Title

The Biology of Reproductive Delays in Mammals: Reproductive Decisions, Energetics, and Evolutionary Ecology

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The Biology of Reproductive Delays in Mammals: Reproductive Decisions, Energetics, and Evolutionary Ecology

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of the requirements for the degree of

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in

Evolution, Ecology and Organismal Biology

by

Teri Jean Orr

December 2012

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“May your trails be crooked, winding, lonesome,

dangerous, leading to the most amazing view.

May your mountains rise into and above the clouds. “

Edward Abby

ABSTRACT OF THE DISSERTATION

The Biology of Reproductive Delays in Mammals: Reproductive Decisions, Energetics, and Evolutionary Ecology

by

Teri Jean Orr

Doctor of Philosophy, Graduate Program in Evolution, Ecology, and Organismal Biology
University of California, Riverside, September 2012
Dr. Kimberly A. Hammond, Chairperson

My dissertation investigates the unique ways organisms put effort (time, energy, and/or nutrients) towards reproduction. My research asked how delays might allow mammals to allocate limited resources to reproduction (i.e., are delays adaptive?) and consisted of several components aimed at understanding the causes and consequences of reproductive delays in mammals. First, I used the phylogenetic comparative method to examine ecological and phylogenetic predictors of obligate delayed implantation in the order Carnivora in 157 species using published data. I evaluated whether average latitude of the geographic range, diet or phylogenetic position are predictors of whether a species exhibits delayed implantation. My results suggest that the presence of delays is positively related to body size and seasonality, and also varies with diet. Second, I dissected museum specimens of male bats (N = 47 species) to measure size of the testes and then indirectly assess if reproductive delays facilitate sperm competition. By reviewing the literature and inspecting museum specimens of bats with different types of delays, including some without delays, I was able to determine that delays might facilitate postcopulatory sexual selection (competition between the sperm from different males). Specifically, I tested and confirmed the prediction that males in species with delays between mating and egg-fertilization have larger testes than males in species that do not delay before fertilization. Third, I empirically evaluated some of the costs and benefits of delays in a species that has pregnancies both with and without delays, *Artibeus jamaicensis*. I compared diet, and food availability between the two pregnancies to evaluate the hypothesis that delays allow females to time the most expensive stage of reproduction, lactation, with periods of greatest food abundance. I found that previous hypotheses that *A. jamaicensis* uses delays to time lactation with fruit abundances may be more complex than previously realized including a dependence upon insects during late pregnancy and some lactational periods. My dissertation contributes important information on adaptive significance of delays in mammals.

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DISSERTATION INTRODUCTION

Allocation of limited resources to reproduction, growth and maintenance is vital for an organism's fitness, and partitioning energy among these competing functions results in trade-offs (Roff, 1992). Traditionally, evolutionary biologists interested in this partitioning of energy and materials have focused on species level patterns (Boggs, 1992) more recently however, the diversity of strategies of energy division relative to reproductive events has received more attention. One extreme response to environmental variation in resources is seen in the presence of reproductive delays, or temporal pauses in reproduction that occur between copulation and fertilization, between fertilization and implantation, or after an embryo has implanted (Mead, 1993). Current literature on the evolution of reproductive delays (hereon 'delays') consists of theory (Isakova, 2006) and large scale species level comparisons (Lindenfors *et al*., 2003). Explanations for the evolution of delays rely on the assumption that delays increase fitness through energetic trade-offs with other functions, such as decreased number of litters per year and increased juvenile survival (Sandell, 1990); yet few empirical studies exist (Racey & Entwistle, 2000). For this reason it is reasonable to suspect that changes in available energy might relate to periods of reproductive delays. If delays were nutritionally or otherwise advantageous we might expect parturition to occur during periods of peak food availability. Indeed, several testable questions arise when considering the posossible origins of delays. Are delays truly advantageous in the wild? What characteristics of an animal's ecology serve as predictors of delays? Furthermore, the potential role of delays in facilitating postcopulatory sexual selection after delays have evolved has not been considered.

My dissertation explores how reproductive delays might allow mammals to allocate limited resources to reproduction (i.e., are delays adaptive?) and in this way understand the causes and consequences of reproductive delays in mammals. I am particularly interested in the intersection between selection (natural and sexual) and reproductive physiology. Because life-history trade-offs are often mediated by an animal's physiology (Ricklefs & Wikelski, 2002), I aim to understand if reproductive delays are an instance where a physiological trait enables females to negotiate existing trade-offs, specifically the temporal allocation of resources to reproduction. The main objective is to ask if, and how, delays might allow mammals to allocate limited resources to reproduction. There are several components aimed at understanding the causes and consequences of reproductive delays in mammals. Following from above are the concepts briefly described below with their associated hypothesis and predictions, and resulted in the 4 chapters of my dissertation. These chapters are conceptually liked under the premise of understanding the biology of reproductive delays from an evolutionary relevant perspective.

Using the comparative method in the well-studied mammal group, Carnivora, I examined ecological predictors and evolutionary origins of delayed implantation in the Order Carnivora in over 200 species. I tested the hypothesis that delays allow females to negotiate seasonal environments, and tested the associated prediction that species at higher latitudes are more likely to have delays. (This aim is discussed in chapter 1 *in-prep* for Evolution). I discovered that animals that are larger in body size, as well as those from seasonal environments are most likely to have delays delays. This study also is the first to compare the performance of phylogenetic logistic regression (Ives and Garland 2010) and phylogenetic generalized linear mixed models (Ives & Helmus, 2011) and included the largest dataset of delays (about 156 species) analyzed thus far.

My review of the literature showed that delays pose an exciting arena within which to investigate this type of sexual selection (an idea that had not been tested before). Next, because delays may have significant impacts on patterns of reproductive competition I dissected museum specimens of male bats from approximately 50 species with varying delay types, to assess through measures of testes size, if delays facilitate sperm competition (an indirect measure of post-copulatory sexual selection). (This aim includes two separate chapters, chapter: 2. literature review/synthesis (*in-revision* for Biological Reviews), and chapter 3. museum specimens (testes sizes, *in-prep* for the American Naturalist))

The assumption that delays are adaptive has not been well tested, thus I examined the costs and benefits of delays in a bat species that has pregnancies both with and without delays, the Jamaican fruit-bat (*Artibeus jamaicensis*). Using this system I evaluated the hypothesis that delays allow females to time the most expensive stage of reproduction, lactation, with periods of food abundance. This study was the basis for a UC Mexus grant that involved extensive fieldwork and resulted in an international collaborative study with researchers at the Universidad Autonoma de Mexico and Instituto Politécnico Nacional in Mexico. (This aim is investigated in chapter 4. shifts to insectivory relative to periods of reproductive demands (pregnancy and lactation) *in-prep* for Functional Ecology.

Organisms allocate energy, materials and time to reproduction in various ways that may have important fitness consequences. For example, rats, when fighting a parasitic infection, may invest more in current offspring because their future reproductive success is less certain (Willis & Poulin, 1999). Similarly, fish may have different fecundities depending on predator regime (Reznick *et al*., 1990). Both theoretical and empirical studies have confirmed that trade-offs occur between life history traits such as survival and reproductive effort, and between growth and reproduction (Reznick & Endler, 1982; Watson, 1984; Roff, 1992; Stearns, 1992; Reznick et al., 1990). Furthermore, if environmental variability or stressors are predictable and reoccurring this can lead to pressures for organisms to develop highly timed reproduction (Badyaev, 2005). Numerous organisms exhibit some sort of reproductive delay, including insects and copepods that lay eggs that do not hatch until the weather is appropriate (Mead, 1993). It is possible that reproductive delays are a way of allocating resources to reproductive effort that has positive effects on fitness. The chapters that follow in this dissertation explore this possibility.

What are reproductive delays?

Periods of extension of different stages of the reproductive cycle are referred to as reproductive delays (Mead, 1993). While the study of patterns in vertebrate reproduction has been of interest to biologists for centuries (Birkhead, 2000), researchers have, historically, approached this area of research from an anthropocentric point of view, assuming that the timing of events from fertilization to birth in all mammals occurred in the same manner as in humans. However in 1843, even before the publication of Darwin's The Origin of Species in 1859, Ziegler observed a unique reproductive physiology in the roe deer (*Capreolus capreolus*; Mead, 1993). In roe deer, implantation of the blastocyst does not occur until 4-5 months after fertilization (Hayssen *et al*., 1994). We now know extensively more about the biology of reproductive delays than Darwin's time.

In mammals, reproductive delays can occur after copulation but before fertilization (delayed fertilization); after fertilization but before implantation occurs (delayed implantation); or during gestation (delayed development). By delaying any of these stages of reproduction, a pregnancy occurs in a different time-frame than it would have without the delay. Is this skew of reproductive timing advantageous? Most researchers suggest that delays allow animals to devote limited resources (energy or materials) to reproduction in a way that is beneficial. Understanding energetic or material costs and benefits of delays is the first step towards evaluating hypotheses concerning the evolutionary origins of this unique life history.

