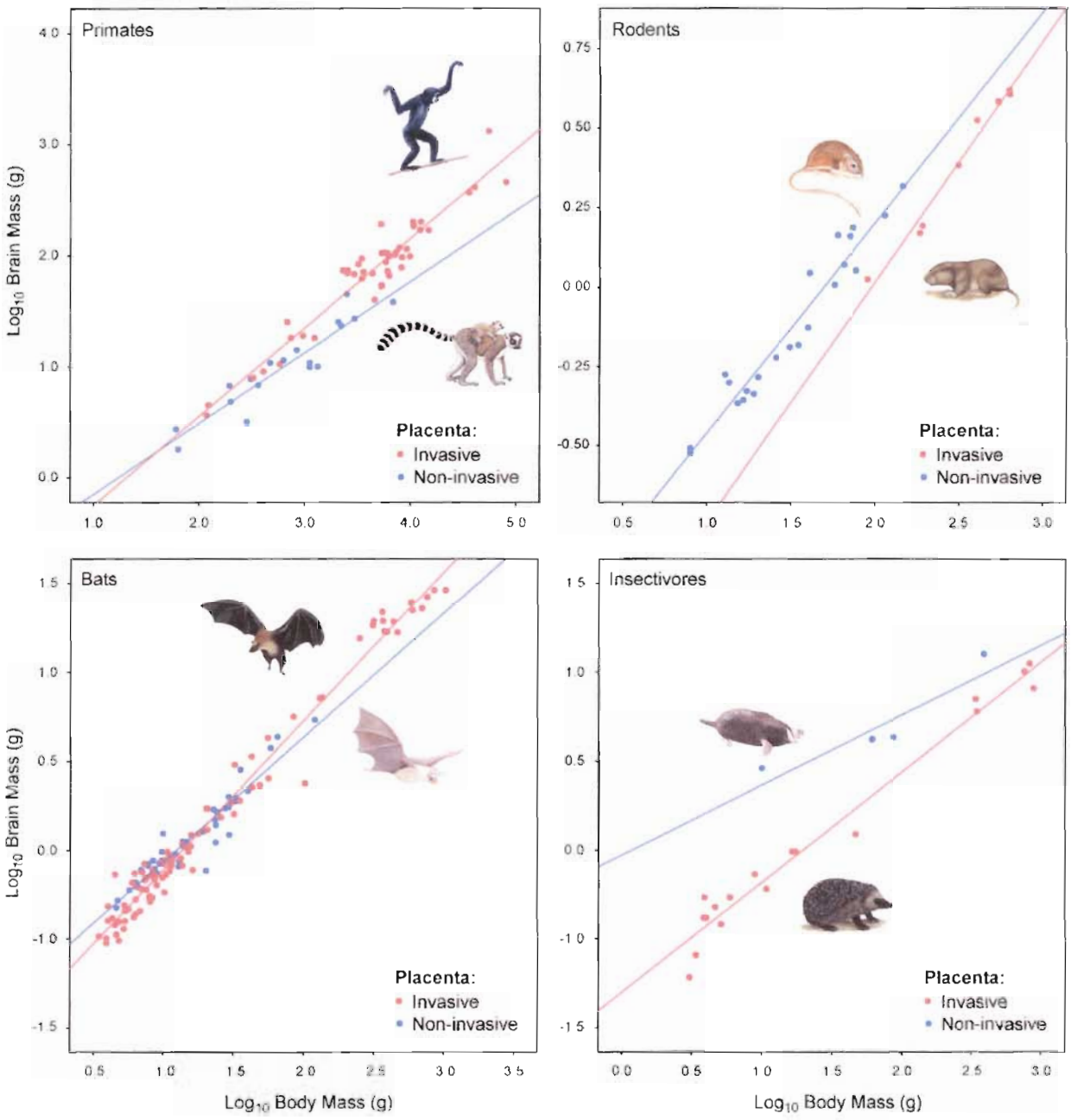


Figure 3. Brain-body allometry in four orders exhibiting placental variability. Species with invasive, hemochorial placentation (red) exhibit steeper allometric slopes than species with non-invasive endotheliochorial or epitheliochorial placentation. The difference in slope is statistically significant (α=0.05) for primates, bats and Lipotyphlans ("Insectivores").

Amongst the bats, Megachiroptera exhibit uniformly invasive placentation, while Microchiroptera exhibit both invasive and non-invasive forms. Average body mass did not differ significantly between groups ($F=2.341$, $p=0.127$). Brain-body allometry differs significantly between invasive ($N=163$) and non-invasive ($N=115$) categories, with the former exhibiting a significantly steeper allometric slope ($\beta=0.839$ versus 0.699; $F=6.074$, $p=0.014$). The same pattern was found when testing independent ($\beta=0.737$ for hemochorial versus 0.680 for non-hemochorial species; $F=6.474$, $p=0.002$).

All rodents exhibit invasive placentation expect for the Heteromyidae (kangaroo rats and pocket mice) which develop a non-invasive form. We tested for a difference in allometric slope between Heteromyidae and their closest sister clade, the Geomyidae (pocket gophers). In this case, data on brain size was available only in the form of endocranial volume rather than brain mass. In contrast with the situation across mammals as a whole, members of the invasive clade ($N=9$) were found to have significantly larger body masses than members of the clade with non-invasive placentation ($N=21$, $F=10.932$, $p=0.001$). The Geomyidae exhibited a steeper allometric slope than their non-hemochorial sister clade ($\beta=0.76$ versus 0.66) though this difference in slope was not found to be statistically significant ($p=0.221$). Under analysis of independent contrasts, the slope for the invasive group was also found to be steeper ($\beta=0.72$ versus 0.56) and this difference approached significance ($F=3.577$, $p=0.071$).

Within Lipotyphla, invasive forms of placentation are found in hedgehogs, gymnures, solenodons and shrews, while moles and desmans develop a non-invasive placenta. The combined non-invasive species (N=6) were found to be significantly larger than the insectivores with invasive placentation (N=18; $F=5.176$, $p=0.034$). As expected, the brain-body allometry of the invasive group was found to be significantly steeper than that of the species with non-invasive placentation ($\beta=0.623$ versus 0.395; $F=6.456$, $p=0.020$). A similar pattern was identified from analysis of independent contrasts ($\beta=0.625$ versus 0.383; $F=4.670$, $p=0.042$).

Amongst primates, strespirhines (lemurs, lorises, galagos and allied species) exhibit non-invasive placentation, while haplorhines (tarsiers, marmosets, monkeys, gibbons and apes) exhibit invasive placentation. The haplorhines have a significantly higher mean body mass ($F=23.424$, $p<0.001$). The brain-body allometry of adult haplorhines is significantly steeper than that of adult strepsirhines ($\beta=0.802$ versus 0.639; N=19 strepsirhines and 51 haplorhines; $F=5.188$, $p=0.026$); for newborn primates the difference in slope is not statistically significant ($p=0.552$), though when newborn brain mass is predicted by adult body mass, the slope of the invasive clade is again significantly steeper than that of the non-invasive clade ($\beta=0.704$ versus 0.520; $F=6.078$, $p=0.020$). The analysis of independent contrasts, however, rendered these results statistically non-significant (though the group of species with invasive placentation always exhibited a steeper slope than the group of species with non-invasive placentation). This lack of significance under independent

contrasts may be a function of low statistical power, given that in Primates there is only one transition between placental types.

These results indicate that invasive hemochorial placentation tends to be associated not with accelerated prenatal brain growth (which would be reflected in a difference in allometric intercept but no difference in allometric slope) but with a steeper brain-body mass allometry. Although there was an overall tendency for the hemochorial species in our dataset to be larger than the non-hemochorial species, bootstrapping tests show that this tendency does not account for the differences in allometry between mammals grouped by placental type. Tests carried out within four orders of mammals demonstrated no systematic correlation between body size and placental type, while often finding a steeper allometric slope in groups of species with hemochorial placentation, though in Primates the result depended on whether the test was carried out using classical statistics or phylogenetically independent contrasts.

Encephalization quotient

Relative brain size in mammals is frequently described in terms of an encephalization quotient, calculated for each species as the ratio of observed brain mass to expected brain mass, the latter based on a global regression of log brain mass against log body mass (Jerison 1973). The steeper allometric slopes found in taxa with invasive placentation do not necessarily translate into higher encephalization quotients, because the intercept of the allometric slope generally appears to be lower in these groups (see Figures 2 and 3).

Across all mammalian species in our dataset, mean encephalization quotient did not differ between placental categories ($F=1.862$, $p=0.173$); nor did it differ within the orders of mammals exhibiting placental diversity (Chiroptera: $p=0.934$; Insectivora: $p=0.667$; Primates: $p=0.122$; Geomyoid rodents excluded since only brain volume, not brain mass, is available). When encephalization quotient was based on a regression of brain mass against body mass within orders rather than globally across all mammals, hemochorial encephalization quotients were significantly higher than non-hemochorial encephalization quotients in the Primates ($F=5.245$, $p=0.025$), but significantly lower in the Insectivores ($F=9.440$, $p=.006$) and not significantly different in the Chiroptera ($F=0.004$, $p=0.951$). These results can be understood by inspection of Figure 2, which shows that species with invasive placentation tend to have relatively large brains at large body sizes, but relatively small brains at small body sizes.

The lack of any consistent association between relative brain size and placental type leads us to interpret differences in allometric slope as the result of differences in the *pattern* of brain growth (for example, the rate of prenatal brain growth or, alternatively, the proportion of brain growth occurring prenatally compared to postnatally) rather than differences in the total relative *quantity* of brain growth. These avenues are explored below.

Proportion of brain and body growth occurring prenatally versus postnatally

If invasive forms of placentation permit more rapid or extended prenatal growth of the brain, then we predict that species with invasive hemochorial

placentation will accomplish a larger proportion of their total brain growth during gestation than is accomplished by non-hemochorial species. The proportion of brain growth occurring prenatally was found to correlate with adult brain size, such that larger-brained species tend to grow a lower proportion of their total brain mass prior to birth (N=113, F=6.435, p=0.013). We adjusted for this pattern by considering the residual proportion of total brain growth occurring prenatally, after regression against log-transformed adult brain mass. Across all mammals, species with non-invasive placentation were found to accomplish on average 12.5% less brain growth prior to birth than species with invasive placentation (mean = 0.35 versus 0.40, N=113, F=6.165, p=0.015). In order to test the significance of this pattern at lower taxonomic resolutions, we repeated the analysis using primate data (primates being the only order of mammals exhibiting placental diversity and with sufficient available neonatal brain mass data for statistical analysis). Consistent with the previous result, the proportion of total brain growth occurring prenatally in strepsirhines was 20% less than that occurring in the haplorhines (mean = 0.40 versus 0.50, N=29 haplorhine and 11 strepsirhine species, F=8.868, p=0.005).

Prenatal brain growth rate

We calculated average prenatal brain growth rate by dividing neonate brain mass by gestation length. Since the log-transformed growth rate was found to correlate in a linear fashion with log adult body size, residuals of the regression against log adult body mass was used as a metric of relative prenatal growth rate. Across all mammals, the average prenatal brain growth rate of

species with invasive placentation appeared to be higher than that of species with non-invasive placentation, independent of body size ($N=85$, $F=3.954$, $p=0.050$). The same trend was found within the primates, the only order of mammals to contain sufficient data for independent analysis. Haplorhine primates, with invasive placentation, exhibited significantly higher prenatal brain growth rates than strepsirhine primates, with non-invasive placentation ($N=20$, $F=50.230$, $p<0.001$).

Precocious and altricial young

Since the different classes of placentation are apparently associated with different prenatal brain growth rates, and since the brain is thought to act as a pacemaker in the rate of prenatal development (Hofman 1983; Sacher and Staffeldt 1974), we expect that the patterns described above will translate into an increase in the degree of precocity exhibited by species with invasive placentation. For 206 mammalian species we gathered data on the timing of five developmental milestones: age at first opening of the eyes; age at opening of the internal auditory meatus; age at first locomotion; age at first ingestion of solid food; and age at the completion of weaning. The first two milestones have occasionally been used as proxies for the level of neurosensory development at birth, in opposition to measures of musculoskeletal development (Grand 1992). It is important to note that precocity can vary across a number of dimensions, human beings, for example, being neurologically precocious (Finlay et al. 2001) but otherwise physically altricial. Similarly, ungulates are often considered to be examples of precocious mammals because of their high level of musculoskeletal

development at birth, yet long gestation lengths in ungulates may mask a slow developmental process. For this reason we consider the timing of developmental milestones since conception rather than since birth.

From a consideration of standard life history variables, gestation length and body size were found to be strong predictors of the timing of all milestones except age at first ingestion of solid food, which varied randomly with respect to candidate explanatory variables and was excluded from further analysis. In combination, body mass and gestation length accounted for 56% of the variance in age at eye-opening, 28% of the variance in age at first locomotion, and 73% of the variance in age at weaning. Body mass was not a significant predictor of age at opening of the auditory meatus, gestation length alone accounting for 65% of the variance. Nevertheless, under a stepwise selection protocol, placental type - independent of body mass and gestation length - was found to be a significant predictor of the age at which eyes open ($p<0.001$) and the age at which the internal auditory meatus opens ($p=0.040$). For both variables, invasive placentation is associated with a more precocial life history, reducing the age since birth at which each milestone is reached. Surprisingly, placental type accounts for more variance than both body mass and gestation length in the age at which eye opening occurred (partial $\eta^2 = 0.224$ versus 0.058 for body mass and 0.209 for gestation length; r^2 for total model = 0.576), and has a stronger effect than body mass on age at ear opening (partial $\eta^2 = 0.058$ versus 0.002; partial η^2 of gestation length = 0.242; r^2 of total model = 0.666). Placentation had a similar effect on the age at which locomotion first occurs, but the fit of the

model to the data was relatively low (partial η^2 = 0.111 versus 0.116 for body mass; gestation partial η^2 = 0.004; r^2 of three-parameter model = 0.326). In predicting the age at which weaning ends, placental type was found to play no role.

These results indicate that while gestation length is the primary determinant of precocity at birth (long gestations tending to result in more-developed young), invasive placentation is also significantly associated with the production of more precocious offspring, especially with respect to age at eye opening, the variable most commonly used as a measure of neurosensory development.

Alternative hypotheses

The results presented above imply that mammals fall into two natural groups which differ in brain-body relations as a result of differences in the physiological structures and mechanisms underlying prenatal brain development. Species with invasive placentation exhibit steeper brain-body allometric slopes than species with non-invasive placentation, accomplish a larger proportion of their brain growth prior to birth, and appear to exhibit more rapid prenatal brain growth and development. Here we consider a pair of alternative hypotheses that might explain these results without proposing a causal role for placental structure.