A review of the literature (Mead, 1993; Hayssen *et al*., 1994) reveals over 100 mammalian species with some form of reproductive delay, including some bears, wallabies, weasels, seals, armadillos, skunks, bats, and others. Of the 21 mammalian orders, 8 exhibit reproductive delays, including Diprotodontia, Cingulata (Edentata), Eulipotyphyla, Scandentia, Carnivora, Rodentia, Chiroptera, and Cetartiodactyla (Artiodactyla and Cetacea). For example, in the long-tailed weasel (*Mustela frenata*), implantation is delayed for 7-9 months, while active gestation lasts an additional 9.5 months. The California leaf-nosed bat (*Macrotus californicus*) has delayed development lasting on average 4.5 months, with active gestation an approximate additional 8.5 months (Hayssen *et al*., 1994). Delayed fertilization occurs in Townsends' big-eared bat (*Corynorhinus townsendii*), whereby females store sperm and ovulation does not occur until after arousal from torpor (Kunz & Martin, 1982).

What are the existing hypotheses regarding the origins of delays?

In a review paper, Hamlett (1935) summarized what he referred to as theories regarding the origins of delays in mammals which include 1) the advantage of being born at a favorable time for both parent and young, 2) delays only occur in old genera that existed during the Pleistocene, because delays assured young were not born during glacial winters, and finally 3) that delays are a byproduct of lower body temperatures of hibernating mammals. Hamlett himself poses a fourth hypothesis which is that delays are not advantageous, but instead fixed inherited phenotypes present in some species. Hamlett's suggestion, in essence, the null hypothesis for the origin of delays is important when considering the evolution of any phenotype (i.e. trait is not adaptive) (Gould & Lewontin, 1979). Namely, the burden of proof should always be on demonstrating the adaptive nature of a trait, and not the other way around (Gould & Lewontin, 1979).

Several additional hypotheses have been published in the last fifty years. Delays may allow females to match the energetic demands of pregnancy and lactation to food abundances and favorable weather (Ferguson *et al*., 2006). More specifically, delays might allow females to wean young when food is available (van der Merwe, 1978). Females might use delays to coincide estrus with periods of mate availably to elicit maximal male competition, thereby providing a forum for female mate choice (Sandell, 1990). Another hypothesis is that delays likely enable the synchronization of breeding, as seen in marine mammals such as pinnipeds (Bartholomew, 1970). Because pinnipeds (Odobenidae, Otariidae, Phcidae etc.) are widely dispersed much of the year, the selective advantage of being able to have parturition occurring in a narrow window during which mating also occurs (shortly thereafter) is important (Bartholomew, 1970). Delays may have been the ancestral state that was lost as species became smaller to counteract other changes in the life history associated with the decrease in body mass due to fecundity selection (Ferguson *et al*., 1996, Lindenfors *et al*., 2003). Recently, it has been suggested that delays might be a genetic byproduct resulting from changes to other aspects of reproductive physiology and thus are not the result of selective pressures for this particular phenotypic trait at all (Isakova, 2006).

Aims of the thesis

The goal of my dissertation is to investigate the potential evolutionary causes of reproductive delays (environmental pressures, life-history energy allocation issues) and the potential consequences for mate choice once delays have evolved in a taxonomic group. My dissertation contributes previously lacking data on individual and seasonal differences of how energy is divided among the different functions; body maintenance, growth or reproduction. I maintain that reproductive delays serve as a life-history strategy and can be explored as such. Furthermore, I explore a previously ignored and somewhat re-vamped (relative to Birkhead & Møller (1993) who largely focused on delayed implantation) hypothesis regarding the role of delays in facilitating post-copulatory sexual selection.

Structure of the thesis

I am first author on all of my dissertation chapters presented above in outline format and in full citations below, which are included here as manuscripts formatted to the standardized University of California Riverside formatting requirements for thesis formatting. Consequently, there is some repetition in introductory material. My various co-authors contributed to conducting the research presented and/or the analysis and in all cases to some degree the writing of the paper. Dr. Anthony Ives helped substantially with the development of a program (PGLMM; Ives & Helmus, 2011) used to analyze the data presented in Chapter 1. However, in all cases I was the lead investigator; from conception, funding to carrying out the research including its analysis and final write-up.

Chapter One: **Orr, TJ,** P Lindenflors, L Dalen, A Angerbjoern, AR Ives and T Garland, Jr. Ecological predictors of delayed implantation in the Carnivora. *In-prep for Evolution*.

Chapter Two: **Orr, TJ,** and M Zuk. Reproductive delays facilitate sperm competition, evidence from museum specimens. *In-revision for submission to the American Naturalist*.

Chapter Three: **Orr, TJ,** and M Zuk. Synthesis: Exploring the possible role of reproductive delays in facilitating post-copulatory sexual selection. *In-revision for submission to Biological Reviews*.

Chapter Four: **Orr, TJ,** and KA Hammond. Reproductive energetics and dietary shifts in a bat with delayed development, *Artibeus jamaicensis*. *In-prep for Functional Ecology.*

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Chapter 1.

Phylogenetic analysis of delayed implantation in the Carnivora

ABSTRACT

Mammalian reproduction exhibits considerable diversity, including delayed implantation (DI), defined as a period of diapause during which the blastocyst remains unattached rather then immediately implanting. This mode of reproduction appears to be limited to nine of the 21 extant mammalian orders, of which the Carnivora is the best-studied. The adaptive significance of DI has been considered many times, yet previous studies have been limited by available statistical methodologies and have treated gestation length as a proxy for DI - a limiting assumption. Using new statistical methods, we assessed if the presence of DI, treated as a presence-absence variable, can be predicted by interspecific variation in body mass, latitude (center of geographic range) or diet type (five categories or simply strict carnivores vs. all others), and if delayed implantation exhibits phylogenetic signal. Our dataset for 157 species is the largest yet employed for this type of analysis. We conducted three different types of analyses: conventional logistic regression, phylogenetic logistic regression, and phylogenetic generalized linear mixed models (PGLMM). The presence of DI exhibited statistically significant phylogenetic signal: related species were more likely to resemble each other than a species drawn at random from the sample. Conventional logistic regression with model comparisons based on the Akaike information criterion corrected for sample size (AICc) indicated that body mass (larger-bodied species were more likely to exhibit DI) and latitude (species at higher latitudes were more likely to exhibit DI) were statistically significant predictors of DI, and also that the presence of DI varied among major clades of Carnivora (N = 13 recognized for analysis). Phylogenetic logistic regression also suggested the importance of body mass and latitude, as well as diet (carnivores were more likely to have DI, with phylogenetic signal present in residual DI in a model that included all three of these as independent variables. PGLMM analyses indicated overwhelming support for a model including body mass and diet coded in five categories, in addition to the presence of phylogenetic signal in residual DI (cumulative Akaike weight or model probability = 0.996). Overall, our results suggest that evolutionary changes in delayed implantation within the Carnivora have been associated with changes in body mass, diet, and/or latitude, some of which covary among species of Carnivora, and that these associations may vary in relation to phylogeny.

Keywords: delayed implantation; Carnivora; comparative method; dependant variables; allometry; grade shifts, comparative method

INTRODUCTION

Predictable and recurring environmental variability or stressors can lead to selection for well-timed reproduction (for example, to coincide with greatest resource abundance or favorable temperatures) (Bronson, 1989; Badyaev, 2005). One extreme response to environmental variation is demonstrated by the presence of **reproductive delays**—temporal pauses in reproduction that occur between copulation and fertilization, between fertilization and implantation, or after an embryo has implanted (Mead, 1993). Reproductive delays are widespread in a variety of taxa, including insects and copepods, both of which lay eggs that do not hatch until the weather is appropriate, birds that lay a series of eggs across weeks that hatch synchronously (Mead, 1993), and many species of mammals, including representatives of at least seven orders (Daniel, 1970; Hayssen, Tienhoven & Tienhoven, 1993; Renfree & Shaw, 2000).

At least 123 mammalian species exhibit a period of reproductive delay (Daniel, 1970; Hayssen, Tienhoven & Tienhoven, 1993; Racey & Entwistle, 2000; unpublished results), which can be classified into three types: 1) after copulation but before fertilization (**delayed fertilization**, due to delayed ovulation and/or sperm storage); 2) after fertilization but before the blastocyst implants (**delayed implantation**), or 3) during gestation (**delayed development**). Delays may be **obligate** (always occur) or **facultative** (Daniel, 1970). Facultative delays are those that may occur due to lactation, changes in temperature, photoperiod or resource (food) abundance (Temte, 1985; Mead, 1993). Here, we focus on obligate delayed implantation because this is the most common, best-studied, and phylogenetically diverse type of reproductive delay in mammals (Hayssen, Tienhoven & Tienhoven, 1993; Lindenfors, Dalen & Angerbjoern, 2003). We examine variables suggested by some of the most current adaptive explanations for obligate DI in the Carnivora.