First, placental type may covary with overall growth during gestation, so that the relationship between placentation and growth rate is not exclusive to the

brain but also maintains with other structures of the developing body. In order to evaluate this possibility we conducted an ad hoc test of the hypothesis that species with invasive placentation not only accomplish a greater proportion of brain growth, but also accomplish a greater proportion of total body growth (exclusive of the brain) than species with non-invasive placentation. However, the proportion of total body growth occurring prenatally did not differ significantly between invasive and non-invasive groups (N=106, F=0.912, p=0.342), and within the primates the same lack of association was found (N=33, F=1.124, p=0.297).

We also found that prenatal brain growth rate was significantly higher for species with invasive placentation that non-invasive placentation. Again, is it possible that invasive placentation increases overall growth rate and its effects are do not operate exclusively or predominantly on the brain? We tested this hypothesis by calculating prenatal body growth rates (exclusive of the brain) for the species in our dataset, and found that while the groups differ in prenatal brain growth rate, they do not differ in prenatal body growth rate (N=85, F=0.314, p=0.577). The difference in the proportion of brain mass grown prenatally does not appear to be the result of species with invasive placentation having longer gestation lengths, since size-corrected gestation length does not differ between the two groups of mammals (N=251, F=1.199, p=0.275).

These additional tests provide evidence that species with invasive placentation, as a result of exhibiting high prenatal brain growth rates, accomplish a larger proportion of their total brain growth during gestation than is

accomplished by species with non-invasive placentation, and that this interaction between placentation and prenatal growth appears to operate exclusively or predominantly on brain development, rather than on development of the body as a whole. As such it is consistent with the notion that variation in placental form may be specifically associated with the development of the brain.

A second alternative hypothesis is that, while species with invasive placentation exhibit more rapid prenatal brain growth, this difference reflects high levels of parental investment not restricted to pregnancy, hence not ultimately the result of placentation. To test this possibility we further tested for a difference in *postnatal* growth rates. The postnatal body growth rate of species with invasive placentation, calculated from the change in mass during lactation and the duration of lactation, was not significantly higher than that of species with invasive placentation, indeed it was significantly lower ($N=47$, $F=5.151$, $p=0.028$). This pattern may provide a partial explanation for the overall larger size of non-invasive species: the majority of body mass growth – irrespective of placental type – occurs postnatally; hence for a mammal of a given newborn mass, species with non-invasive placentation (hence higher postnatal growth rates) may tend to achieve a larger adult body mass.

A similar comparison of postnatal brain growth rates is difficult due to the lack of mammalian data on brain mass at weaning. In an attempt to circumvent this difficulty we assumed that, for female mammals, the age of sexual maturity is proportional to the age at which adult size is reached. We then calculated brain growth rate between birth and adulthood and compared this metric (corrected for

its covariance with adult body mass) across mammals grouped by placental type. The results were similar, the postnatal brain growth rate of species with non-invasive placentation being significantly higher than that of invasive species independent of body mass (N=88, F=7.746, p=0.007).

The alternative hypotheses presented in this section – that the effects of placentation are not unique to the brain, and that invasive placentation is a correlate of overall growth rate including postnatal as well as prenatal growth – are not supported by our dataset. These results indicate that variation in placental invasiveness is associated with variation in the quantity of maternal resources invested specifically in the brain, and specifically during pregnancy.

Table 2. Principal hypotheses, tests and results considered in Chapter 2.
The significance of results is noted as follows: ** = $p<0.05$; * = $p<0.1$.

Hypothesis	Test	Result
The slope of the brain-body allometry in species with invasive placentation differs from that in species with non-invasive placentation	Traditional statistics, all mammals, with bootstrapping	Invasive placentation slope > noninvasive placentation slope (**)
	Traditional statistics, Chiroptera	Invasive placentation slope > noninvasive placentation slope (**)
	Phylogenetically independent contrasts, Chiroptera	Invasive placentation slope > noninvasive placentation slope (**)
	Traditional statistics, Geomyidae and Heteromyidae (Rodentia)	Invasive placentation slope > noninvasive placentation slope
	Phylogenetically independent contrasts, Geomyidae and Heteromyidae (Rodentia)	Invasive placentation slope > noninvasive placentation slope (*)
	Traditional statistics, Lipotyphla	Invasive placentation slope > noninvasive placentation

		slope (**)
	Phylogenetically independent contrasts, Lipotyphla	Invasive placentation slope > noninvasive placentation slope (**)
	Traditional statistics, Primates	Invasive placentation slope > noninvasive placentation slope (**)
	Phylogenetically independent contrasts, Primates	Invasive placentation slope > noninvasive placentation slope
Species with invasive placentation accomplish more brain growth prenatally than species with noninvasive placentation	Traditional statistics, all mammals	Species with noninvasive placentation accomplish an average of 12.5% more brain growth prenatally (**)
	Traditional statistics, Primates	Species with noninvasive placentation accomplish an average of 20% more brain growth prenatally (**)
Species with invasive placentation exhibit higher prenatal brain growth rates than species with non-invasive placentation	Traditional statistics, all mammals	Species with invasive placentation exhibit higher prenatal brain growth rates (**) while prenatal body growth rates and gestation lengths do not differ.
Species with invasive placentation are more neurologically precocious at birth	Traditional statistics, all mammals	The eyes and ears of young with invasive open significantly earlier than the eyes and ears of young with noninvasive placentation (**)

Discussion

Physiological mechanisms

What placental physiological mechanism might be responsible for divergence in brain-body allometry and associated traits between species with invasive versus non-invasive placentation? One possible explanation is that the invasive placenta is able to transfer nutritional resources at a faster rate than the

non-invasive placenta, permitting an increase in parental investment during pregnancy. Comparative studies of prenatal nutrition do not indicate any consistent differences in the placental consumption of carbohydrates or proteins between species with invasive placentation versus non-invasive placentation. Placentally non-invasive species such as sheep exhibit a placental glucose utilization rate similar to that found in placentally invasive species such as human beings; and the amino acid composition of newborn carcasses is similar in the rat (with invasive placentation), the pig (with non-invasive placentation) and even the chick (with no viviparity) (Père 2003). As a result, we conclude that absolute levels of fetal energy consumption during gestation – which depend largely upon carbohydrate transfer from mother to fetus – may not be a major constraint on brain growth rate in normal pregnancies and may not be involved in generating the observed allometric patterns described above.

Studies of fatty acid nutrition during pregnancy, to the contrary, demonstrate a more pronounced distinction between species with invasive versus non-invasive placentation. In studies of pregnancy in species with invasive placentation – such as primates (Dancis et al. 1976; Haggarty et al. 1997; Hendrickse et al. 1985; Hull and Elphick 1978; Portman et al. 1969), rodents (Hershfield and Nemeth 1968; Honda et al. 1990; Hummel et al. 1975; Koren and Shafrir 1964; Thomas and Lowy 1982; Thomas and Lowy 1983; Thomas and Lowy 1984) and lagomorphs (Edson et al. 1975; Elphick et al. 1975; Elphick and Hull 1977a; Elphick and Hull 1977b; Gilbert et al. 1984; Stephenson et al. 1990) – maternal fatty acids are found to be readily and rapidly transferred

to the fetus. Under normal nutritional conditions, the rate of placental transport is responsive to changes in maternal serum lipid concentration, such that the levels of fatty acid in fetal and maternal tissues are correlated. Under fasting conditions, the rate of placental transport may increase and it shows signs of selectivity for essential and long-chain fatty acids over non-essential fatty acids. In all cases, fetal levels of serum fatty acid are considerably higher than maternal levels, and in those species whose neonatal body composition has been studied, lipids derived directly from the mother (as opposed to being synthesized by the fetus) constitute a majority of the total lipid content of the carcass.

In contrast, studies of pregnancy in species with non-invasive, (epitheliochorial) placentation – such as bovids (Elphick et al. 1979; Leat and Harrison 1980; Shand and Noble 1979) and swine (Elphick et al. 1980; Père 2001; Thulin et al. 1989) – or (also non-invasive) endotheliochorial placentation – such as felines (Elphick and Hull 1984) – find that the transfer of fatty acids from mother to fetus is minimal or non-existent. Placental uptake rate is not correlated with maternal serum fatty acid concentration, and there is no evidence of placental selectivity for essential or long-chain fatty acids. Fetal serum fatty acid level is as low as one percent of that of the mother, and it appears that only trace amounts of lipid derived directly from the mother (as opposed to being synthesized by the fetus) are present in the neonatal body.

In all species, maternally-derived carbohydrates form the main energy supply for the developing fetus, and their transfer rate does not appear to covary with placental type. Lipids, however, are of particular importance to the brain not

because they provide an energy supply but because they perform important structural roles. Lipids constitute around one half of the dry matter of the mammalian brain, the most structurally- and metabolically-important being long chain polyunsaturated derivatives of essential fatty acids, such as docosahexaenoic acid and arachidonic acid (Crawford et al. 1976).

Fatty acids required for brain development may be synthesized by the fetal liver or extracted from the mother via the fetally-derived placenta. However, the essential fatty acids, being of dietary origin, must follow the latter route (Père 2003). The mechanisms by which they do so fall into three broad classes:

- During pregnancy the majority of fatty acids circulate in the maternal serum as phospholipids, triglycerides or cholesterol ester, in each of which the fatty acid is attached to another molecule by an ester bond (Berghaus et al. 2000; Hoving et al. 1994; Otto et al. 1997). A minority of fatty acids circulate as free non-esterified molecules associated with a carrier protein, albumin, and a tiny fraction circulate in an unbound form (Benassayag et al. 1999; Patel et al. 1997). Esterified fatty acids are unable to cross directly from maternal serum to fetal tissue, but fetal lipoprotein receptors bind them to the placental surface where they are hydrolysed to yield non-esterified fatty acids (Haggarty 2004). These are transferred from mother to fetus by a process of diffusion driven by a concentration gradient in unbound albumin (Stephenson et al. 1993), which is up to 20% more concentrated on the fetal side of

the placental barrier than on the maternal side (Benassayag et al. 1999).

- The transfer of non-esterified fatty acids from mother to fetus is further promoted by the existence of fetal fatty acid binding/transport proteins borne by the maternal-facing membranes of the placenta (Dutta-Roy 2000).Such proteins are common in organs with high metabolic requirements, and are thought to facilitate the transmembrane and cytoplasmic transport of long-chain molecules (Van Nieuwenhoven et al. 1996). In humans, the placenta is unusual in bearing its own tissue-specific surface fatty acid binding protein that is not expressed elsewhere in the body. Unlike other forms, which will bind with any fatty acid, placental fatty acid binding protein binds selectively with essential fatty acids and their long-chain polyunsaturated derivatives, particularly docosahexaenoic acid, arachidonic acid and linoleic acid (Campbell and Dutta-Roy 1995; Campbell et al. 1996; Campbell et al. 1998; Haggarty et al. 1997). Consequently, its presence exclusively on the maternal face of the placenta serves to drive a selective enrichment of long-chain polyunsaturated fatty acids known to play a central role in brain development, and protect the supply of polyunsaturated fatty acids to the fetus during critical periods of development.

- Finally, two putative functions of placentally-derived leptin may contribute to the uptake and metabolism of fatty acids by the placenta. First, the hormone may play a role in mobilizing maternal lipid reserves, making them available for fetal use in late pregnancy (Hoggard et al. 2001). Second, it may increase the rate at which maternal esterified fatty acids are hydrolysed at the placental surface through the hormone's enhancement of placental nitric oxide production (White et al. 2006).

Despite the fact that these mechanisms have been elucidated from the study of only a handful of primates, rodents and lagomorphs (all of which have invasive placentation), it is of interest that all of them involve the binding of maternal serum fatty acids on the placental surface, and hence require direct contact between the maternal bloodstream and fetal epithelium. Such an arrangement is found only in species exhibiting the hemochorial (invasive) form of placentation. This notion is consistent with the placental perfusion and radioactive labelling experiments described above. These results suggest that divergence in mammalian brain-body allometry may in part be the result of differences in the ability of species of different placental type to effect prenatal fatty acid transport from maternal blood to developing fetal brain. Such differences may also account, in part, for the tendency of species with invasive placentation to be smaller than those with non-invasive placentation. Recent comparative research indicates that small mammals tend to exhibit higher long-chain polyunsaturation of cell membrane fats than large mammals (Hulbert and

Else 2005). If invasive placentation provides a better supply of such fatty acids during development then it may be favoured by natural selection not only in species of large brain size but also in species of low body size (see Chapter 3).

Concluding remarks

Previous studies have generally viewed mammalian brain-body allometry as a unitary relationship, with a single allometric slope, albeit differentiated by "grades", describing the correlation between brain and body size from mouse to elephant (Jerison 1973). Studies which seek correlations between life history or ecology and encephalization quotient (i.e., Armstrong 1983; Eisenberg and Wilson 1978; Iwaniuk et al. 2001; O'Shea and Reep 1990; Worthy and Hickie 1986) implicitly assume that a single unitary allometric slope is a valid description of mammalian brain-body relationships – for it is this single unitary allometric slope by which species are judged to be highly or poorly encephalized. Our analysis of prenatal effects on brain-body allometry in 471 mammalian species indicates that mammals, with respect to encephalization, are actually structured into two fundamentally distinct groups differentiated by the physiological source of fatty acids destined for early brain development. The notable difference in allometric exponent between species with invasive versus non-invasive placentation is consistent across both neonate and adult data, it is qualitatively similar across major taxonomic groups, and it is independent of differences between clades in absolute body size. Our findings draw into question the validity of generalized measures of relative brain size that are

supposedly applicable to all mammals, and they suggest that previous studies of encephalization that do not account for placental type may usefully be revisited.

Our analysis of brain growth rates suggests that the steeper allometric slope found in species with invasive placentation is the result of earlier and more rapid prenatal investment in encephalization. This interpretation is in agreement with mathematical models of allometry as a time-independent representation of two growth curves, which predict that retardation in the growth of an organ or structure should result in a reduction of the allometric exponent (Vincinius and Mirazon Lahr 2003). For this reason, the results of our analysis of growth rates and allometry are consistent with the hypothesis that evolutionary transitions in placentation are associated with heterochronic shifts in the growth schedule of the brain, and that invasive placentation is associated with earlier brain development.

How might such heterochronic shifts fit in with existing hypotheses of brain size evolution? There have historically been a number of coexisting hypotheses. First, a number of ecological and life history parameters have been found to be involved in the development of large brains, including longevity (Allman et al. 1993a; Allman et al. 1993b; Hakeem et al. 1996; Sacher 1975), diet (Fish and Lockwood 2003) and metabolic expenditure (Aiello 1997; Aiello and Wheeler 1995; Leonard and Robertson 1994). These views have often been seen as alternatives to – or competitors with – the social brain hypothesis, which argues for a causal connection between the level of social complexity and the brain size characteristic of a species (Brothers 1990; Byrne and Whiten 1988; Dunbar

2003a; Dunbar 2003b; Humphrey 1976). However, recent life history theoretical treatments rooted in primatology incorporate these diverse views into a single model of simultaneous selection acting on brain size and longevity, in which the cost of initial brain growth and development during infancy, and the maintenance of neural tissue over the lifespan of the animal, are balanced against the future social and ecological benefits accruing from the possession of a large brain later in life (Kaplan et al. 2003a; Kaplan et al. 2003b; Kaplan and Robson 2002). In this view, improvements in diet quality and reduced mortality rates result in selection for increased longevity, which in turn favours greater investment in the storage and processing of information – especially at early life stages and during an extended childhood learning phase, where expenditure is balanced against future adult reproductive success.