What advantages might delays provide to mammals? Several hypotheses have been presented. H1)-Delayed implantation may provide an advantage by allowing females to bear young at a favorable time from the standpoint of the mother (Sandell, 1990) and/or the young (Fries, 1880; Prell, 1930). This hypothesis is based on the idea that delayed implantation might provide a way to remove the otherwise tight correlation between mating and parturition that would occur if gestation length were fixed. Thus, by delaying implantation, parturition itself could be timed to certain environmental events, despite females mating at various times. Consequently, delayed implantation (DI) can allow females to match the high energetic demands of pregnancy and lactation with periods of peak food abundance and/or favorable weather (van der Merwe, 1978; Ferguson, Higdon & Larivière, 2006). H2)-In addition, delays enable the synchronization of breeding, as seen in pinnipeds (Carnivora) (Bartholomew, 1970). H3)-Delays have been suggested to only occur in old genera that existed during the Pleistocene, because delays assured young were not born during glacial winters (Hamlett, 1935). H4)-Alternatively, delays may allow females to synchronize estrus with periods of mate availability, helping to ensure they will achieve copulation and also eliciting maximal male competition, thus providing a forum for female mate choice (Sandell, 1990). This explanation, as well as the series of hypotheses listed above, all suggest that delays are an adaptive response to environmental variability (Hamlett, 1935; Bartholomew, 1970; Sandell, 1990; Ferguson, Virgl & Larivière, 1996).

Alternatives to hypotheses focusing on the benefits of DI include the possibilities that it may be selectively neutral, a byproduct of other traits or even present negative effects. H5)-For example, it is possible that delays were the ancestral condition in Carnivora and were lost due to fecundity selection as certain taxa became smaller - possibly to counteract other changes in the life history associated with the decrease in body mass (Lindenfors, Dalen & Angerbjoern, 2003). Lindenfors, Dalen & Angerbojoern suggest that as certain Carnivora lineages evolved decreased body sizes and started having more litters and pups per year, DI became disadvantageous and was subsequently lost (Lindenfors, Dalen & Angerbjoern, 2003).

DI may also be a result of other aspects of a species' physiology. For example, delays were once thought to be a byproduct of lower body temperatures associated with hibernation in mammals (Hamlett, 1935; Racey, 1979). It has also been suggested that delays are incidental to genomic rearrangements relating to other traits that are associated with timing of reproduction, whereby an alteration of timing results in delays (Isakova, 2006). In essence, the selective neutrality hypothesis in regards to reproductive delays represents the null and is important to keep in mind when considering the evolution of any phenotype (Gould & Lewontin, 1979).

The Carnivora are a good lineage for studies of DI because they have been well-studied relative to other mammalian orders. Furthermore, over half of the mammalian species known to possess DI are in the Carnivora (Thom, Johnson & Macdonald, 2004). Indeed, all previous comparative treatments of delayed implantation in mammals have focused on the Carnivora, with, to our knowledge, only one exception that considered members of the order Chiroptera (Bernard & Cumming, 1997). Delayed implantation is thought to be the ancestral state in the Carnivora (Lindenfors *et al.*, 2003), so analyses that consider this lineage alone cannot address the origins of DI. Because DI can be experimentally induced even in mammalian species that do not usually possess delays, it has been suggested that DI is ubiquitous and thus basal for all mammals (Ptak *et al*., 2012). If DI is an ancestral trait, then in some extant species it may be a "fixed" phenotype, with little or no individual phenotypic variation on which selection could act and/or little or no additive genetic variance that would allow it to be lost even if selection favored its loss.

With the exception of one study (Lindenfors, Dalen & Angerbjoern, 2003) all comparative studies of DI in the Carnivora have solely considered the Mustelidae. Most previous attempts to evaluate the predictors of DI in Carnivora have suffered from three separate issues: mis-conceptualization of delays as a continuous-valued rather than binary trait; statistical analyses that did not incorporate information on phylogenetic relationships and/or variable treatment of DI as the independent versus more appropriate dependant variables. Although some studies have treated DI as binary (Lindenfors, Dalen & Angerbjoern, 2003) and even in some cases as the dependant variable using logistic regression (Thom, Johnson & Macdonald, 2004), most studies have examined DI as continuous (Ferguson, Higdon & Larivière, 2006). This issue of treating delays as continuous or independent variables may have because for many years the only phylogenetically based statistical methods available to analyze binary variables examined them as the independent variable e.g., independent contrasts (Felsenstein, 1985; Garland *et al*., 1992, 1993) and phylogenetic generalized least-squares approaches (e.g., Grafen, 1989; Lavin *et al*., 2008). Thus, the major limitation to investigating DI in a statistically sound way was due to the lack of methods available to analyze binary variables while simultaneously considering phylogenetic relationships.

We took advantage of the recently developed phylogenetic logistic regression (Ives & Garland, 2010) to examine the predictors of delayed implantation within the Carnivora. We chose to examine some independent variables suggested by previous research (see above: H1, H3, H4, H5) to have roles in the evolutionary origin and/or maintenance of DI. Specifically, we tested H1: DI allows females to negotiate seasonal environments, i.e., to time certain stages of reproduction to seasonality (Ferguson, Higdon & Larivière, 2006). Thus, we expected species inhabiting higher latitudes would have a higher occurrence of delayed implantation. Furthermore, if seasonality provides a selective advantage for DI, then there may also be a relationship between diet type and DI. Thus, we also asked if species with certain diets were more or less likely to have reproductive delays. Because, H4: the hypothesis that delays may allow females to synchronize estrus with seasonally available mates also relates to seasonality, our analyses of latitude (and thus H1) will simultaneously examine one of the predictors of H4.

To examine H3, we evaluated clade as a predictor of DI with the logic that, assuming DI is the ancestral state (Lindenfors, Dalen & Angerbjoern, 2003), its retention might be more likely in certain lineages, irrespective of other factors that may influence the selective mileau for DI (Hamlett, 1935). Finally, we examined the proposed loss of DI with reduction in body mass (H5) (Lindenfors, Dalen & Angerbjoern, 2003; Ferguson, Higdon & Larivière, 2006). For our results to be consistent with this hypothesis, we would expect DI to be less common in smaller-bodied species.

METHODS

Delayed implantation (DI) was treated as a binary dependent variable (delayed implantation = 1, no delay = 0). By analyzing 157 species of Carnivora, we were able to evaluate the role of five potential predictors of DI: $\log_{10}$ body mass, latitude, diet type (coded two different ways), and clade (family) membership.

Latitude served as a proxy for seasonality, with species at higher latitudes experiencing more seasonality than those at lower ones. Diet was coded two ways: Diet6 with five categories (carnivore [meat only], insectivore, piscivore, herbivore, and omnivore). Diet7 included two categories: carnivorous (including diets comprised of meat, insects or fish) or not carnivorous (omnivores with most of their diets comprised of plant material [>50%] and herbivores). These two diet categories (Diet6 and Diet7) were used because detailed information on percentages of various items in the diet was generally unavailable.

We examined associations between DI and phylogeney in two ways. We used the phylogenetic topology and branch lengths (see Figure 1.1) in statistical analyses to describe the expected variance-covariance matrix of residual DI and we also coded for clade membership (Family13, Figure 1.1) based on obvious monophyletic groupings in our set of species. This categorical independent variable allowed us to test the possibility that delayed implantation is largely restricted to certain clades (H3) (Gartner *et al*., 2010).

Variables in models:

DI3 (dependent)—delayed implantation coded as 0,1 (1=present, 0=absent)

Mass—log10 body mass of mean female mass (g) for a given species

Lat—absolute value of mean distance from the equator of a species' range

Diet6—Diet coded into 5 categories: herbivore, omnivore, carnivore, insectivore, piscivore

Diet7— Diet coded into 2 categories. 1=carnivorous, 0=herbivorous or omnivorous with 'plant' >50% of diet

Data collection

Data on the presence or absence of obligate delayed implantation were collected from the literature, including mammalian species accounts (Mead, 1989; Hayssen, Tienhoven & Tienhoven, 1993) and the unpublished data of Lindenfors (Appendix 1.1). If duration of delay was less than 2 weeks, then we did not consider the species to have delayed implantation (Ferguson, Virgl &

Larivière, 1996). One species, *Vulpes velox*, is polymorphic for delays throughout its range but only possesses delays in a small portion of its range (Hayssen, Tienhoven & Tienhoven, 1993; Ferguson, Virgl & Larivière, 1996; Lindenfors, Dalen & Angerbjoern, 2003), thus it was categorized as not having delays.