Fundamental to such theories of primate brain size evolution is the notion that energetic expenditure on brain development can be shifted to an earlier time in the life history of the individual, and that the duration of the brain's growth phase can be increased by extension of gestation and infancy (Kaplan and Robson 2002; Walker et al. 2006). We argue that the physiological mechanisms of resource acquisition by the brain during its early growth are hence important but previously unexplored components of mammalian brain size evolution. The results presented here support our more general view that evolutionary transitions in placental structure may be one of the mechanisms by which modifications to the timing and rate of eutherian brain growth evolve, just as the evolution of extended placentation was initially involved in the diversification of

mammalian reproduction into its eutherian and Metatherian modes, the former being associated with large brains and the latter with small ones. To the extent that maternal-fetal conflict was involved in the origin of invasive placentation, and the subsequent evolution of placental form and function as eutherian mammals diversified (Crespi and Semeniuk 2004; Haig 1993), it may play an important role in the macroevolution of mammalian allometry and life history.

CHAPTER 3: PRENATAL EFFECTS ON BRAIN SIZE EVOLUTION: LIFE HISTORY, ECOLOGY AND PARENT-OFFSPRING CONFLICT

Introduction

The evolution of mammalian brain size has generally been explained in terms of a trade-off between the cognitive benefits of large complex brains and the high energetic costs of growing and sustaining them. The cognitive benefits of large brains are implied by the existence of positive correlations between brain size and social complexity in various mammalian taxa (Barton 1996; Dunbar 1992; Gittleman 1986; MacLaren et al. 1992; Sawaguchi and Kudo 1990; Shultz and Dunbar 2006); between brain size and intensity of mammalian mating competition (Jones and MacLarnon 2004); between brain size and problem-solving ability in primates (Deaner et al. 2007); and by the identification of an apparent survival advantage in novel environments enjoyed by large-brained birds (Sol et al. 2005). However, the energetic costs of brain growth and maintenance are high (Laughlin et al. 1998), such that the ability of a mammal to sustain those costs from dietary sources may limit attainable brain size (Fish and Lockwood 2003; Mann et al. 1988) unless alternative expensive tissues can be sacrificed in the brain's favour (Aiello 1997; Aiello and Wheeler 1995). Of central evolutionary concern, then, is whether the growth and development of a large brain will yield sufficient "returns" over the course of an animal's life history to offset the high initial energetic investment.

As an investment, neural tissue is unusual in that its value appreciates over time. Possession of a complex brain with the capacity for learning and negotiating complex social interactions or physical environments is of limited value at birth, when innate behaviours such as suckling and seeking protection are likely of greater significance to survival. However the value of neural capital increases throughout the life span of an individual as new knowledge, skills and information are acquired (Kaplan et al. 2003a). This phenomenon is expected to yield synergistic evolutionary pressures relating brain size, longevity and parental investment. First, high levels of encephalization provide a context in which longevity is favoured by natural selection, since the overall return on neural investment accrues over time; conversely, since knowledge, skills and the ability to learn may promote the survival of an animal over long durations, natural selection is expected to favour large brains in long-lived animals (Kaplan et al. 2003a; Kaplan et al. 2003b; Kaplan and Robson 2002). Second, the relatively high reproductive value of adult animals relative to young ones under conditions of high encephalization will, under economic models, encourage the production of smaller litter sizes and an increase in per capita investment in offspring (Chesnais 1992); and at the same time, these higher levels of investment in offspring during the prenatal period and infancy provide a context in which longevity and high levels of encephalization are both facilitated and selected for. Such synergistic processes are thought to have played a role in the evolution of the large brains, intergenerational transfers of skills and knowledge during

extended childhood, and longevity in hominids and other primates (Kaplan et al. 2003a; Kaplan et al. 2003b; Kaplan and Robson 2002; Walker et al. 2006).

To the extent that evolutionary transitions toward larger brain sizes are dependent upon the existence of mechanisms for early maternal investment in the brain during pregnancy, the mammalian placenta is expected to be an important physiological mechanism upon which patterns of brain size evolution with the mammals depend. The placenta, an organ of fetal genotype which controls and regulates resource transfer from mother to offspring during pregnancy, and which varies markedly in structure and function between eutherian taxa, has been identified as an arena in which genetic conflicts over the rate and magnitude of resource allocation manifest themselves (Crespi and Semeniuk 2004; Haig 1993). Specifically, invasive forms of placentation may be associated with enhanced fetal mobilization of maternal resources, and the fetal manipulation of maternal energy budgets by secretion of hormones and other substances into her bloodstream, resulting in improved resource acquisition by the fetus during pregnancy (Haig 1993; Petry et al. 2007). Maximum likelihood reconstructions of placental characters indicate that the ancestral eutherian placental condition was of an invasive form, and the occurrence of non-invasive placentation in extant taxa is the result of 9 to 11 independent evolutionary transitions occurring within eight mammalian orders (Elliot and Crespi 2006; Wildman et al. 2006).

Adult cognitive abilities depend in part upon processes of brain development during gestation. Mammals *in utero* are highly sensitive to a wide

variety of stressors, which can result in alterations to brain morphology and behaviour (Weinstock 2001) including deficits in social interaction, brain symmetry, anxiety, circadian rhythm and sleeping. Of special relevance to the study of placentation are the effects on brain development of prenatal nutritional status (in part determined by the extractive properties of the placenta; (Père 2003)) and gestation length (in part determined by the timing of placental hormone secretions; (Jenkin and Young 2004)). In human and animal models, pregnancies compromised in length and nutritional status are associated with reductions in regional brain volumes (Peterson 2003), cognitive impairments such as reduced learning or memory (Briscoe et al. 2001; Erhard and Boissy 2004; Gomez-Pinilla and Vaynman 2005; Isaacs et al. 2001; Landon et al. 2007; Lefebvre et al. 1988; Morgane et al. 1993; Peters 1979), and mental illness (Dauncey and Bicknell 1999).

Evolution of life history is one possible mechanism by which mammals may accommodate the nutritional properties of different types of placenta; similarly, the demands of life history and ecology (such as the production of a large number of offspring or the production of offspring with large brains) may selectively promote placental evolution. The importance of brain development to mammalian fitness, and the predictions of the evolutionary economics model of brain size and life span, lead us to predict coevolution between aspects of life history, placentation and brain size.

In this chapter I continue my analysis of differences in comparative brain growth resulting from variation in the prenatal interactions of mother and fetus, by

focusing upon the relationship between placental invasiveness, brain size and the slow-fast life history continuum (Promislow and Harvey 1990). The slow-fast continuum - describing variation in life history ranging from a slow syndrome characterized by slow metabolism, long lifespan and large size, to a fast syndrome characterized by the opposite – incorporates the major axes of life history variation in mammals and other taxa (Bennett and Owens 2002; Bielby et al. 2007; Promislow and Harvey 1990; Sibly and Brown 2007). In particular, I focus upon the theoretical expectation of a positive correlation between slow life histories and large brain sizes. We expect that mammals are better able to reap the cognitive rewards of slow life histories when they are equipped with a mechanism for the rapid prenatal transfer of large quantities of nutritive resources. Hence we predict both that invasive placentation will be selectively favoured in species with extensive brain growth and longevity, and that correlations between life history slowness and encephalization will be stronger in species with invasive placentation than in species with non-invasive placentation.

In addition, we consider the role of parent-offspring conflict in the evolution of patterns of mammalian brain growth. Under invasive placentation, control of the rate and quantity of fatty acid and other nutrient transfer is likely exercised to a great extent by the fetus, which can not only extract materials directly from the blood stream of the mother, but also secrete hormones directly back into the her circulatory system that act to mobilize maternal resources in favour of the fetus (Haig 1993; Petry et al. 2007). Under non-invasive placentation, on the other hand, control is likely more in the hands of the mother, since the fetus lacks the

capacity to directly modify the physiology of maternal-fetal nutritional systems. Postnatal maternal investment in the form of milk composition and provision will be largely under the control of the mother regardless of placental type. The optimal level of prenatal investment likely differs between mothers and their offspring (Trivers 1974). We expect that species with non-invasive placentation will exhibit an amelioration of parent-offspring conflict due to the greater control of a single actor (the mother) throughout both prenatal and postnatal phases of growth. This amelioration is expected to be evidenced by consistency between prenatal and postnatal growth rates; species with invasive placentation, to the contrary, should be characterized by relatively high prenatal growth rates and relatively low postnatal growth rates, since parturition should result in a partial transfer of control (from fetus to mother) over the rate of resource exchange.

Materials and methods

Data on the life history and placentation of placental mammals were obtained from the study of brain-body allometry presented in Chapter 2 (Table 1, Appendix 1). A practical difficulty in analysing these data is the existence of strong covariance among all of the variables. Typically, life history variables are analysed after being corrected for their correlation with body mass. In order to avoid making an *a priori* judgement of how best to "correct" brain mass in these analyses we first used structural equation modelling to establish the relationships between the nine Ln-transformed life history variables of our dataset, then used a stepwise selection protocol to fit brain mass into the model (SPSS 2006). Structural equation modelling can be considered a form of multiple regression

which explicitly models associations between traits by considering each variable to be measured by more than one empirical indicator, and which offers more flexible assumptions regarding collinearity (Bentler and Stein 1992). The nine variables (body mass at birth, adulthood and weaning; duration of gestation and weaning, age at first reproduction, and lifespan; litter size and annual number of offspring per year) were taken to be the manifest, or measurable variables in the model. In addition, we consider three latent, or unobservable, variables of wide currency in life history theory, namely size, the position of a species' life history along the slow-fast continuum (slowness), and reproductive output, each of which is indicated by a number of the manifest variables. Previous empirical and theoretical work (Roff 1992; Stearns 1992) leads us to expect co-occurrence of large size, slow life history and low fecundity, a view which was modelled using S.E.M. and contrasted with the more naive model in which each life history variable is simply correlated with log body mass independently.

Our hypothesis predicts that bivariate correlations between life history variables in mammals differ depending upon the form of placentation exhibited by each species. Specifically, we predict that the correlation between brain size and variables related to the slow-fast life history continuum (gestation length, weaning period, age at first reproduction, and lifespan) will be more pronounced in mammals with invasive placentation than in mammals with non-invasive placentation. Similarly, we expect that the apparent effects of placental invasiveness on the prenatal nutritional status of young will be manifest as

differences in slope and/or intercept of correlations between brain size and variables representing offspring quantity and growth rate.

In order to explore these bivariate correlations, the life history variables were analysed using traditional regression analysis (SPSS 2006) and phylogenetically independent contrasts (Felsenstein 1985; Maddison and Maddison 2005). In the traditional analysis, we corrected for the covariance of life history variables with body mass by considering the residuals of the regression of each variable against log maternal body mass. In order to compare the relationship between life history variables under independent contrasts, the ancestral placental state under maximum likelihood was inferred for each node of a recent species-level mammalian phylogeny (Bininda-Edmonds and Cardillo 2007), and independent contrasts generated for each log-transformed life history variable were grouped by the placental type inferred for that node of the tree. These sets of independent contrasts were then analysed using regression forced through the origin. Where necessary, contrasts in body mass were partialed out of these analyses or residuals were analysed in order to correct for the covariance of contrasts in life history variables with contrasts in body mass.

Finally, the relationships between prenatal and postnatal growth rates of brain and body were examined. Again, the residuals of the regression of log growth rates against log maternal body mass were used, in order to remove the confounding effects of body size variation. Average prenatal growth rates are simply the ratio of weight of brain/body at birth to gestation length, but average postnatal growth rates are more difficult to estimate since data on brain and body

size at weaning are available for few species. As an alternative, we use the ratio of adult brain or body mass minus neonatal brain or body mass, to female age at first reproduction; the latter is considered a proxy for the approximate age at which adult size is attained.

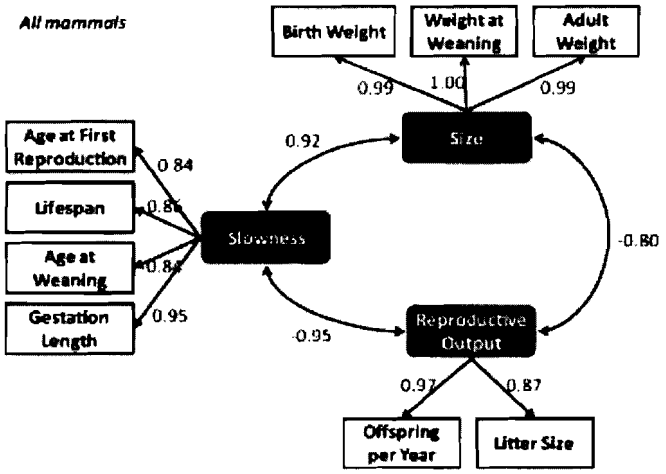
Results

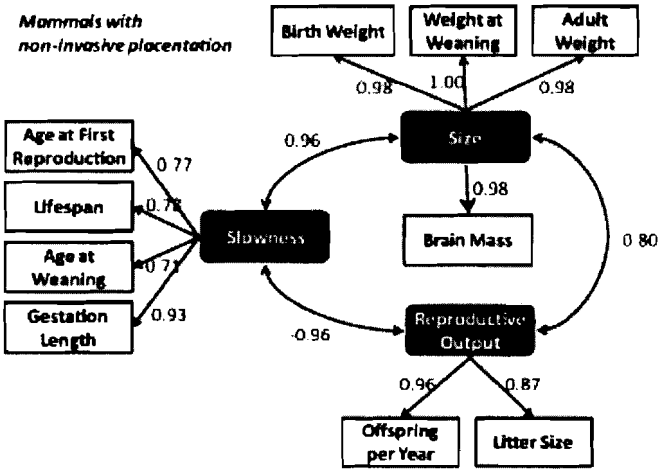
Structural equation modelling of mammalian life history

The structural equation model of mammalian life history based on theoretical considerations of the trade-off between growth, fecundity and the lifespan, illustrated in Figure 4 (top), was found to fit the data significantly better than a theoretically-naïve null model in which each life history variable is simply correlated with body mass in the absence of any latent variables (Akaike Information Criterion = 945.8 versus 2989.0, Comparative Fit Index = 0.926 versus 0.749; N=1438). Regression weights on each path of the diagram are consistent with theoretical expectations that larger mammalian species have slower life histories and lower fecundity.

Log-transformed adult brain mass was fit into the life history model under a stepwise selection protocol in which weak correlations between brain size and each of the three latent variables were excluded from the analysis until a significant reduction in fit was observed. The best fitting models for species grouped as placentally invasive or non-invasive are illustrated in Figure 4 (middle and bottom). The principal difference between the two models is that slow life histories are positively correlated with adult brain mass, independent of the

confounding effects of body mass, only in the group of species with invasive placentation. These results are consistent with the hypothesis that evolutionary extension of the lifespan and the adoption of a slow life history may evolve so as to increase the evolutionary payoff accruing to mothers following costly invasive placentation and relatively high levels of early parental investment, and accruing to offspring following the devotion of resources to brain growth rather than reproduction.


All mammals
Birth Weight
Weight at Weaning
Adult Weight
0.99
1.00
0.99
Age at First Reproduction
Lifespan
Age at Weaning
Gestation Length
0.84
0.86
0.88
0.95
Slowness
0.92
Size
-0.80
-0.95
Reproductive Output
0.97
0.87
Offspring per Year
Litter Size


Mammals with non-invasive placentation
Birth Weight
Weight at Weaning
Adult Weight
0.98
1.00
0.98
Age at First Reproduction
Lifespan
Age at Weaning
Gestation Length
0.77
0.78
0.71
0.93
Slowness
0.96
Size
0.98
Brain Mass
0.80
-0.96
Reproductive Output
0.96
0.87
Offspring per Year
Litter Size

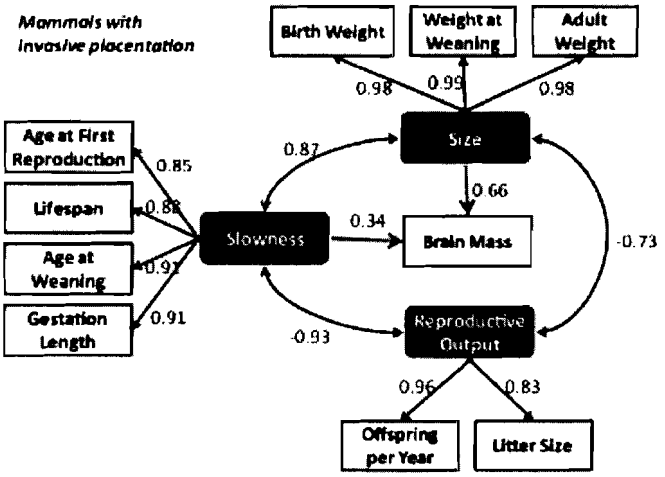

Mammals with invasive placentation
Birth Weight
Weight at Weaning
Adult Weight
0.98
0.99
0.98
Age at First Reproduction
Lifespan
Age at Weaning
Gestation Length
0.85
0.88
0.91
0.91
Slowness
0.87
Size
0.66
0.34
Brain Mass
-0.73
-0.93
Reproductive Output
0.96
0.83
Offspring per Year
Litter Size

Figure 4. Structural equation models of mammalian life history and brain size. Top: all mammals; middle: mammals with non-invasive placentation; bottom: mammals with invasive placentation. Latent variables are black, manifest variables are white. Single headed arrows represent hypothesized causal relationships while double-headed arrows represent correlation. The "slowness" of a mammal's life history appears to interact with brain size only amongst species with invasive placentation.

The slow-fast life hisory continuum

Our analysis of bivariate correlations between relative brain size (as measured by the residual of the regression of log adult brain size against log adult body size) and a number of variables reflecting the position of each species along the slow-fast life history continuum, found differences in regression slope related to placentation consistent with those illustrated by the SEM model presented above. Slow life histories, as measured by residual gestation length, residual weaning period and residual age at first female reproduction, are statistically more strongly (positively) correlated with residual brain mass in species with invasive placentation than in species with non-invasive placentation (gestation length: B = 0.489 in species with invasive placentation versus 0.318 in species with non-invasive placentation, $F=16.973$, $N=239$, $p<0.001$; weaning period: B = 0.562 versus 0.378, $F=56.840$, $N=188$, $p<0.001$; age at first reproduction: B = 0.501 versus 0.354, $F=30.234$, $N=211$, $p<0.001$; ANCOVA). Relative lifespan was found to be positively correlated with relative brain size amongst species with invasive placentation, but this association was marginally non-significant in species with non-invasive placentation (for species with

invasive placentation B = 0.603, R^2=0.498, N=75, p<0.001, F=34.683; for species with non-invasive placentation B=0.215, R^2=0.215, N=121, p=0.079, F=3.132).

These tests were repeated using phylogenetically independent contrasts based on a recent species-level phylogeny of the mammals (see Materials and Methods). No significant correlation was found to exist between residual contrasts in brain size and residual contrasts in gestation length, weaning age or age at first reproduction, and when categorized by placental invasiveness the two groups of mammals did not differ significantly in this respect (p=0.563, 0.435, and 0.664 respectively). Residual contrasts in lifespan, on the other hand, were significantly positively correlated with their corresponding residual contrasts in brain mass in the group of species with invasive placentation (B=0.237, p=0.017, N=67) but not in the group of species with non-invasive placentation (B=0.013, p=0.107, N=96), and the interaction between placenta and lifespan was a significant component of the regression model (p=0.002, F=6.545). Similar results were obtained from analysis of body and brain weight at birth. The previous section identified an apparent restriction of the association between slow life histories and large brains to those species with invasive placentation. This pattern is also recovered using traditional linear regression, as well as independent contrasts, demonstrating that it is not an incidental by-product of the phylogenetic structure of the mammalian dataset or statistical methods used. Together, these results are consistent with the hypothesis that lifespan and brain size may be linked through an economic mechanism that involves evolution of the placenta as a mediator of early parental investment.

Offspring quantity and quality

Since neural tissue is metabolically expensive to grow and maintain, it is expected to be involved in mediating the mammalian life history trade-off over "offspring quality" versus "offspring quality" (Bell and Koufopanou 1986; Roff 1992; Stearns 1992). Figure 5b indicates that, among the group of species with invasive placentation, those of large size tend to have larger brains than their non-invasive counterparts, but those of small size tend to have smaller brains than their non-invasive counterparts. However, Figure 5a also indicates that small placentally-invasive species give birth to significantly more offspring per year than placentally-non-invasive species of similar size, while large placentally-invasive species give birth to significantly fewer offspring. Hence the trade-off between offspring quality and quantity appears to be more pronounced in taxa with invasive placentation, such that these species are found at both extremes of the mammalian spectrum, exhibiting both the largest litters and largest brains found in mammals.

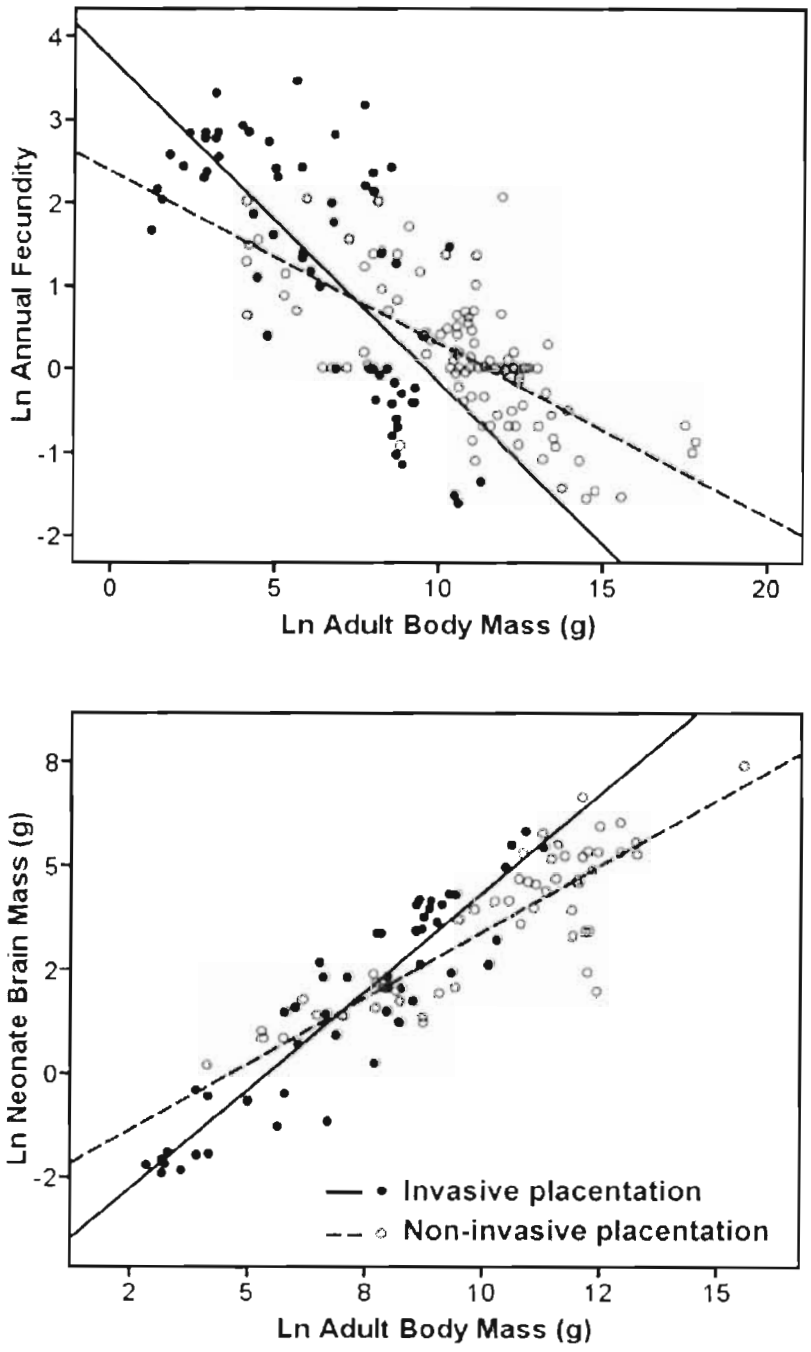


Figure 5. Fecundity and brain size in mammals exhibiting invasive versus non-invasive placentation. Differences in slopes of regression lines between invasive and non-invasive groups are statistically significant (log annual fecundity: $p=0.001$; log neonate brain mass: $p<0.001$). Among mammals of small size, species with invasive placentation exhibit larger litters but smaller neonatal brains than species with non-invasive placentation; among mammals of large size, species with invasive placentation exhibit smaller litters but larger neonatal brains than species with non-invasive placentation.

Growth rates before and after birth

The timing of life history events such as gestation and lifespan cannot be considered in isolation from the rate of growth. The evolution of short gestation lengths or fast life histories, for example, may be "compensated" by increased growth rates during gestation, mitigating any consequences of adult brain size evolution. The relationship between growth rates before and after birth is of special interest, since this differential will likely determine the effects of evolutionary changes to the nutritive qualities of placentation, which naturally have no direct effect on growth after birth.

In order to explore these issues we first compared the average prenatal and postnatal brain and body growth rates in the two groups of mammals. In order to correct for the covariance of growth rates with maternal body mass, we used the residual of each growth rate against maternal body mass. Prenatal brain growth rates may be higher in the mammals with invasive placentation (mean residual brain growth rate = 0.16) than in mammals with non-invasive placentation (mean residual brain growth rate = -0.14), a difference that is marginally non-significant ($p=0.074$, $F=3.285$, $N=75$). On the other hand, mean prenatal body growth rate (exclusive of the brain) show no indication of a difference between the two groups (mean residual body growth rate = -1.35 (invasive) versus 1.5 (non-invasive), $p=0.579$, $F=0.311$; $N=75$).

In contrast to prenatal brain growth rates, postnatal brain growth rates were found to be significantly lower in species with invasive placentation (mean

residual brain growth rate = -0.22) than in species with non-invasive placentation (mean residual brain growth rate = 0.20), a statistically significant difference ($p=0.003$, $F=9.058$, $N = 86$). The same pattern was found for postnatal body growth rate (mean residual body growth rate = -0.20 (invasive) versus 0.18 (non-invasive), $p=0.031$, $F=4.789$; $N=86$).

Bivariate correlations between prenatal and postnatal brain and body growth rates (with the effect of maternal body mass partialed out of the data) are described in Table 3. Within both placental groups, species with rapid body growth also experience rapid brain growth (residual brain and body growth rates are positively correlated before and after birth). For mammals with non-invasive placentation only, species with high prenatal body growth rate also tend to have high postnatal body growth rate. Interestingly, there was no such correlation in species with invasive placentation; furthermore, the postnatal brain and postnatal body growth rates of species with invasive placentation are significantly *negatively* correlated with prenatal brain growth rate. Broadly similar results arise from analysis of independent contrasts (N=76 contrasts; Table 4). Positive correlations between residual prenatal/postnatal brain and body growth rates are found only in species with non-invasive placentation, suggesting a relative disconnection between control of growth rates before versus after birth in species with invasive placentation. Consistent with the parent-offspring conflict hypothesis, the correlation between prenatal brain growth rate and postnatal body growth rate is positive in species with non-invasive placentation but negative in species with invasive placentation.

Table 3. Pearson's R of the bivariate correlations between residual growth rates of prenatal brain, prenatal body, postnatal brain and postnatal body, split by placental type. Correlations significant at alpha = 0.1 are labelled with a single asterisk, while correlations significant at alpha = 0.05 are labelled with a double asterisk. Residuals arise from the regression of each log transformed growth rate (in average grams per day) against log adult body mass. For both placental groups, residual brain and body growth rates are positively correlated before and after birth, and for species with non-invasive placentation, prenatal and postnatal residual body growth rates are also positively correlated. Interestingly – and suggestive of parent-offspring conflict – the postnatal brain and body growth rates of species with invasive placentation are significantly *negatively* correlated with prenatal brain growth rates.

Residual Growth Rate Correlation	Invasive Placentation	Non-invasive Placentation
Prenatal Brain x Prenatal Body	0.338*	0.816**
Postnatal Brain x Postnatal Body	0.797**	0.644**
Prenatal Brain x Postnatal Brain	-0.422**	0.040
Prenatal Body x Postnatal Body	-0.013	0.333*
Prenatal Body x Postnatal Brain	0.007	0.178
Prenatal Brain x Postnatal Body	-0.689**	-0.031

Table 4. Pearson's R of the bivariate correlations between independent contrasts in residual growth rates of prenatal brain, prenatal body, postnatal brain and postnatal body, split by placental type. Correlations significant at alpha = 0.1 are labelled with a single asterisk, while correlations significant at alpha = 0.05 are labelled with a double asterisk. Positive correlations between residual prenatal/postnatal brain and body growth rates are found only in species with non-invasive placentation, suggesting a relative disjunction between control of growth rates at different times in species with invasive placentation. Consistent with parent-offspring conflict hypothesis, the correlation between prenatal brain growth rate and postnatal body growth rate is positive in species with non-invasive placentation but negative in species with invasive placentation.