Latitudinal range data were taken from Davies *et al*. (2007) and supplemented with data from mammalian species accounts (as noted in Appendix 1.1). Using these data, we computed the absolute value of species range mid-points (i.e. average distance from the equator to the mid-point of a given species' range). Diet data were collected from Muñoz-Garcia & Williams (2005) and several additional sources (see Appendix 1.1).

Body mass data were collected from the literature (Mead, 1989; Hayssen, Tienhoven & Tienhoven, 1993; Ernest, 2003; Lindenfors, *unpublished data*). We recorded male and female body masses, but only mean female masses were used in analyses when data on both sexes were available. Body mass was $\log_{10}$ transformed prior to analyses. A more detailed and comprehensive list of references and data is provided in the (Appendix 1.1).

Phylogeny

We used a supertree (Nyakatura & Bininda-Emonds, 2012). This tree was made using weighted parsimony and supermatrix analyses, and includes species-level resolution with few polytomies as well as estimated branch-lengths in million of years before present. Taxonomy followed Wilson and Reeder (2005) and the tree was manipulated in Mesquite (using PDAP and PDTREE, Midford, Garland & Maddison, 2005; Maddison & Maddison, 2006). The tree used in our analyses included three additional species not present on Nyakatura and Bininda-Emond's supertree (2012; Figure 1.1). These taxa (swift fox [*Vulpes velox*], kit fox [*Vulpes macrotis*]), Cameroon clawless otter [*Aonyx congicus*]) were all once considered subspecies of species in the tree (*Vulpes lagopus* and *Aonyx capensis*, respectively), but have been recognized recently as distinct species (*Aonyx*; Jacques *et al*., 2009; and *Vulpes*; Wozencraft, 2005), and so were added in Mesquite.

Conventional statistical analyses

We used conventional logistic regression, phylogenetic logistic regression (Ives & Garland, 2010), and phylogenetic linear mixed models (PGLMM: Helmus & Ives, 2011; this paper). For the first and third of these approaches, we employed an information-theoretic approach based on Akaike's information criterion (AIC) (Burnham & Anderson, 2002) to choose among models. All possible combinations of our independent variables were considered, unless noted otherwise (in cases where models failed to converge). The non-phylogenetic analog of each model was conducted in SPSS (conventional logistic regression); and in PLogReg using (conventional logistic regression with Firth). The simplest models included only one predictor of delayed implantation. Next, we examined composite models that included more than one predictor variable

Akaike information criterion (AIC) values were calculated using maximum likelihood values: AIC=(-2*ln ML Likelihood) + (2* number of parameters in the model). AIC comparisons followed the general rules outlined by Burnham and Anderson (2002). Thus, we used AIC comparisons to simultaneously evaluate the effects of phylogeny and ecological predictors on the occurrence of delayed implantation (e.g. Gartner *et al*., 2010; Oufiero *et al*., 2011). Models with lower AIC (or AIC$_c$) values are better.

$$R^2 = 1 - \left\{ \frac{L(M_{Intercept})}{L(M_{Full})} \right\}^{2/N}$$

Because logistic regression does not apply a standard goodness-of-fit approach that minimizes variance, a statistic equivalent to the R^2 value used by ordinary least-squares regression cannot be calculated. Instead, pseudo R^2 values are estimated, which share a similar range from 0 to 1 (although some types of pseudo R^2s never reach 1). SPSS provides two specific pseudo R^2 estimates: Nagelkerke and Cox and Snell's. Nagelkerke R^2 can reach values of 1, while Cox and Snell's R^2 cannot. Both of the quasi R^2 values are based on comparing a null to a fitted model. The denominator of this ratio is similar to a sum of squared errors of a null model (predicting a dependant variable with no independent variables) and the numerator is the sum of squared errors of the fitted model. Thus, the ratio of these indicates the degree to which the model parameters improve with prediction of the null (smaller ratios indicate a greater improvement thus a higher the R-square).

*Note that the maximum value of Cox & Snell's pseudo R-squared is not 1. Instead, if the full model predicts the outcome perfectly and has a likelihood of 1, Cox & Snell's is then 1-$L(M_{Intercept})^{2/N}$, which is less than one. Nagelkerke adjusts the Cox and Snell's R^2 to include 1 by dividing Cox and Snell's R^2 by 1-L(Mintercept)$^{2/N}$. If the full model predicts the outcome perfectly, it will take a likelihood value of 1 (Nagelkerke R^2=1).

$$R^2 = \frac{1-\left\{\dfrac{L(M_{Intercept})}{L(M_{Full})}\right\}^{2/N}}{1-L(M_{Intercept})^{2/N}}$$

When $L(M_{full}) = 1$, then $R^2 = 1$;

When $L(M_{full}) = L(M_{intercept})$, then $R^2 = 0$.

Phylogenetically informed statistics

Phylogenetic analyses were performed in Matlab. We first tested for phylogenetic signal, the tendency for related species to resemble each other (Blomberg & Garland, 2002; Blomberg, Garland & Ives, 2003). We used the PHYSIG_LL.m program provided by Blomberg et al. (2003) to calculate the K statistic, the randomization test for phylogenetic signal based on the mean squared error (MSE), and the likelihood of the specified phylogenetic tree (and an assumed model of Brownian-motion like trait evolution) fitted to the data versus the likelihood of a star phylogeny fitted to the data. The K statistic (obtained with the PHYSIG_LL.m program) provides a metric of phylogenetic signal for continuous-valued traits by evaluating a trait relative to the characters evolving along a phylogeny under Brownian motion. Lower values indicate lower signal, a value of unity indicates the amount expected under Brownian motion evolution along the specified tree, and values above unity indicate even more signal than expected. Note that even when the amount of phylogenetic signal is substantially less than unity, as indicated by the K statistic, it can still be highly statistically significant via the randomization test of Blomberg et al. (2003), depending on sample size. The programs used for phylogenetic logistic regression (PLogReg: Ives & Garland, 2010) and for phylogenetic linear mixed models (PGLMM: Ives & Helmus, 2011; this study) (see below) provide tests for phylogenetic signal in a binary dependent variable, DI in the present case. PLogReg estimates a value *a* (Ives & Garland, 2010) that ranges from -4 to 4, with increasing phylogenetic signal as values approach 4 and less signal (phylogenetic signal) as values of *a* approach -4. PGLMM estimates a value σ_s^2 that is zero when there is no phylogenetic signal and increases with phylogenetic signal.

Three different analyses were used to determine the best predictors of DI: phylogenetic logistic regression (PLogReg.m; Ives & Garland, 2010), phylogenetic generalized linear mixed models (PGLMM; Helmus & Ives, 2011; this study), and a subset of models run in Regressionv2.m (Lavin *et al*., 2008). We constructed all possible independent models using our five variables either as single or co-predictors (1, 2, 3 or 4 independent variables in a model) of DI with the other variables, first singly, then two at a time, eventually containing every possible combination. Each of these models was analyzed three times using conventional and phylogenetic statistics (SPSS, Matlab: PLogReg, PGLMM) for a total of 92 separate analyses and an additional six models in Regressionv2.m.

Phylogenetic Logistic Regression (PLogReg).—We repeated our analyses as described above using standard logistic regression (*i.e.,* star phylogeny), logistic regression with a Firth correction, and finally a phylogenetically informed logistic regression (phylogenetic logistic regression). Thus, our values from both programs could be compared to verify accuracy of outputs. Phylogenetic logistic regression is based on an evolutionary model of binary traits whereby character states switch between 0 and 1 (Ives & Garland, 2010).

In phylogenetic logistic regression, we used all three of the available treatments: the star phylogeny with and without a Firth correction and phylogenetic logistic regression (Ives & Garland, 2010). However, because the first of these, assuming a star phylogeny, is the same as SPSS, upon verification that values matched those given by both programs we reported only those given by conventional statistics run in SPSS. Each analysis (logistic regression with the Firth correction and phylogenetic with the Firth correction) was run using bootstrapping to obtain confidence interval estimates and thus calculate p-values based on 2,000 iterations of the data (see Ives &

Garland, 2010). All continuous-valued independent variables were standardized in PLogReg to have a mean of 0 and variance of 1. We ran models with 2,000 bootstraps, unless the categorical variable of clade (with 13 categories and hence coding as 12 dummy variables) caused problems with convergence. Several models failed to converge and others failed to complete bootstrapping thus these models could not be completed (see Results). To test for phylogenetic signal in residual DI, the *a* statistic was used (Ives & Garland, 2010). To test for phylogenetic signal in raw DI, the *a* statistic was again used, and PLogReg was run with no independent variables.