Residual Growth Rate Correlation	Invasive Placentation	Non-invasive Placentation
Prenatal Brain x Prenatal Body	-0.339*	0.290
Postnatal Brain x Postnatal Body	0.600**	0.681**
Prenatal Brain x Postnatal Brain	-0.190	0.511**
Prenatal Body x Postnatal Body	-0.013	0.497**
Prenatal Body x Postnatal Brain	0.117	0.361
Prenatal Brain x Postnatal Body	-0.336**	0.541**

Discussion

Our analysis of the relationship between brain size and the slowness of mammalian life history support our view that evolutionary increases in brain size may be accomplished by an extension of the lifespan and the adoption of a slow life history, but only in the context of physiological mechanisms such as invasive placentation which permit high levels of early parental investment in the brain growth of offspring. In the absence of such a mechanism, as under non-invasive placentation, high levels of investment in early brain development may be

hampered, reducing the fitness benefits that might otherwise result from the adoption of slower life histories. If longevity is a means by which mammals increase the fitness benefits accruing from cognitive development and offset the costs of early investment in the development of a brain, then selection for longevity will likely be reduced in strength among species with non-invasive placentation, which make a relatively small initial investment in early brain development. Hence we find that a correlation between brain size and longevity is evident only in those species with invasive placentation making large early investments in neural capital.

As the ancestral state of the common ancestor of rodents, lagomorphs and primates, invasive placentation is also found in numerous species with fast life histories. In our view, animals exhibiting invasive placentation but with a fast life history are unlikely to devote high levels of prenatal maternal investment to brain development, because a rapid life history does not provide sufficient time for a sufficient return on the resources invested in early brain growth. Nevertheless, invasive placentas are still expected to make a relative excess of resources – especially the energetically-expensive fatty acids – available to young during pregnancy, in comparison with non-invasive placentas (Père 2003). An interesting characteristic of the data presented in Figure 2 is that while the brain mass/body mass and fecundity/body mass regression slopes differ between species grouped as placentally invasive or non-invasive, the intercepts of those slopes also differ, such that small species with invasive placentation exhibit relatively small brains and large litter sizes, while large species with

invasive placentation exhibit relatively large brains and small litter sizes. We suggest that these life-history extremes reflect alternative strategies for the prenatal investment of resources provided by invasive placentation: investment in "quality", or large brains, in the context of large body size and slow life history, or "quantity", or large litters, in the context of small body size and fast life histories.

The relationship between fecundity and the slow-fast life history continuum has previously been identified as an inverse correlation between litter size and gestation length (Read and Harvey 1989). One possible explanation for these patterns is that competition within the litter results in a race between littermates to acquire maternal resources (Haldane 1932). Competition among littermates to extract the greatest quantity of maternal resources is likely to conflict with the mother's interest in maximizing litter size while preventing the energetic costs of doing so from inflating to unsustainable levels (Trivers 1974). Our analysis of prenatal and postnatal growth rates provides a possible means of testing for evidence of such parent-offspring conflict. Under invasive placentation, control of the rate and quantity of fatty acid and other nutrient transfer is likely exercised to a great extent by the fetus, which can not only extract materials directly from the blood stream of the mother, but also secrete hormones directly back into the her circulatory system that act to mobilize maternal resources in favour of the fetus (Haig 1993; Petry et al. 2007). Under non-invasive placentation, on the other hand, control is likely more in the hands of the mother, since the fetus lacks the capacity to directly modify maternal blood chemistry. Postnatal maternal investment in the form of milk composition and provision, on the other hand, will

be largely under the control of the mother irrespective of placental type. Conflict over the allocation of resources under invasive placentation will thus be evidenced by high prenatal and perhaps low postnatal growth rates compared to the rates found in species with non-invasive placentation.

The results presented in Table 3 are broadly consistent with the hypothesis of parent-offspring conflict over allocation of resources. Thus, among species with non-invasive placentation, those with high prenatal body growth rates also tend to have high postnatal body growth rates, which is expected based on our view that mother exerts control over both prenatal and postnatal nutrient transfer in these animals. However, this correlation is absent in species with invasive placentation, indicative of the independence of prenatal and postnatal growth patterns consistent with a transfer of control from fetus to mother at birth. Furthermore, the postnatal brain and body growth rates of species with invasive placentation are significantly *negatively* correlated with prenatal brain growth rate. In other words, postnatal growth rates controlled largely by the mother are balanced against prenatal resource extraction controlled largely by the fetus. In comparison with fetuses with non-invasive placentation, or with invasive placentation but moderate rates of resource extraction, fetuses using invasive placentation to extract resources at rates higher than the maternal optimum may tend therefore to suffer reduced maternal provision after birth.

The apparent existence of parent-offspring conflict over the allocation of resources from mother to offspring before birth may help to explain one lingering

problem: if invasive placentation provides benefits for its bearers under conditions of both slow and fast life histories, then why do many taxa exhibit non-invasive placentation, and why has non-invasive placentation arisen independently within a number of mammalian clades? One possibility raised by the results presented above is that non-invasive placentation is a means whereby fetuses and mothers have evolved to avoid conflict over resource transfer that is costly to one or both of the parties. Avoidance of parent-offspring conflict may be beneficial in species that produce single offspring rather than litters, in species with high levels of intra-litter relatedness, or in species in which mothers cannot sustain high rates of nutrient transfer during pregnancy, or cannot sustain pregnancies over a long period of time. An ad hoc analysis of existing databases of mammalian mating systems and litter sizes do not support the hypothesis that singleton births or monogamous mating systems (which increase intra- and inter-litter relatedness and are hypothesized to reduce parent-offspring conflict) are concentrated in clades with non-invasive placentation. An alternative possibility which remains to be considered in detail is the role of diet. Data on diet composition in primates, rodents and Lipotyphlans suggests that the origin of non-invasive placentation may be associated with the adoption of a nutritionally impoverished or seasonally-restricted diet. Haplorhine primates (with invasive placentation) tend to feed on a variety of vegetable and animal matter including flowers, fruit, leaves, insects and vertebrates, while strepsirhine primates (with non-invasive placentation) tend to be more specialized predators, especially upon tree gums, nectar, arthropod prey, and/or a seasonally-restricted

range of fruit (Andriant and Rahandra 1973; Atsalis 1999; Corbin and Schmid 1995; Donati and Borgognin-Tarli 2006; Fietz and Ganzhorn 1999; Fietz and Tataruch 2003; Genin 2003; Ossi and Kamilar 2006; Radespiel and Reimann 2006; Schülke 2003; Tarnaud 2006a; Tarnaud 2006b; Tecot and Overdorff 2006; Ullrey et al. 2003; Vasey 2004). In rodents, the only taxon exhibiting non-invasive placentation is Heteromyidae (kangaroo rats, kangaroo mice and pocket mice). These species inhabit unpredictable environments characterized by extremes in temperature and food availability (Murray et al. 2006). In most genera, such as Dipodomys or Perognathus, the grains of a small number of grass species comprise up to 90% of nutritional intake by volume (Alcoze and Zimmerman 1971; Bradley and Mauer 1971; French 1974; Lemen and Freeman 1986; Messerve 1976; Reichman 1975; Soholt 1973). In Microdipodops, insect prey constitute a similar proportion of the diet (Harris 1986; Lemen and Freeman 1986). Such dietary specialization is extreme in comparison to the more generalist habits of the sister taxa of Heteromyidae, the Geomyidae (pocket gophers) whose extremely varied diet incorporates the leaves, shoots, roots and tubers of a wide range of forbs, grasses and shrubs (Miller 1964; Williams and Cameron 1986). Similarly, within Lipotyphla, the only clade to have evolved non-invasive placentation is Talpidae (the moles and desmans). Over 75% of animals predated upon by moles of the genus *Talpa* are earthworms (Beolchini and Loy 2003), in contrast with a highly varied, generalist diet in shrews (Dickman 1995; Punzo 2003; Ritzi et al. 2005; Whitaker 2006) and hedgehogs (Jones et al. 2005; Nowak 1999).

These findings are consistent with our view that non-invasive placentation may have been selected for in part because of its dampening effects on parent-offspring conflict under nutritionally-straitened conditions. In order to test these hypotheses fully it will be necessary to obtain information on the diet of pregnant and non-pregnant females in a variety of taxonomic groups. Other nutritional matters, such as the effect of capital breeding versus income breeding the quality of fetal brain fatty acid composition, are also attractive lines of future research.

Conclusions

The life histories of mammals are characterized by two fundamental trade-offs: first, the investment of resources in growth and maintenance versus reproduction, and second, the investment of resources in offspring quality versus offspring quantity (Bell and Koufopanou 1986; Roff 1992; Stearns 1992). The analyses and discussion presented here suggest that placental structure plays an important role in determining the outcome of such trade-offs (Figure 6), and show that analysis of mammalian brain evolution cannot proceed in the absence of hypotheses regarding the physiological underpinnings of resource distribution between different components of life history. One important implication of this

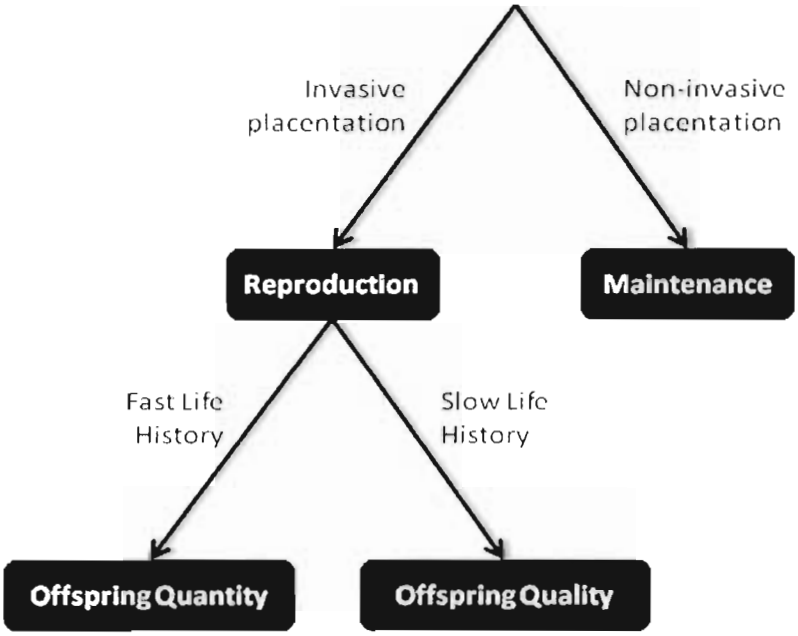


Figure 6. Hypothesized relationships between placental invasiveness and the slow-fast life history continuum, in connection with life history tradeoffs over reproduction/maintenance and offspring quality/quantity.

work is that studies searching for universal life-history and allometric patterns or trends within Eutheria need to consider the existence of two fundamentally different groups of eutherian mammals which differ in their modes of maternal-offspring interaction. Data on the ontogeny of brain size throughout the lifespan of species with different forms of placentation, perhaps made available through technologies such as magnetic resonance imaging of living individuals and museum specimens of known age (Marino and Murphy 2001), would be of great value in exploring this idea further. In addition, our work yields predictions that the processes of maternal fat deposition prior to pregnancy, diet during

pregnancy, and milk composition after birth, will differ significantly between species with invasive versus non-invasive placentation, consistent with the parent-offspring conflict hypothesis. Species with invasive placentation should, for example, exhibit higher levels of pre-maternal fat deposition and diets of higher energetic quality, but less nutritive lactation behaviour, than species with non-invasive placentation. Our results also suggest that the acquisition and analysis of cross-species physiological data on prenatal and postnatal fat usage would be an invaluable means of better understanding the physiological basis of life history evolution in mammals. More generally, our findings indicate that parent-offspring conflict, and its physiological underpinnings in placental development, exert profound effects on the evolution of mammalian life histories, which should be accounted for in future studies of how mammalian life history, physiology, development and ecology coevolve together.

CHAPTER 4: PLACENTAL INVASIVENESS MEDIATES THE EVOLUTION OF HYBRID INVIABILITY IN MAMMALS

Introduction

Speciation is a consequence of the evolution of barriers to gene flow between diverging populations. Studies in vertebrates indicate that barriers resulting from behavioural, ecological, life-history, and geographical divergence are common in disparate groups, including mammals, reptiles, birds and amphibians. Despite the commonality of mechanisms generating speciation within vertebrates, components of reproductive isolation such as hybrid inviability evolve at markedly different rates in each vertebrate group. For example, amphibians have been shown to evolve hybrid inviability ten times more slowly than eutherian mammals (Wilson et al. 1974), and birds have been shown to do so around three times more slowly (Fitzpatrick 2004). Furthermore, different taxonomic groups of eutherian mammals exhibit considerable diversity in the rate at which hybrid inviability has evolved (Fitzpatrick 2004). Here we provide evidence that these marked differences in the rate of evolution of hybrid inviability in mammals can in part be accounted for by differences among clades in the degree of placental invasiveness, that is, how deeply the placenta invades the maternal uterine lining. In particular, clades with invasive placentation (contacting the maternal bloodstream, Figure 7) evolve hybrid inviability much

more slowly, apparently as a result of increased downregulation of the maternal immune system during pregnancy.

Mammals are unique among the vertebrates in being almost universally viviparous. Unlike in the vast majority of other oviparous vertebrates, prenatal nutrition of eutherian mammals is accomplished by a chorioallantoic placenta, which is in close contact with both the fetal and maternal blood circulatory systems and which consequently provides an interface at which fetal antigens, including those of paternal origin, are presented to the maternal immune system (Bainbridge 2000). Pregnancy therefore represents an immunological paradox for the mammalian mother in that the conceptus can be considered a natural allograft that must be recognized as foreign but not rejected in order for pregnancy to proceed (Medawar 1953). While the initiation of pregnancy requires maternal recognition of the blastocyst as foreign tissue, the progression of pregnancy into later stages is accompanied by a transformation of the maternal immune environment that protects the developing fetal allograft from rejection.

Different taxonomic groups of eutherian mammals vary in the extent to which they have evolved means to suppress the immunological consequences of proximity between mother and fetus. Physiological research suggests that the maternal immune response is more strongly suppressed in taxa exhibiting invasive hemochorial placentation (in which maternal circulatory blood spaces are enclosed directly by fetal tissues) than in taxa exhibiting non-invasive endotheliochorial or epitheliochorial placentation (in which maternal blood is separated from fetal surface antigens by cell layers of maternal origin) (Baker et

al. 1999; Meeusen et al. 2001). Placental invasiveness is also associated with an increasingly strong downregulation of the adaptive inflammatory immune response (Hunt et al. 2000; Meeusen et al. 2001) and with reduced MHC antigen expression on the placental surface (Ait-Azzouzene et al. 1998; Baker et al. 1999).

Failure of pregnancy, both within and between species, is commonly due to inappropriate immunological interactions between mother and fetus (Clark 2003; Croy et al. 1982; MacLaren et al. 1992; Ruffing et al. 1993). On the basis of the apparent higher degree of downregulation of the maternal immune response in species with invasive placentation, we hypothesize that species with invasive placentation are less likely to abort interspecific conceptuses (or conceptuses from divergent populations) because of immunological incompatibility between mother and offspring. Consequently, our first prediction is that species with hemochorial (invasive) placentation should evolve hybrid inviability more slowly than species with epitheliochorial or endotheliochorial (noninvasive) placentation, because they experience reduced zygote loss during gestation.

Differences among mammal clades and placental forms in the rate of evolution of hybrid inviability should translate into effects on reproductive isolation and speciation. Allopatric speciation is widely regarded as the norm in vertebrates because their ability to travel relatively large distances promotes gene flow between divergent populations or incipient species. Sympatry combined with the ability to hybridize is known to result in the loss of species via

fusion, demonstrated, for example, by concern over the future of endangered animals such as the dingo *Canis lupus dingo* and the wildcat *Felis sylvestris* in the face of widespread hybridization with domesticated sister taxa. We expect a sustained period of allopatry to be found more frequently in sister species that have the ability to maintain interspecific pregnancy by virtue of their placental morphology, because sympatry of divergent populations within such species should more readily result in the fusion of populations by hybridization and the loss of incipient species. Consequently, our second prediction is that hemochorial sister species will, on average, exhibit a lower degree of sympatry than epitheliochorial or endotheliochorial sister taxa. We test our two predictions using data on placental type, ability of pairs of species to hybridize, genetic distances between pairs of species that can hybridize, and species geographic ranges.

Materials and methods

Database of hybridisable mammals

We used the compendium of mammalian species known to hybridize successfully by Gray (1972). An interspecific pregnancy was judged to be successful if it resulted in the birth of life offspring; we make no assumption about the likelihood of gene flow resulting from such hybridizations, though many such hybridizations are known to result in fertile or semifertile adults.

Measurement of genetic distances

We obtained genetic distance data via GenBank fro mammals known to hybridize successfully. We report genetic distances as the percentage of sites

that have undergone substitutions, using two putatively neutral loci (cytochrome b and 12S ribosomal RNA). In order to obtain a phylogenetically independent sample of hybridisable species pairs within clades where all species can hybridize, only the most divergent non-overlapping hybridisable species within each clade were included in the analysis; this resulted in a dataset unaffected by the bias caused by some clades being more heavily sampled than others. Genetic distances were calculated according to Kimura's two-parameter method (Kimura 1980), as implemented in the *distmat* program of EMBOSS (Rice et al. 2000). Distances calculated using this method are based on the number of transitions/transversions apparent from comparison of a pair of nucleotide sequences, corrected for the probability of multiple substitutions at a single site.

Measurement of range sympatry

We obtained species range maps from a number of sources including NatureServe (Patterson et al. 2003) and the African Mammal Databank (IEA 2006). Maps not available in digital format were digitized before analysis. Species range area was calculated by counting pixels in Adobe Photoshop. Range maps were overlaid to calculate the area of overlap between species ranges. Sympatry was measured by dividing the area of overlap between the range of two species by the area of the smaller species range. This measure results in a value between 0 (no overlap) and 1 (the smaller range is entirely enclosed by the larger range). Before analysis, measures of sympatry were normalized by arcsine transformation.

Analysis of hybridisable species pairs

We used ANOVA to test for the difference in the mean genetic distance found between pairs of hybridisable species grouped by placental type. In order to account for the phylogenetic nonindependence of data, we also repeated the analysis using only the most divergent non-overlapping pairs of hybridisable species within each clade that contained multiple pairs of hybridizab le species, as described in a previous section. We used data from studies of the mammalian molecular clock to test the alternative hypothesis that the observed patterns of genetic divergence between hybridizing sister species can be explained by differences in the age of each clade rather than differences in placentation. Finally, we also analyzed the genetic distance data using overall mean values for higher taxa (families or subfamilies) that were homogenous for placental type; this highly conservative analysis assumes that the effects of placental type on hybridizability are independent only at the highest taxonomic levels at which placental variation is found.

Analysis of sympatry data

We based our analysis of sympatry on the comparison of sister species. Where possible, sister species were determined from published phylogenetic sources. For 14 of our sister pairs, no published phylogeny could be found, so phylogenies for undisputed monophyletic groups were reconstructed using genetic sequences available in GenBank. For a number of our species pairs, no published phylogeny was available, nor were genetic sequences available within GenBank. Twenty-five such pairs were included in our analysis on the basis of

taxonomy, given that they occur in genera containing only two species. We used ANOVA to test for a difference between the mean sympatry of sister species grouped by placental type. To assess the extent to which our results are robust across different clades, we repeated the analysis separately for each order of mammals exhibiting both invasive and noninvasive placental forms. We used genetic distance data (as a surrogate for time since divergence) to test the alternative hypothesis that the observed patterns in range sympatry can be explained by differences in the age of each sister pair.

Results

To test the hypothesis that species with invasive placentation evolve hybrid inviability more slowly than species with noninvasive placentation, we examined 208 pairs of mammalian species (representing seven orders and 23 families) known to be hybridizable (capable of bringing interspecific pregnancies to term and birthing live offspring; (Gray 1972)). We predicted that mean genetic distances would be lower between pairs of species exhibiting noninvasive placentation than they are between hybridizable pairs of species exhibiting invasive placentation, because the former group must, in general, be more closely related if they are to be immunologically compatible in utero.

As predicted, hybridizable pairs of species with noninvasive placentation are much less genetically distant than hybridizable pairs of species with invasive placentation (mean = 10.28 ± 1.84 and 6.69 ± 0.92 substitutions per hundred base pairs of cytochrome *b* for species with invasive and noninvasive placentation, respectively; $P < .001$, $N = 159$, $F = 46.5$; mean = 6.00 ± 1.27 and

2.84 ± 0.51 substitutions per hundred base pairs of 12S for species with invasive and noninvasive placentation, respectively; $P < .001$, $N = 143$, $F = 50.0$). This analysis may overrepresent certain laboratory, farm, and zoo animals that have been subject to many attempted hybridizations and are thus present in multiple pairs of species in our data set. In order to circumvent this possible bias, we repeated the analysis using only the most divergent pairs of taxa within each monophyletic group (Figure 7). The results with this subset of most divergent species pairs were consistent with the previous analysis (mean = 12.64 ± 1.79 and 7.37 ± 1.2 substitutions per hundred base pairs of cytochrome *b* for species with invasive and noninvasive placentation, respectively; $P < .001$, $N = 61$, $F = 26.7$; mean = 7.97 ± 2.7 and 3.71 ± 0.83 substitutions per hundred base pairs of 12S for species with invasive and noninvasive placentation, respectively; $P < .001$, $N = 35$, $F = 14.74$). Finally, these results are not biased by the relative ages of clades with invasive versus noninvasive placentation; analysis of molecular clock data for orders and suborders shows that these clades do not differ in estimated divergence dates ($P > .30$ by ANOVA, using topologies and branch length data from recent mammalian trees (Hasegawa et al. 2003; Murphy et al. 2001; Springer et al. 2003), comparing the placentally invasive Haplorhine primates, Lipotyphla, Rodentia, and Lagomorpha with the non-invasive Strepsirhine primates, Carnivora, Perissodactyla, and Artiodactyla).

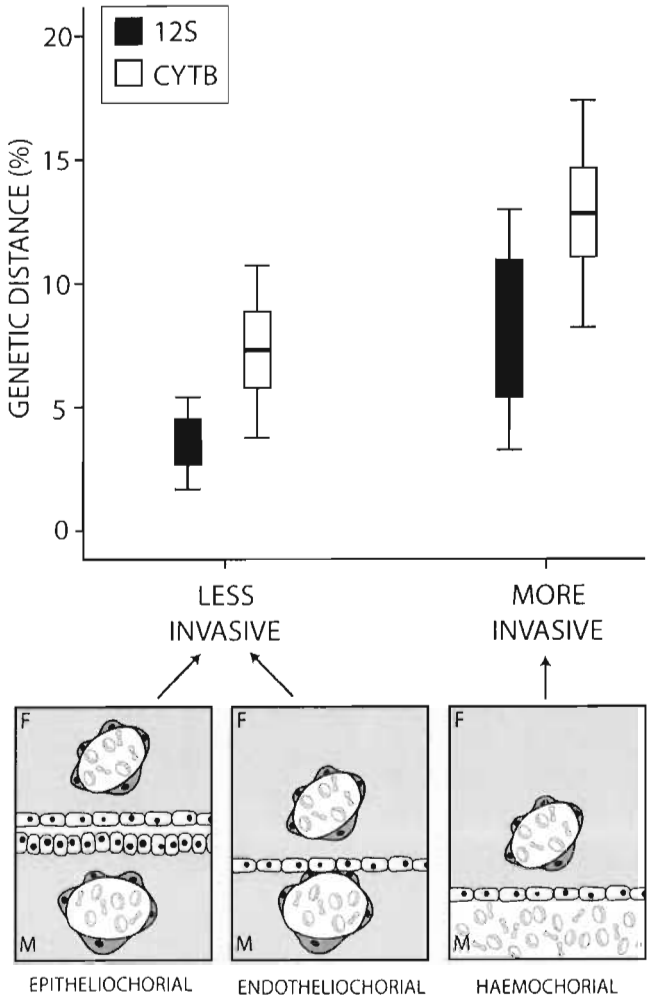

12S
CYTB
GENETIC DISTANCE (%)
20
15
10
5
0
LESS INVASIVE
MORE INVASIVE
F
M
EPITHELIOCHORIAL
ENDOTHELIOCHORIAL
HAEMOCHORIAL

Figure 7. Maximum genetic distance at which hybridization occurs between species with invasive or non-invasive placentation. Placental invasiveness is quantified in terms of the number of maternal cell layers separating fetal tissues from the maternal circulatory system (see Mossman 1987; Wildman et al. 2006). With high invasiveness, there is a higher genetic distance between pairs of hybridizable mammal species. In species with epitheliochorial placentation—including all strepsirhine Primates, Cetartiodactyla, Perissodactyla, Pholidota, and some Lipotyphla—the fetal and maternal component of the interhemal membrane both typically consist of endothelium, connective tissue, and epithelium, making a total of six cell layers between the two circulatory systems. In species with endotheliochorial placentation—including all Carnivora, Serenia, and Proboscidea, some Rodentia, most Insectivora and Xenarthra, and the sole member of Tubulidentata—the maternal epithelium and connective tissue are stripped away during development of the placenta, resulting in an interhemal interface consisting of just four cell layers. Finally, in species with hemochorial placentation—including all haplorhine Primates, Lagomorpha, Dermoptera, and Hyracoidea, most members of Rodentia and Chiroptera, and many members of Afrotheria—all of the maternal cell layers are removed during development of the placenta, and maternal blood spaces are directly enclosed by the fetal chorion. The hemochorial placenta is thus unique in that it involves the presentation of fetal surface antigens directly to the blood circulatory system of the mother, unimpeded by cell layers of maternal origin. Bottom, schematic representation of blood flow relations between maternal (M) and fetal (F) sides of the three types of placenta. Open nucleated cells represent epithelial tissue; dark gray nucleated cells represent endothelial tissue; light gray areas represent connective tissue; open dumbbell-shaped cells represent hemocytes. Top, box plot showing cytochrome b and 12S genetic distance between hybridizable pairs of mammals with less invasive versus more invasive placentation. Horizontal bars show mean values; boxes show 95% confidence intervals; error bars show 1 SD either side of the mean. The mean value for mammals with less invasive placentation is approximately half that for mammals with more invasive placentation, indicating that the former species evolve reproductive isolation via hybrid inviability more rapidly than the latter

Data within each clade on the evolution of hybrid inviability and on placental type are not statistically independent under usual models of character evolution, such as Brownian motion (Felsenstein 1985; Martins 2000). Given that only four major clades in our analysis exhibit invasive placentation (Haplorhine primates, Lipotyphla, Rodentia, and Lagomorpha) and four major clades show non-invasive placentation (Strepsirhine primates, Carnivora, Perissodactyla, and Artiodactyla), the tests conducted above may be subject to unsuitably high risk of Type I error. However, analyses using mean values for each of these eight major groups yielded essentially the same results, with hemochorial (invasive) taxa exhibiting roughly twofold higher genetic distances for hybridizable species pairs (cytochrome *b*, mean genetic distance 6.41 ± 1.46 vs. 3.01 ± 0.95, Mann-Whitney *U*-test, $P = .021$, $N = 8$; 12S, mean genetic distance 11.52 ± 1.92 vs. 6.29 ± 1.12, Mann-Whitney *U*-test, $P = .034$, $N = 7$; both results also remain significant if the related groups Perissodactyla and Artiodactyla are combined). These results demonstrate the strength and among-taxon consistency, across major eutherian orders, of the differences in hybridization effects between placental type.

An explicitly phylogeny-based test of our hypothesis requires inferring a species-level phylogeny for the bulk of eutherian mammals, inferring the transitions between invasive and non-invasive placentation, and relating these transitions to changes in the genetic distances at which species can hybridize. Nine to 11 evolutionary transitions in placental type can be inferred on such a phylogeny, but data on hybridization distances are currently available for taxa spanning only two of these transitions, and in both cases, the clades with invasive

versus non-invasive placentation are much more distant than sister lineages (Figure 8; see also Wildman et al. (Wildman et al. 2006) who inferred 11 such transitions). Both inferred changes in hybridization distance are in the predicted direction, but robust phylogeny-based tests of our hypothesis require data on hybridization from additional taxonomic groups that closely bracket the transitions in placental type. Until then, our analysis of this prediction can be considered as a species-level test that describes current patterns of trait association (Ricklefs and Stark 1996) subject to the presumptions that the immunological effects of invasive placentation on hybrid inviability exhibit macroevolutionary lability and that unobserved, phylogenetically distributed third variables do not drive the observed patterns (Ridley 1989). Given the >100 million years of mammalian radiation represented here, the wide range of taxa already included in our analyses, and the striking magnitude (around twofold) of the differences between placental types in hybridization distance, we believe that the strength of these species-level results should compel more comprehensive tests.