Phylogenetic Generalized Linear Mixed Models (PGLMM).—PGLMM is an implementation of a Bernoulli Generalized Linear Mixed Model (Gelman & Hill, 2007; McCulloch *et al*., 2008; Bolker *et al.*, 2009) that incorporates phylogenetic covariances. It was developed for the analysis of community data, although here we apply it to trait data. It allows the computation of the likelihood and can therefore be used in model selection.

Suppose trait y takes values $Y_i = 0$ or $Y_i = 1$ for n species with known phylogenetic relationships. The PGLMM for the case of a single independent variable x is

$$\begin{aligned} &\Pr(Y_i=1) = \mu_i \\ &\mu_i = \mathrm{logit}^{-1}(b_0 + b_1 x_i + \varepsilon_i) \\ &\boldsymbol{\varepsilon} \sim \mathrm{Gaussian}(\mathbf{0}, \sigma_s^2 \mathbf{C}) \end{aligned} \tag{1}$$

The Bernoulli probabilities μ_i are treated as random variables, with the distribution of logit(μ_i) containing the random variable ε that contains phylogenetic information. The logit function, logit(μ) = log(μ (1 – μ)), takes values from $-\infty$ to $+\infty$ as μ varies from 0 to 1. The value of ε can be thought of as a continuously valued, phylogenetically inherited, but unmeasured trait given by a Gaussian random variable with covariance matrix $\sigma_s{}^2\mathbf{C}$. The construction of the covariance matrix

$\sigma_s^2\mathbf{C}$ can be performed under different assumptions about the evolutionary process to translate the phylogeny into anticipated covariances between ε_i for different species; the simplest process is Brownian motion evolution, in which case the off-diagonal elements of **C** are proportional to the shared branch lengths of species on the phylogeny. The diagonals of **C** are the root-to-tip distances for each species, and these distances can be equal in length (for an ultrametric tree with contemporaneous tips) or unequal (e.g., if a tree with time-calibrated branch lengths were used and some species in the phylogeny had become extinct in the distant past).

In this model, σ_s^2 measures phylogenetic signal (relative to **C**), and a test for phylogenetic signal is whether $\sigma_s^2 > 0$. There is no non-phylogenetic random term in the distribution of logit(μ_i), so in the absence of phylogenetic signal, the PGLMM reduces to Generalized Linear Model (GLM)(McCullagh & Nelder, 1989). If there were no phylogenetic signal, then a non-phylogenetic random term in logit(μ_i) would be redundant, because variability in the data is already captured in the Bernoulli probability $\Pr(Y_i=1) = \mu_i$. The random term ε_i is only needed to account for phylogenetic covariance (the off-diagonal elements of **C**).

We performed parameter estimation and computed the likelihood for model selection by combining Penalized Quasi-Likelihood (PQL) and Maximum Likelihood (ML) in a two-step process. This approach is presented in Ives and Helmus (2011), in a different context, along with extensive numerical explorations investigating the statistical properties of the approach. First, the regression coefficients are estimated via PQL, conditional on the working estimates of the variances. Second, the variances are recalculated using ML with the estimates conditional on the updated estimates for coefficients. These steps are sequentially iterated to convergence.

Phylogenetic generalized least squares and RegOU.-- The Matlab Regressionv2.m allows for phylogenetic generalized least squares and also phylogenetic regressions in which residual trait variation is modeled as evolving under an Ornstein-Uhlenbeck (OU) process, with continuous and/or categorical independent variables (Lavin *et al*., 2008). We used Regressionv2.m with latitude and body mass as dependent variables, predicted by delayed implantation (0 vs. 1) or diet, which is similar to several previous analyses of DI (Ferguson, Higdon & Larivière, 2006; see also Brashares *et al*., 2000). For each of our models, we used a star phylogeny (as assumed in conventional statistics, i.e., OLS), the tree as indicated in Figure 1 (PGLS), and the RegOU transformation of the tree that is intended to mimic stabilizing selection and provides a parameter *d* that indicates how closely the transformed tree matches the original tree, with values closer to 1 demonstrating increasing hierarchy and resemblance to the original tree and a value of zero indicating a star (Felsenstein, 1988; Garland *et al*., 1993; Blomberg, Garland & Ives, 2003; Lavin *et al*., 2008; Gartner *et al*., 2010). By comparing the fit of alternative regression models we simultaneously tested for phylogenetic signal in the residuals and whether the trait differed in relation to DI or diet.

RESULTS

Variation and phylogenetic signal in delayed implantation

The absence of obligate delayed implantation is more prevalent then it's presence among the Carnivora (Lindenfors, Dalen & Angerbjoern, 2003), including for the 157 species in our analysis (N=37 no delay, N=120 delay). Furthermore, DI had strong phylogenetic signal (p=0.0015 based on bootstrapping of a model in PLogReg that included no independent variables). In the

PGLMM program with no independent variables, the estimated S was 0.0839, with a 95% confidence interval of 0.0324 to 0.1816. In PGLMM models that included independent variables, residual DI also had statistically significant phylogenetic signal, although this result was less consistent in phylogenetic logistic regressions (see below).

Variation and phylogenetic signal in potential predictors of delayed implantation

Body mass ranged from 50 grams in the least weasel, *Mustela nivalis* to 716,667 grams in the Northern elephant seal, *Mirounga angustriostris*, with an average of 3,990 grams (SE+-5,824 g) for all taxa we examined. The average geographic range midpoint was 23.68° from the equator (minimum 0.14°, and max 70°). Members of the five diet categories listed from most to least common in our data set were Carnivores (N=91), omnivores (N=40), insectivores (N=13), piscivores (N=12), and herbivores (N=1). Number of species in our Diet7 categorization that had carnivorous diets (N=133) greatly exceeded those that were non-carnivorous (N=24).

Phylogenetic signal was present for $\log_{10}$ body mass (K=0.565; randomization test for statistical significance based on the mean squared error, $P< 0.001$; ln maximum likelihood for the specified phylogeny was -90.63 versus -174.09 for a star phylogeny). Latitude had lower phylogenetic signal (K=0.151) that was still statistically significant based on the randomization test (P=0.0005). However, for latitude, the ln maximum likelihood for the specified phylogeny was lower (-688.15) than for a star phylogeny (-671.56). This seeming discrepancy between the randomization test based on the MSE and the fit of the phylogenies can occur for reasons discussed elsewhwere (see Fusco *et al.,* 2011).

Conventional logistic regression.—In conventional logistic regression, the four top models (based on smallest AICc values) had a combined Akaike weight of 0.9972 and all four were highly statistically significant based on a χ^2 Omnibus test of Model Coefficients (all $p<1*10^{-9}$). All four top models included latitude and Family13. Body mass and Diet7 (carnivorous or not) were included in two of the top four models (see Table 1.1). The best model, which included body mass, latitude, Diet7, and Family13, had an Akaike weight of 0.6544, substantially higher than the next-best model (0.3248), which lacked body mass as an independent variable. Tallied Akaike weights for the individual variables were: Family13 (0.9999), latitude (0.9992), Diet7 (0.9368), and $\log_{10}$ mass (0.6986). In all four top models, latitude, body mass, and Diet7 were statistically significant independent variables ($P<0.05$) or marginally non-significant, based on coefficients and odds ratios tests, whereas Family13 was not.

Table 1.2 presents results of conventional logistic regression with the Firth correction, obtained with the PLogReg program (Ives and Garland, 2010). Models could not be compared via AICc with this program because it does not provide likelihood values.

Phylogenetic logistic regression.—As PLogReg does not provide likelihood values, we considered better models to be those with a greater number of statistically significant independent variables. However, this strategy could not include Family13 because we have no omnibus test for the significance of the 12 dummy variables into which it is must be coded for analyses in PLogReg. In addition, models that included other independent variables in addition to Family13 did not converge.

Ignoring Family13, our "best" model (see Table 1.3) included body mass, latitude, and Diet7, which is generally consistent with the results of conventional logistic regression, where the best model included these independent variables plus Family13 (Table 1.1). Additionally, for this model, the 95% confidence interval for α did not include -4, thus indicating statistically significant phyogenetic signal in residual DI.