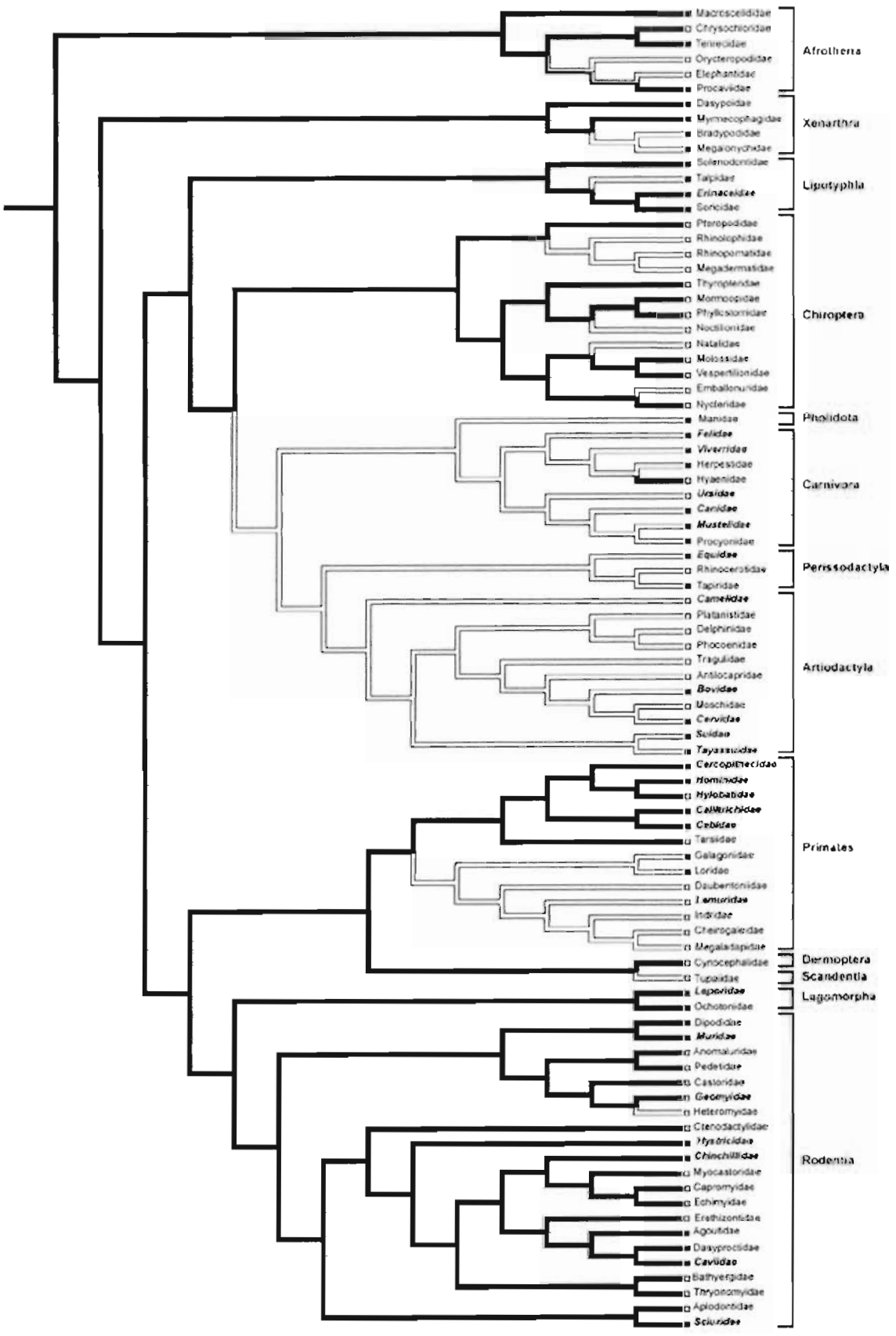

Macroscelididae
Chrysochloridae
Tenrecidae
Orycteropodidae
Elephantidae
Procaviidae
Afrotheria
Dasypoidae
Myrmecophagidae
Bradypodidae
Megalonychidae
Xenarthra
Solenodontidae
Talpidae
Erinaceidae
Soricidae
Lipotyphla
Pteropodidae
Rhinolophidae
Rhinopomatidae
Megadermatidae
Thyropteridae
Mormoopidae
Phyllostomidae
Noctilionidae
Natalidae
Molossidae
Vespertilionidae
Emballonuridae
Nycteridae
Chiroptera
Manidae
Pholidota
Felidae
Viverridae
Herpestidae
Hyaenidae
Ursidae
Canidae
Mustelidae
Procyonidae
Carnivora
Equidae
Rhinocerotidae
Tapiridae
Perissodactyla
Camelidae
Platanistidae
Delphinidae
Phocoenidae
Tragulidae
Antilocapridae
Bovidae
Moschidae
Cervidae
Suidae
Tayassuidae
Artiodactyla
Cercopithecidae
Hominidae
Hylobatidae
Callitrichidae
Cebidae
Tarsiidae
Galagonidae
Loridae
Daubentoniidae
Lemuridae
Indridae
Cheirogaleidae
Megaladapidae
Primates
Cynocephalidae
Dermoptera
Tupaiidae
Scandentia
Leporidae
Ochotonidae
Lagomorpha
Dipodidae
Muridae
Anomaluridae
Pedetidae
Castoridae
Geomyidae
Heteromyidae
Ctenodactylidae
Hystricidae
Chinchillidae
Myocastoridae
Capromyidae
Echimyidae
Erethizontidae
Agoutidae
Dasyproctidae
Caviidae
Bathyergidae
Thryonomyidae
Aplodontidae
Sciuridae
Rodentia

Figure 8. Distribution of our placental and hybridization data on a phylogenetic tree of eutherian mammals. This tree of mammalian families was inferred from five genes—RAG-1 (recombination activating gene 1), RAG-2 (recombination activating gene 2), cytochrome *b*, VWF (von Willebrand's factor), and IRBP (interphotoreceptor binding protein)—and 405 species using maximum likelihood, and it is fully compatible with other recent mammalian trees (i.e., see Wildman et al. 2006). Placental invasiveness data was taken from a number of published sources (Bernirschke and Miller 1982; Carter et al. 2004; Gopalakrishna and Karim 1979; King 1993a; Luckett 1974a; Mess 2003; Mossman 1987; Rasweiler 1993b). Placental invasiveness was mapped onto the branches of the tree by maximum likelihood reconstruction using Mesquite (Maddison and Maddison 2005), and the reconstructions correspond very closely with those of Wildman et al. (2006). Solid branches represent more invasive placentation (hemochorial), and open branches represent less invasive placentation (endotheliochorial or epitheliochorial). Families of mammals whose names are written in bold are those for which hybridizability data and genetic distance data are available. Families whose names are written in regular type are those for which data were not available and are not included in this study. Each family has a small square, either solid or open, to the left of its name. Solid squares denote the availability of species range data (resulting in a measure of sympatry) for sister species within each family; open squares denote the current unavailability of such data.

An additional means of testing our hypothesis that placental invasiveness mediates aspects of speciation is to use independent lines of evidence. The hypothesis also predicts that a sustained period of allopatry should be found more frequently in sister species that have the ability to maintain interspecific pregnancy by virtue of their placental morphology, because sympatry of divergent populations within such species should more readily result in the fusion of populations by hybridization and the loss of incipient species. We tested this prediction by gathering data on species ranges for 166 pairs of sister taxa of known placental type (representing 11 orders and 46 families of nonaquatic, nonvolant mammals) and testing for a mean difference in allopatry between groups with invasive versus noninvasive placentation. We followed Barraclough and Vogler (2000) in measuring the degree of allopatry between two species as the area of range overlap divided by the area of the smaller species range, normalized by arcsine transformation. By considering only recent biological sister

species, as determined from published phylogenies or analyses of published DNA sequences, we avoided the phylogenetic inference of ancestral species ranges from present-day species ranges, which has come under criticism (Losos and Glor 2003), and we thus minimized the potentially confounding effect of range shifts since speciation.

Consistent with our prediction, hemochorial sister species were on average less sympatric (mean 0.22 ± 0.06) than grouped epitheliochorial and endotheliochorial (non-invasive) species (mean 0.55 ± 0.1; $P<0.01$, $N = 166$, $F = 33.46$; ANOVA). This difference does not result from the possibility that sister species with invasive placentation tend to be younger than those with noninvasive placentation and have had less time for their species ranges to become sympatric: cytochrome b and 12S genetic distance, surrogates for time since divergence (Gissi et al. 2000), did not differ between invasive and non-invasive groups (cytochrome b: $P = .51$, $N = 58$, $F = 0.4$; 12S: $P = .26$, $N = 50$, $F = 1.3$; ANOVA), irrespective of their ability to hybridize. Hemochorial species were also less sympatric, on average, in the two orders (Primates and Insectivora) where both invasive and noninvasive placental forms are represented in our data set (primates: $P = .001$, $F = 12.41$; insectivores: $P = .007$, $F = 14.42$; ANOVA), which suggests that the overall mean difference was robust to taxon-specific effects other than placentation. Such reduced range overlap between sister species pairs and invasive placentation is an expected biogeographic consequence of their slower evolution of hybrid inviability.

Discussion

Our analyses suggest that invasive placentation engenders an approximate halving of the rate at which eutherian mammal species evolve hybrid incompatibility, compared with species with noninvasive placentation. The results provide evidence for the importance of physiological adaptations to placental viviparity in determining the rate at which different clades of mammals evolve reproductive isolation. In particular, the balance between placental antigenicity and maternal immune response may differ between taxa with invasive versus noninvasive placentation, such that immune system downregulation in taxa with invasive placentation allows for viable hybrid production at much greater genetic distances. The lower degree of sympatry between sister species in hemochorial clades provides independent support for this hypothesis and suggests that placental invasiveness has important implications for modes of speciation. The evolution of placental form and function, under conditions of ongoing conflict over resource allocation between mother and offspring (Crespi and Semeniuk 2004; Haig 1993; Wildman et al. 2006; Zeh and Zeh 2000), may thus generate strong effects on rates and patterns in the evolution of reproductive isolation. Further studies of eutherian phylogeny, comparative reproductive immunology, the causes of hybrid inviability in mammals, and placental evolution should illuminate the mechanisms driving these results.

CHAPTER 5: CONCLUSIONS

In the introductory chapter I describe a number of important evolutionary questions which this thesis is intended to explore. First, there is the question of what role physiology (specifically, the placenta) plays in the evolution of life history trade-offs. Life history trade-offs have been of interest to evolutionary biologists for many years. However, the notion of trade-off has often been used to describe negative associations between traits without evidence of how those traits interact functionally and causally (Zera and Harshman 2001). In my thesis I attempt to ground trade-offs over resource allocation during pregnancy in physiological outcomes resulting from the degree of placental invasiveness of the fetus. I find that variation in the rate and magnitude of fatty acid transport across the placenta is a possible causal mechanism underlying hitherto unrecognized systematic differences in the brain-body allometry of mammals with invasive versus non-invasive placentation. Similarly this variation appears to influence the evolutionary economics of life history evolution, especially the outcomes of evolution along the slow-fast life history continuum. Chapter 3 discusses some potential future research that might be conducted to further test and elaborate the hypotheses presented in my thesis. In particular we should test for associations between placental type and maternal gestational strategies such as income breeding (in which resources used for reproduction are acquired during the reproductive period) versus capital breeding (in which resources used for

reproduction are acquired from existing maternal energy stores), for example through measurement of maternal body fat composition before and after pregnancy. Shifts in maternal diet that occur with the onset of the reproductive period should also illustrate the importance of maternal access to fatty acids to fetal brain growth. Such future studies may also be able to focus on within-species variation, for example using human medical data on the outcome of individual pregnancies. Studies of the molecular evolution of genes involved in fatty acid transport are an additional potential future line of research, my prediction being that these genes undergo periods of accelerated evolution along branches of the phylogenetic tree upon which placentation undergoes structural transformations.

A second, related question is the nature of allometry in mammals. In Chapter 2, I present evidence that some of the between-taxon variation in brain-body allometry results from differences in the timing and magnitude of brain growth resulting from variation in the invasiveness of the placenta. These results lead me to question the validity of considering the realized brain size of each mammalian species to be a departure from a single mammalian allometric slope, or a member of a taxon-level "grade". Instead, variation in brain-body allometry is likely the result, in part, of differences in the physiological basis of resource acquisition by the fetus during pregnancy. This view might be tested profitably in other taxa exhibiting placental variation, such as the fish or reptiles.

The fact that allometry and life history trade-offs are constrained by the physiological basis of resource allocation to different functions and life stages is,

in itself, not particularly surprising. To the extent that this physiological basis varies between taxa, however, a functional understanding of life history trade-offs is necessary if we are to understand the evolution of alternative strategies within a diverse group of species such as mammals. Of particular interest in the case of placentation is that placental invasiveness is not merely an external constraint limiting the availability of resources in such a way as to generate conflict between physiological functions over a finite pool of resources. In addition, the placenta may be involved in conflict between parents and offspring. In Chapter 3, I suggest that placental invasiveness is not merely a cause of life history trade-offs, but that the evolution of non-invasive placentation may also be a result of selection against the escalation of conflict between mother and offspring or between littermates. This hypothesis yields direct predictions regarding the evolution of non-invasive placentation that could be tested using in-depth studies of mammalian clades exhibiting placental variation, such as the primates or insectivores. The existence of parent-offspring conflict is also expected to leave a molecular signature, namely the accelerated rate evolution of genes involved in parent-offspring interaction along branches of the phylogenetic tree exhibiting invasive placentation, in comparison with that of branches exhibiting non-invasive placentation. The role of genomic imprinting of placentally-expressed genes has also been described in terms of parent-offspring conflict, yet these views remain to be tested in a broad comparative context.

A final question in my introductory chapter relates to the role of placentation in generating reproductive incompatibility between divergent

populations or species of mammal. I provide an explanation rooted in reproductive immunology to account for divergence in hybridization rate and allopatry in sister-species with invasive versus non-invasive placentation. Again, testing such hypotheses in a focal group of species within which we can study hybridization in a comprehensive way may be a useful way of proceeding. Direct experimental measurement of hybridization potential within sister-clades of viviparous versus oviparous fish, for example, would permit the hypothesis of Chapter 4 to be tested independently. The mammalian data, on the whole, supports our view that placental structure may influence the ability of species to hybridize, and may therefore influence the rate at which speciation occurs in sympatry or between neighbouring allopatric species separated by a hybrid zone.

Together, the results presented in my thesis underscore the importance of functional studies in generating hypotheses and explaining the origin and outcome of life history trade-offs, allometry, and other aspects of mammalian evolution. Fostering a deeper understanding of the physiology of parent-offspring interactions, incorporating methods derived from the study of molecular evolution, and conducting within-species studies of pregnancy outcome, are the best ways of testing and refining these ideas further.

APPENDICES

Appendix 1

Occurrence of invasive (hemochorial) and non-invasive (endotheliochorial or epitheliochorial) placentation in eutherian mammal species.