PGLMM.—The best PGLMM model (i.e. lowest AICc value) included body mass and Diet6 (5 categories). This model, with an Akaike weight of 0.9980, was substantially 'better' than the next-best model that only included only body mass (Akaike weight = 0.0010). Summed Akaike weights for the individual variables in this model indicate that body mass (0.9993) and Diet6 (0.9980) have similar importance. Estimates and standard errors of the coefficient for $\log_{10}$ body mass in the best model indicate that it is not statistically significant (95% confidence interval includes 0). A similar statistic is not available for the 5-category Diet6 variable. However, the model with mass and Diet6 can be compared with a model lacking any independent variables by a likelihood ratio test. The former has a quasi-likelihood of -392.4 versus only -407.4 for the latter, indicating that the former fits the data significantly better than a model with no independent variables (χ^2 = 30.0744, d.f. = 5, P < 0.0001). Moreover, the model with only body mass (likelihood = -403.6) as an independent variable fits significantly better than the null model variables (χ^2 = 7.7226, d.f. = 1, P = 0.0055), as does the model with only Diet6 (likelihood = -416.2, χ^2 = 17.4424, d.f. = 4, P = 0.0016). Finally, we can compare the model with only mass and the model with only Diet6 to the model with both mass and Diet6. In both cases, the differences in ln likelihoods are highly statistically significant (χ^2 = 22.3518, d.f. = 4, P < 0.0002 and χ^2 = 47.5168, d.f. = 1, P < $1*10^7$, respectively), thus indicating that the fuller model provides better fit. Taken together, we interpret these results to mean that the effects of body mass and Diet6 are somehow interacting to predict DI in the best-fitting model (see Table 1.4). The estimate of the parameter S was always significantly different from zero in the top 13 models, indicating phylogenetic signal in residual DI.

Phylogenetic ANOVA of body mass and latitude.—Body mass and latitude were analyzed as dependent variables in Regressionv2.m (Lavin *et al*., 2008) to determine whether they differed between species that did and did not exhibit DI, and also to test for confounding with diet (Table 1.5). Body mass did not differ significantly between species with and without DI in the best-fitting models (PGLS and RegOU). Latitude, however, was significantly higher in species with DI (Fig. 1.2).

Body mass did not vary significantly in relation to either of our diet categorizations in the much-better fitting phylogenetic models (Table 1.5). The RegOU model was the best fitting for analysis of latitude in relation to both diet categories. In these RegOU models, latitude differed statistically in relation to Diet7 (two categories) but not in relation to Diet6 (five categories).

DISCUSSION

As is apparent from Figure 1, delayed implantation in the Carnivora is not randomly distributed with respect to phylogenetic relationships, as has been previously noted (Lindenfors, Dalen & Angerbjoern, 2003). This non-randomness is demonstrated by the fact that our PGLMM analyses (Table 1.4) indicated significant phylogenetic signal in residual DI, i.e., after accounting for the predictive ability of body mass and diet (as well a latitude, although this was not an important predictor of DI in the PGLMM models). Consistent with this result, all of the better-fitting models in conventional logistic regression included our variable coding for clade membership

(Family13; see Table 1.1). Thus, some clades were more likely to possess delays relative to others.

Body mass was an important predictor of DI in the best models in all of our analyses (conventional logistic regression, phylogenetic logistic regression, and PGLMM). The slope of the relationship indicates that larger-bodied species were more likely to have delays relative to smaller ones. We also found that DI occurs more in species that inhabit higher latitudes, and hence more seasonal environments. Delays thus could provide females with a selective advantage by allowing them to fine-tune the timing of reproductive events, including pregnancy, lactation (H1), and mating (H4) relative to seasonal variation.

The relation of diet to DI has not previously been examined in the Carnivora. Although included in the top PGLMM and conventional logistic regression models, diet was the independent variable least consistently in the best models (both Diet6 and Diet7). Which way of categorizing diet better predicted DI varied among analysis types?

Diet type and latitude are associated both with phylogeny and with each other. For example, a conventional contingency analysis of both Diet6 and Diet7 with Family13 is statistically significant in both cases (Diet6; Pearson’s R $p=0.002$, Pearson's χ^2 $p<4*10^{-42}$ and Diet7; Pearson’s R $p>0.7$, Pearson's χ^2 $p<8*10^{-15}$). These associations complicate the analysis of which "independent variables" best predict delayed implantation. However, our general conclusion is that body mass followed by diet and then latitude all account for some of the variation in the presence of delayed implantation in the Carnivora, and that DI is also associated with phylogeny beyond these effects. We do not yet provide analyses that would allow us to determine if clades with origins in the glacial periods are more likely to have delays (H3), but this would be an interesting future direction. Our results are consistent with the hypothesis that delays may have been lost in some smaller-bodied species (H5), perhaps due to fecundity selection (Lindenfors, Dalen & Angerbjoern, 2003). However, DI also may be associated with latitude and hence variation in seasonality (H1, H4) (Ferguson, Virgl & Larivière, 1996; Thom, Johnson & Macdonald, 2004; this study, results of conventional and phylogenetic logistic regressions, but not PGLMM).

The present comparative study provides an important and overdue evaluation of the putative predictors of delayed implantation, while treating delays in a perhaps more biologically and statistically appropriate manner; as a binary dependent variable. Furthermore, the phylogenetically informed statistical analyses applied here have not previously been used in the study of DI. The new PGLMM approach presented here was particularly useful because it allowed both model-selection and hypothesis testing with particular independent variables.

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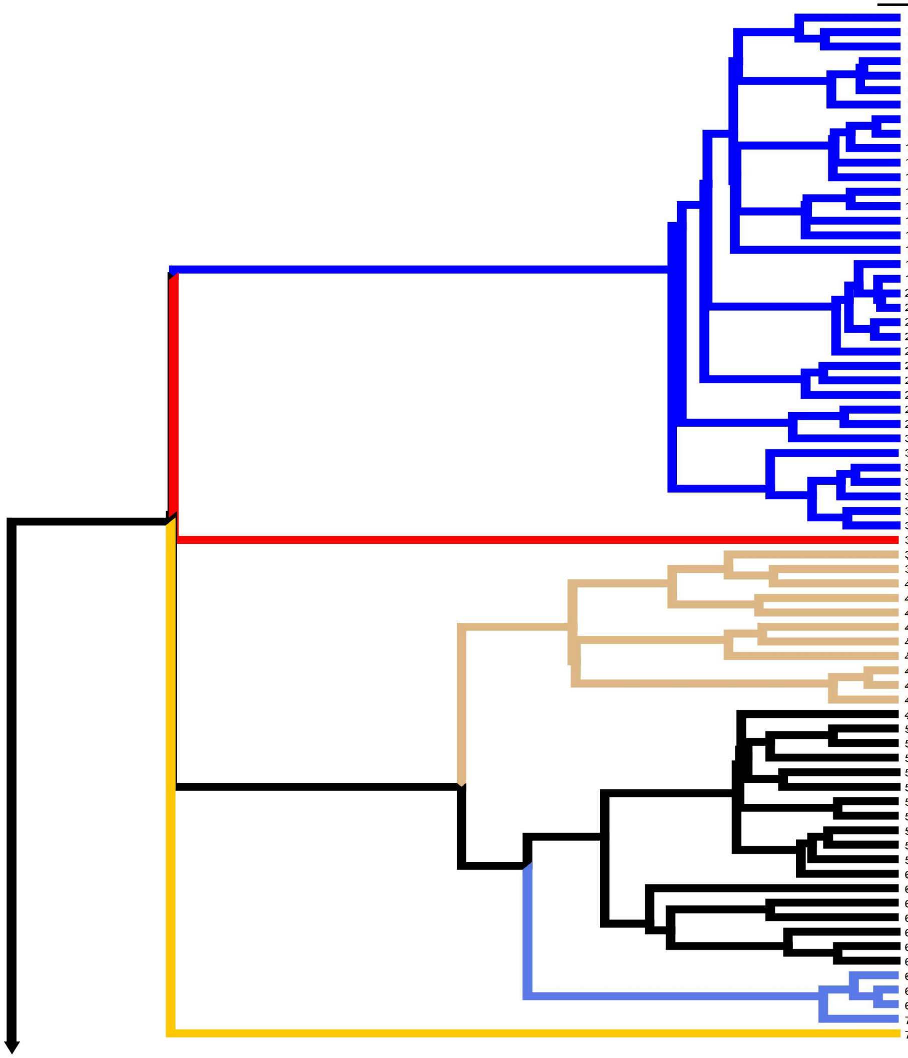
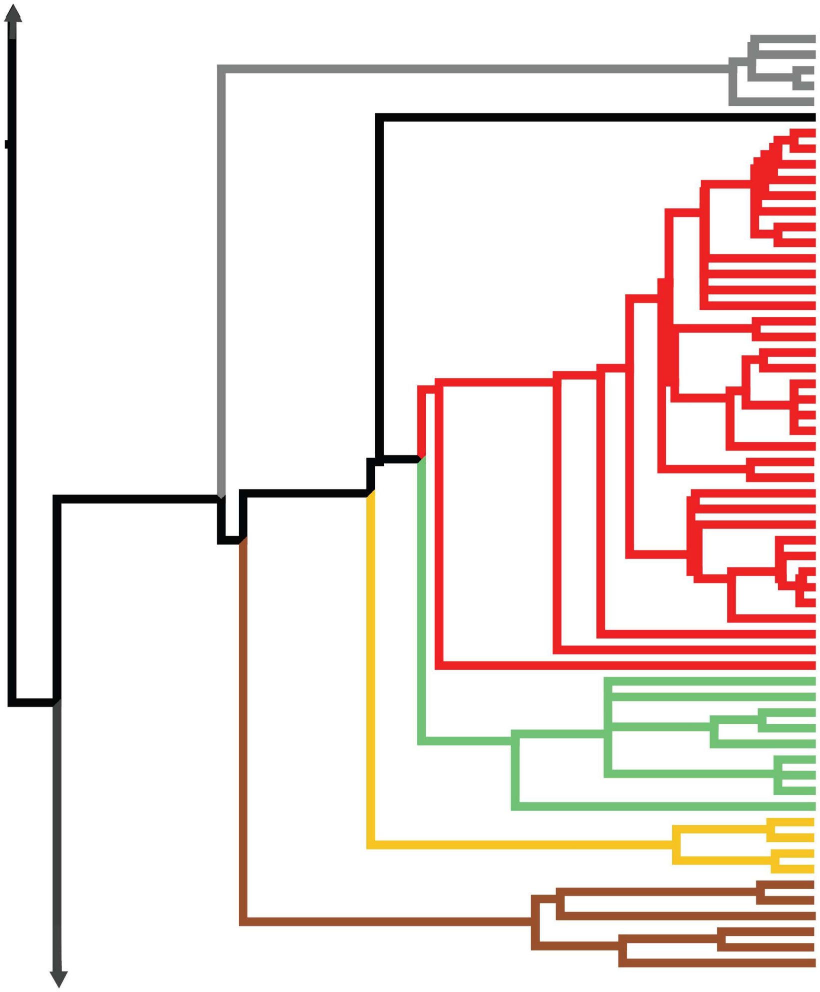
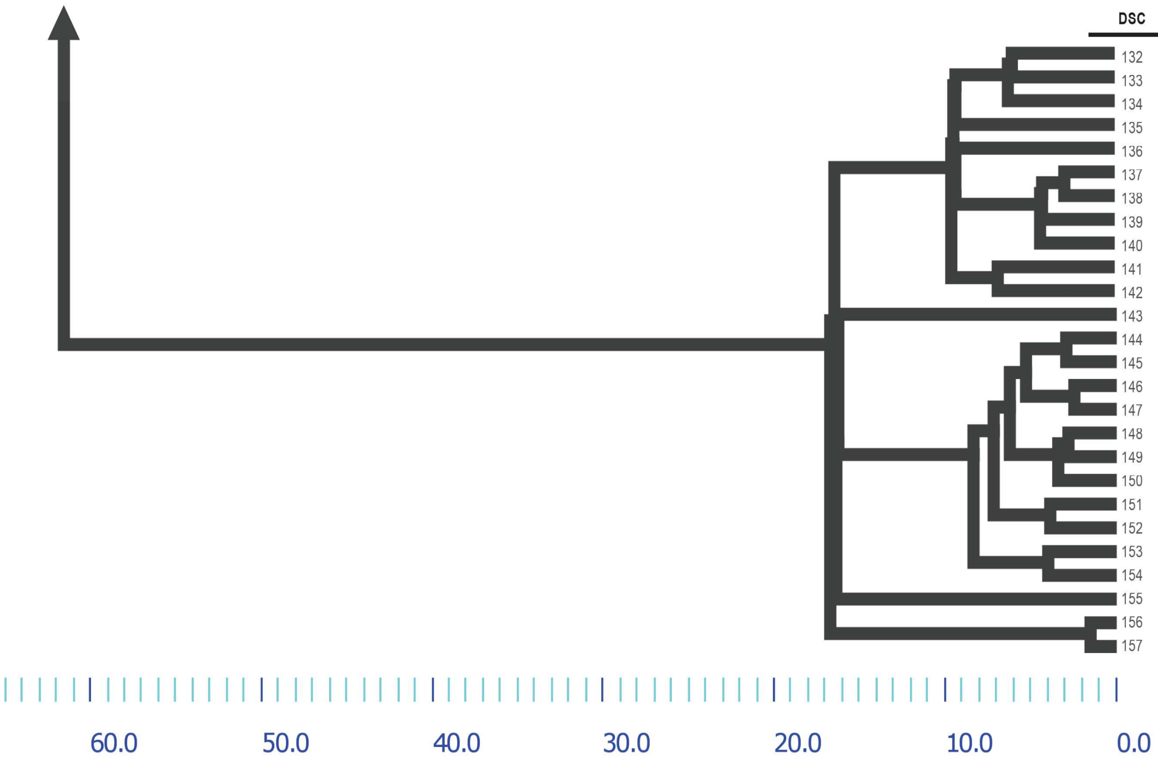
Figure 1.1.—Carnivora phylogeny used in analyses (Nyakatura & Bininda-Emonds, 2012). The tree below is split into three panels. Arrrows indicates where the three parts of the tree attach. Clade coding is indicated by different colors (consistent with those used in the appendix). The blue scale bar at the bottom of the third tree is in millions of years before present.



DSC Order	Family13	Mesquite Name	DI	Diet6	Diet7
1	1	aj_Acinonyx_jubatus	0	4	1
2	1	zq_Puma_concolor	0	4	1
3	1	hy_Puma_yagouaroundi	0	4	1
4	1	l8_Lynx_canadensis	0	4	1
5	1	l9_Lynx_lynx	0	4	1
6	1	la_Lynx_pardinus	0	4	1
7	1	lb_Lynx_rufus	0	4	1
8	1	FB_Felis_bieti	0	4	1
9	1	fs_Felis_silvestris	0	4	1
10	1	fm_Felis_margarita	0	4	1
11	1	fc_Felis_chaus	0	4	1
12	1	fn_Felis_nigripes	0	4	1
13	1	p7_Prionailurus_bengalensis	0	4	1
14	1	p8_Prionailurus_viverrinus	0	4	1
15	1	PQ_Prionailurus_planiceps	0	0	1
16	1	pu_Prionailurus_rubiginosus	0	4	1
17	1	om_Felis_manul	0	4	1
18	1	oc_Leopardus_colocolo	0	1	1
19	1	og_Leopardus_geoffroyi	0	4	1
20	1	O2_Leopardus_guigna	0	4	1
21	1	lt_Leopardus_tigrinus	0	4	1
22	1	lo_Leopardus_pardalis	0	4	1
23	1	lw_Leopardus_wiedii	0	4	1
24	1	OJ_Leopardus_jacobitus	0	4	1
25	1	CB_Catopuma_badia	0	4	1
26	1	ct_Catopuma_temminckii	0	4	1
27	1	pm_Pardofelis_marmorata	0	4	1
28	1	cc_Caracal_caracal	0	1	1
29	1	py_Profelis_aurata	0	4	1
30	1	ls_Leptailurus_serval	0	4	1
31	1	n2_Neofelis_nebulosa	0	4	1
32	1	p1_Panthera_leo	0	4	1
33	1	pp_Panthera_pardus	0	4	1
34	1	po_Panthera_onca	0	4	1
35	1	pt_Panthera_tigris	0	4	1
36	1	uu_Uncia_uncia	0	4	1
37	2	pd_Prionodon_pardicolor	0	4	1
38	3	ab_Arctictis_binturong	0	1	0
39	3	ph_Paradoxurus_hermaphroditus	0	1	0
40	3	pl_Paguma_larvata	0	1	0
41	3	hd_Hemigalus_derbyanus	0	1	0
42	3	cn_Cynogale_bennettii	0	1	0
43	3	c5_Civettictis_civetta	1	1	0
44	3	vz_Viverra_zibetha	0	4	1
45	3	vi_Viverricula_indica	0	4	1
46	3	gm_Genetta_maculata	0	4	1
47	3	g5_Genetta_tigrina	0	4	1
48	3	g4_Genetta_genetta	0	4	1
49	4	ad_Atilax_paludinosus	1	1	0
50	4	ck_Cynictis_penicillata	0	2	1
51	4	ps_Paracynictis_selousi	0	2	1
52	4	ia_Ichneumia_albicauda	0	2	1
53	4	go_Galerella_sanguinea	0	4	1
54	4	hi_Herpestes_ichneumon	0	4	1
55	4	he_Herpestes_edwardsi	0	4	1
56	4	hj_Herpestes_javanicus	0	4	1
57	4	cg_Crossarchus_obscurus	0	2	1
58	4	ho_Helogale_parvula	0	1	0
59	4	mb_Mungos_mungo	0	2	1
60	4	ss_Suricata_suricatta	0	2	1
61	4	ci_Cryptoprocta_ferox	0	4	1
62	4	eg_Eupleres_goudotii	0	2	1
63	4	ff_Fossa_fossana	0	4	1
64	4	ge_Galidia_elegans	0	4	1
65	4	g2_Galidictis_fasciata	0	4	1
66	4	md_Mungotictis_decemlineata	0	2	1
67	5	c8_Crocuta_crocuta	0	4	1
68	5	pb_Hyaena_brunnea	0	4	1
69	5	hh_Hyaena_hyaena	0	1	1
70	5	zj_Proteles_cristata	0	2	1
71	6	n3_Nandinia_binotata	0	1	0