ORDER	FAMILY	PLACENTA	MODEL SPECIES	SELECTED REFS
Afrotheria	Chrysochloridae	Hemochorial	Eremitalpa granti	(Gabie 1959; Gabie 1960)
Afrotheria	Elephantidae	Endotheliochorial	Loxodonta Africana, Elephas maximus	(Allen 2006; Perry 1974; Wooding 2005)
Afrotheria	Macroscelididae	Hemochorial	Elephantulus rufescens, Petrodromus tetradactylus, Rhynchocyon chrysopygus, Rhynchocyon petersi	(Bernirschke 2006; Cutler et al. 1998; Oduor-Okelo 1980; Oduor-Okelo 1984b; Oduor-Okelo et al. 2004)
Afrotheria	Orycteropodidae	Endotheliochorial	Orycteropus afer	(Taverne and Bakker-Slotboom 1970)
Afrotheria	Procaviidae	Hemochorial	Heterohyrax brucei, Procavia capensis	(Bernirschke 2006; Oduor-Okelo 1980; Starck 1959; Sturgess 1948)
Afrotheria	Tenrecidae	Hemochorial	Potamogale velox, Hemicentetes semispinosus, Setifer setosus, Echinops telfairi, Micropotamogale lamottei	(Bluntschli 1938; Carter 2005a; Carter 2005c; Carter 2006; Carter et al. 2004; Hill 1939; Strauss 1943)
Artiodactyla	Antilocapridae	Epitheliochorial	Antilocapra americana	(Bernirschke 2006; O'Gara 1969; Wurster and Bernirschke 1967)
Artiodactyla	Bovidae	Epitheliochorial	Bos, Bison, Kobus, Connochaetes, Redunca, Madoqua, Aepyceros, Rupicapra, Ovis, Capra, Gazella, Bubalus, Pudu, Naemorhedus etc.	(Amoroso et al. 1953; Bernirschke 2006; Boshier and Holloway 1977; Kayanja and Epelu-Opio 1976; King 1993b; King et al. 1982; Mossman 1987; Wilson and Kerr 1969; Wislocki 1941)
Artiodactyla	Camelidae	Epitheliochorial	Lama pacos, Lama glama, Camelus	(Abd-Elnaeim 1999; Bernirschke 2006;

			bactrianus, Camelus dromedarius, Vicugna vicugna	Fowler and Olander 1990; Ghazi et al. 1994; Morton 1961; Olivera 2003a; Olivera 2003b; Skidmore et al. 1996; Steven 1980)
Artiodactyla	Cervidae	Epitheliochorial	Odocoileus, Elaphurus, Cervus, etc.	(Bernirschke 2006; Harrison and Hamilton 1952; Mossman 1987; Sinha 1969; Wurster and Bernirschke 1967)
Artiodactyla	Giraffidae	Epitheliochorial	Giraffa camelopardalis	(Bernirschke 2006; Deka et al. 1980)
Artiodactyla	Hippopotamidae	Epitheliochorial	Choeropsis liberiensis, Hippopotamus amphibious	(Bernirschke 2006; MacDonald and Bosma 1975)
Artiodactyla	Moschidae	Epitheliochorial	Moschus moschiferus	(Bernirschke 2006)
Artiodactyla	Suidae	Epitheliochorial	Sus scrofa, Phacochoerus aethiopicus, Hylochoerus meinertzhageni	(MacDonald and Bosma 1975)
Artiodactyla	Tayassuidae	Epitheliochorial	Tayassu pecari, Catagonus wagneri	(Bernirschke 2006; Dieffenbach 1907; MacDonald and Bosma 1975)
Artiodactyla	Tragulidae	Epitheliochorial	Tragulus javanicus	(Bernirschke 2006; Kimura 2004)
Carnivora	Ailuridae	Endotheliochorial	Ailurus fulgens	(Bernirschke 2006)
Carnivora	Canidae	Endotheliochorial	Canis familiaris, Vulpes vulpes, Canis adustus, Canis mesomelas, Otocyon megalotis.	(Kehrer 1973; Mossman 1987; Stoffel et al. 1998)
Carnivora	Felidae	Endotheliochorial	Felis catus, Panthera leo, Panthera tigris, etc.	(Kehrer 1973; Leiser and Koob 1993; Tiedemann 1979)
Carnivora	Hyaenidae	Hemochorial	Crocuta crocuta	(Wynn et al. 1990)
Carnivora	Mephitidae	Endotheliochoiral	Spilogale putorius	(Sinha 1976; Sinha and Mean 1978)
Carnivora	Mustelidae	Endotheliochorial	Mustela putorius furo, Enhydra lutris, Mustela vison, Aonyx cinerea	(Bernirschke 2006; Gulamhusein and Beck 1975; Pfarrer 1999; Sinha and Mossman 1966)
Carnivora	Nandiniidae	Endotheliochorial	Nandinia	(Mossman 1987)
Carnivora	Odobenidae	Endotheliochorial	Odobenus rosmarus	(Bernirschke 2006)
Carnivora	Otariidae	Endotheliochorial	Arctocephalus pusillus, Callorhinus ursinus	(Rand 1955)
Carnivora	Phocidae	Endotheliochorial	Leptonychotes weddelli, Phoca vitulina, Halichoerus	(Amoroso and Matthews 1952;

			grypus	Davies 1950)
Carnivora	Procyonidae	Endotheliochorial	Procyon lotor, Nasua narica	(Bernirschke 2006; Creed and Harrison 1965)
Carnivora	Ursidae	Endotheliochorial	Ursus americanus, Thalarctos maritimus, Melursus ursinus	(Bernirschke 2006; Wimsatt 1974; Young 1969)
Carnivora	Viverridae	Endotheliochorial	Arctictis, Paradoxus, Genetta, Helogale, Ichenumia, Cynictis, etc.	(Moghe 22B; Young 1978)
Cetacea	Delphinidae	Epitheliochorial	Tursiops truncatus, Globicephala melaena, Cephalorhynchus commersoni, Lissodelphis borealis	(Bernirschke 2006; Morton and Mulholland 1961; Wislocki and Enders 1941)
Cetacea	Monodontidae	Epitheliochorial	Delphinapterus leucas	(Bernirschke 2006)
Cetacea	Phocoenidae	Epitheliochorial	Phocaena phocaena	(Wislocki 1933)
Cetacea	Platanistidae	Epitheliochorial	Platanista gangetica, Platanista indi, Pontoporia blainvillei	(Harrison 1972; Pilleri and Gihr 1976a; Pilleri and Gihr 1976b)
Chiroptera	Emballonuridae	Endotheliochorial	Taphozous melanopogon	(Bhigwade 1990)
Chiroptera	Hipposideridae	Hemochorial	Hipposideros fulvus	(Bhigwade 1990)
Chiroptera	Megadermatidae	Endotheliochorial	Megaderma sp.	(Bhigwade 1990)
Chiroptera	Molossidae	Hemochorial and endotheliohorial	Molossus ater, Tadarida brasiliensis, Tadarida tragata	(Bernirschke 2006; Gopalakrishna and Badwaik 1990; Rasweiler 1991; Rasweiler 1993a)
Chiroptera	Mormoopidae	Hemochorial	Pteronotus parnellii	(Badwaik and Rasweiler 1998)
Chiroptera	Natalidae	Endotheliochorial	Natalus sp.	(Carter and Enders 2004)
Chiroptera	Noctilionidae	Endotheliochorial	Noctilio albiventris	(Rasweiler 1993a)
Chiroptera	Phyllostomidae	Endotheliochorial	Glossophaga soricina	(Rasweiler 1993a)
Chiroptera	Pteropodidae	Hemochorial	Pteropus giganteus, rousettus leschenaulti, Cynopterus sphinx	(Bhigwade 1990; Bhigwade et al. 2000; Karim and Bhatnagar 1996)
Chiroptera	Rhinolophidae	Endotheliochorial	Rhinolophus rouxi	(Bhigwade 1990)
Chiroptera	Rhinopomatidae	Endotheliochorial	Rhinopoma hardwickei	(Bhigwade 1990)
Chiroptera	Thyropteridae	Hemochorial	Thyroptera tricolor spix	(Wimsatt and Enders 1980)
Chiroptera	Vespertilionidae	Hemochorial	Corynorhinus mexicanus, Miniopterus schreibersii, Myotis lucifugus, Myotis	(Bhigwade 1992; Gopalakrishna and Karim 1972; Kimura and Uchida 1984; Rasweiler 1993a;

			moluccarum, Pipistrellus mimus,	Sanchez Hernandez 2003)
Dermoptera	Cynocephalidae	Hemochorial	Cynocephalus volans	(Starck 1959)
Insectivora	Erinaceidae	Hemochorial	Erinaceus europaeus	(Morris 1957)
Insectivora	Solenodontidae	Hemochorial	Solenodon paradoxus	(Wislocki 1940)
Insectivora	Soricidae	Hemochorial	Suncus murinus, Blarina brevicauda, Sorex fumeus	(Owers 1960; Wimsatt et al. 1973; Wimsatt and Wislocki 1947)
Insectivora	Talpidae	Epitheliochorial or Endotheliochorial	Scalopus aquaticus, Talpa europaea	(Carter 2005b; Malassine and Leiser 1984; Prasad et al. 1979)
Lagomorpha	Leporidae	Hemochorial	Oryctolagus cuniculus	(Samuel et al. 1975)
Lagomorpha	Ochotonidae	Hemochorial	Ochotona princeps	(Harvey 1959b)
Perissodactyla	Equidae	Epitheliochorial	Equus burchelli, Equus asinus, Equus caballus, Equus grevyi, Equus kiang	(Allen and Short 1997)
Perissodactyla	Rhinocerotidae	Epitheliochorial	Diceros bicornis, Rhinoceros unicornis, Ceratotherium simum	(Benirschke and Lowenstine 1995; Bernirschke 2006)
Perissodactyla	Tapiridae	Epitheliochorial	Tapirus sp.	(Bernirschke 2006; Schauder 1945)
Pholidota	Manidae	Epitheliochorial	Manis	(Mossman 1987)
Primates	Atelidae	Hemochorial	Ateles geoffroyi	(Bernirschke 2006)
Primates	Callithrichidae	Hemochorial	Callithrix jacchus, Callimico goeldii	(Jollie 2005; Smith and Moore 1990)
Primates	Cebidae	Hemochorial	Cebus, Alouatta, Mandrillus, Lagothrix, etc.	(Bernirschke 2006) (Mossman 1987)
Primates	Cercopithecidae	Hemochorial	Cercopithecus, Papio, Presbytis, Colobus, Pygathrix, Trachypithecus, Macaca etc.	(Bernirschke 2006; Burton 1980a; Burton 1980b; McLean 2001; Owiti 1986)
Primates	Daubentoniidae	Epitheliochorial	Daubentonia madagascariensis	(Bernirschke 2006; Hill and Burne 1922)
Primates	Galagidae	Epitheliochorial	Galago crassicaudata, Galago senegalensis	(King 1984; Njogu 2005)
Primates	Hominidae	Hemochorial	Pan, Pongo, Homo, Gorilla	(Bernirschke 2006) (Mossman 1987)
Primates	Indriidae	Epitheliochorial	Indri indri, Propithecus verreauxi	(Hill 1932)
Primates	Lemuridae	Epitheliochorial	Microcebus murinus, Eulemur	(Bernirschke 2006;

			fulvus, Lemur catta, Varecia variegatus	Strauss 1978)
Primates	Lorisidae	Epitheliochorial	Loris tardigradus	(Hill 1932)
Primates	Pitheciidae	Hemochorial	Pithecia pithecia	(Bernirschke 2006)
Primates	Tarsiidae	Hemochorial	Tarsius spectrum	(Luckett 1974b)
Rodentia	Agoutidae	Hemochorial	Agouti paca	(Bonatelli 2002; Bonatelli 2005; Miglino 2002)
Rodentia	Anomaluridae	Hemochorial	Anomalurus	(Luckett 1971)
Rodentia	Aplodontiidae	Hemochorial	Aplodontia rufa	(Harvey 1959a)
Rodentia	Bathyergidae	Hemochorial	Bathyergus janetta	(Luckett and Mossman 1981)
Rodentia	Castoridae	Hemochorial	Castor canadensis	(Fischer 1971)
Rodentia	Caviidae	Hemochorial	Cavia porcellus, Kerodon rupestris	(Bernirschke 2006; Kaufmann and Davidoff 1977; Oliveira 2006)
Rodentia	Chinchillidae	Hemochorial	Chinchilla laniger, Lagostomus maximus	(King and Tibbets 1976)
Rodentia	Ctenodactylidae	Hemochorial	Ctenodactylus gundii	(Luckett 1980)
Rodentia	Dasyproctidae	Hemochorial	Dasyprocta azarae	(Becher 1921a; Becher 1921b; Miglino 2002)
Rodentia	Dinomyidae	Hemochorial	Dinomys brannicki	(Bernirschke 2006)
Rodentia	Dipodidae	Hemochorial	Jaculus jaculus	(King and Mossman 1974)
Rodentia	Erethizontidae	Hemochorial	Erethizon dorsatum	(Perrotta 1959)
Rodentia	Geomyidae	Hemochorial	Geomys bursarius	(Mossman 1987; Mossman and Strauss 1963)
Rodentia	Heteromyidae	Endotheliochorial	Dipodomys, Microdipodops	(King and Tibbets 2005)
Rodentia	Hydrochaeridae	Hemochorial	Hydrochaerus hydrochaeris	(Miglino 2002)
Rodentia	Hystricidae	Hemochorial	Hystrix cristata	(Luckett and Mossman 1981)
Rodentia	Muridae	Hemochorial	Clethrionomys, Dicrostonyx, Lemmus, Microtus, Peromyscus, Phodopus, Rattus, Mus, Mesocricetus, etc.	(Carpenter 1982; Kertschanska 2000; King and Hastings 1977; Ogura 1991)
Rodentia	Myocastoridae	Hemochorial	Myocastor coypus	(Hillemann and Gaynor 1961)
Rodentia	Octodontidae	Hemochorial	Octodon degus	(Bosco 2006; Kertschanska et al. 1997)
Rodentia	Pedetidae	Hemochorial	Pedetes capensis	(Fischer and Mossman 1969)
Rodentia	Sciuridae	Hemochorial	Spermophilus	(Carter and Enders

			lateralis	2004)
Rodentia	Thryonomyidae	Hemochorial	Thryonomys swinderianus	(Oduor-Okelo 1984a; Oduor-Okelo and Gombe 1991)
Rodentia	Zapus	Hemochorial	Zapus sp.	(King and Mossman 1974)
Scandentia	Tupaiidae	Endotheliochorial	Tupaia	(Bernirschke 2006; Luckhardt et al. 1985)
Sirenia	Trichechidae	Endotheliochorial	Trichechus manatus	(Enders and Carter 2004)
Xenarthra	Bradypodidae	Endotheliochorial	Bradypus variegatus	(Amorim 2004; Bernirschke 2006)
Xenarthra	Cyclopedidae	Hemochorial	Cyclopes didactylus	(Wislocki 1928b)
Xenarthra	Dasypodidae	Hemochorial	Cabassous chacoensis, Chaetophractus villosus, Dasypus hybridus, D. novemcinctus, Tolypeutes matacus	(Adamoli 2001)
Xenarthra	Megalonychidae	Endotheliochorial	Choloepus hoffmanni, Choloepus didactylus	(Bernirschke 2006; Wislocki 1928a)
Xenarthra	Myrmecophagidae	Hemochorial	Myrmecophaga jubata, Myrmecophaga tridactyla, Tamandua tetradactyla	(Becher 1931; Bernirschke 2006; Walls 1939)

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