DSC	Fam13	Mesquite Name	DI	D6	D7
72	7	hm_Helarctos_malayanus	1	1	0
73	7	ua_Ursus_americanus	1	1	0
74	7	u1_Ursus_arctos	1	1	0
75	7	um_Ursus_maritimus	1	4	1
76	7	mu_Melursus_ursinus	1	1	0
77	8	a9_Ailurus_fulgens	1	3	0
78	9	a1_Aonyx_capensis	0	4	1
79	9	a2_Aonyx_congicus	0	1	1
80	9	ac_Aonyx_cinerea	0	1	1
81	9	l5_Lutrogale_perspicillata	0	4	1
82	9	l3_Lutra_lutra	0	0	1
83	9	lm_Hydrictis_maculicollis	0	0	1
84	9	l2_Lontra_canadensis	1	0	1
85	9	ll_Lontra_longicaudis	1	0	1
86	9	el_Enhydra_lutris	1	0	1
87	9	gv_Galictis_vittata	0	4	1
88	9	pa_Poecilogale_albinucha	0	4	1
89	9	zp_Pteronura_brasiliensis	0	0	1
90	9	mv_Mustela_frenata	1	4	1
91	9	zg_Neovison_vison	1	4	1
92	9	mj_Mustela_altaica	0	4	1
93	9	za_Mustela_nivalis	0	4	1
94	9	zd_Mustela_putorius	1	4	1
95	9	mx_Mustela_lutreola	1	4	1
96	9	my_Mustela_nigripes	0	4	1
97	9	ze_Mustela_sibirica	0	4	1
98	9	mq_Mustela_erminea	1	4	1
99	9	is_Ictonyx_striatus	0	4	1
100	9	vp_Vormela_peregusna	1	4	1
101	9	eb_Eira_barbara	0	1	1
102	9	mp_Martes_pennanti	1	4	1
103	9	g6_Gulo_gulo	1	4	1
104	9	ma_Martes_americana	1	4	1
105	9	m1_Martes_foina	1	1	1
106	9	mn_Martes_martes	1	4	1
107	9	mz_Martes_zibellina	1	4	1
108	9	M2_Martes_melampus	1	1	1
109	9	mf_Martes_flavigula	1	1	0
110	9	m3_Meles_meles	1	1	1
111	9	tt_Taxidea_taxus	1	1	1
112	9	mc_Mellivora_capensis	1	4	1
113	10	bg_Bassaricyon_gabbii	0	1	0
114	10	b1_Bassariscus_astutus	0	1	0
115	10	nn_Nasua_narica	0	1	0
116	10	n1_Nasua_nasua	0	1	0
117	10	NO_Nasuella_olivacea	0	1	0
118	10	pe_Procyon_cancrivorus	0	1	0
119	10	pk_Procyon_lotor	0	1	1
120	10	PX_Procyon_pygmaeus	0	1	0
121	10	p6_Potos_flavus	0	1	0
122	11	m6_Mephitis_macroura	1	2	1
123	11	m7_Mephitis_mephitis	1	1	1
124	11	sp_Spilogale_putorius	0	4	1
125	11	s1_Spilogale_pygmaea	0	2	1
126	12	At_Arctocephalus_townsendi	1	0	1
127	12	nc_Neophoca_cinerea	1	0	1
128	12	or_Odobenus_rosmarus	1	2	1
129	12	cq_Cystophora_cristata	1	0	1
130	12	p4_Pusa_sibirica	1	0	1
131	12	ml_Mirounga_leonina	1	0	1



DSC	Family13	Mesquite Name	DI	Diet6	Diet7
132	13	*vf_Vulpes_ferrilata*	0	4	1
133	13	*zk_Lycalopex_culpaeus*	0	4	1
134	13	*c3_Cerdocyon_thous*	0	4	1
135	13	*c4_Chrysocyon_brachyurus*	0	1	0
136	13	*cm_Canis_mesomelas*	0	4	1
137	13	*cl_Canis_latrans*	0	4	1
138	13	*c2_Canis_lupus*	0	4	1
139	13	*cs_Canis_simensis*	0	4	1
140	13	*cj_Cuon_alpinus*	0	2	1
141	13	*l6_Lycaon_pictus*	0	4	1
142	13	*sv_Speothos_venaticus*	0	4	1
143	13	*np_Nyctereutes_procyonoides*	0	1	1
144	13	*vb_Vulpes_bengalensis*	0	4	1
145	13	*v2_Vulpes_corsac*	0	4	1
146	13	*vr_Vulpes_rueppellii*	0	1	1
147	13	*v4_Vulpes_vulpes*	1	4	1
148	13	*Al_Vulpes_lagopus*	0	4	1
149	13	*vd_Vulpes_macrotis*	0	4	1
150	13	*vv_Vulpes_velox*	0	4	1
151	13	*va_Vulpes_cana*	0	1	1
152	13	*v5_Vulpes_zerda*	0	1	1
153	13	*v1_Vulpes_chama*	0	4	1
154	13	*v3_Vulpes_pallida*	0	4	1
155	13	*o3_Otocyon_megalotis*	0	1	1
156	13	*uc_Urocyon_cinereoargenteus*	0	4	1
157	13	*ul_Urocyon_littoralis*	0	4	1

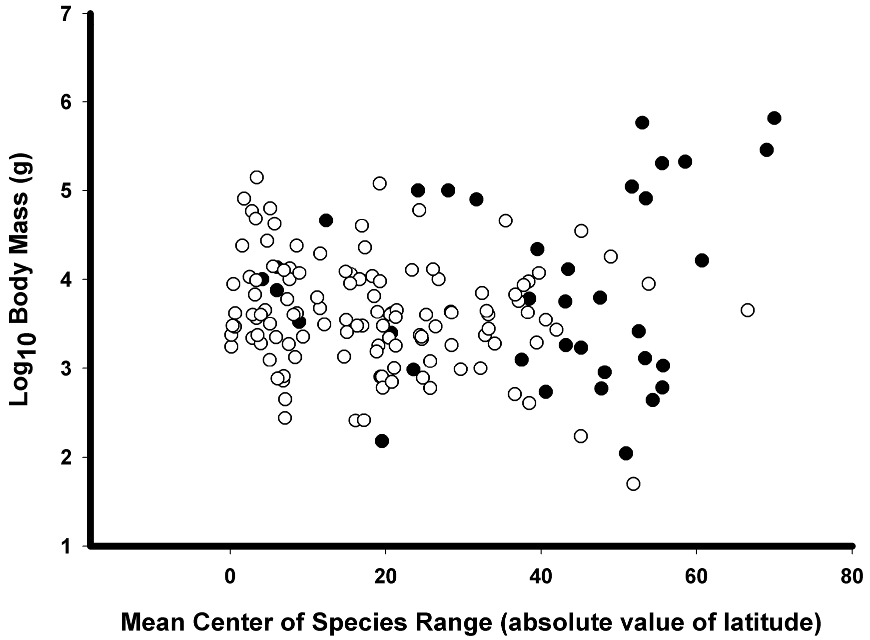


Figure 1.2.— Body mass in relation to mean center of species range distance and reproductive physiology type. Mean center of species range distance is taken from Davies *et al*. (2007) and is the distance from the equator (absolute value of latitude). Open circles indicate absence of obligate DI while solid black circles indicate species with DI. Regressions of log$_{10}$ body mass on latitude were not statistically significant (OLS, PGLS, RegOU [*d*=0.90]; all P>0.5). However, DI was a significant predictor of latitude (OLS, PGLS, RegOU [*d*=0.22]; all P< 0.000003) and of log$_{10}$ body mass (OLS only; P=0.042).

TABLE 1.1. Conventional logistic regression models sorted by AICc (lowest to highest). Results from the SPSS Logistic Regression procedure. R^2 values are Naglekerke R^2 values which as a pseudo R^2 value are less reliable than other R^2 values and thus should be interpreted with caution.

Analysis	Ind_Vars	Model	ModelNum	N_sp	2LLikli	LogLikl	NIndVars	N_Param	AIC	AICc
SPSS	Mass + Latitude + Diet7 + Family13	Reg_Log	23	157	51.6296	-25.8148	15	16	83.63	87.52
SPSS	Latitude + Diet7 + Family13	Reg_Log	21	157	55.7962	-27.8981	14	15	85.80	89.20
SPSS	Mass + Latitude + Family13	Reg_Log	14	157	59.5577	-29.7789	14	15	89.56	92.96
SPSS	Latitude + Family13	Reg_Log	9	157	63.7414	-31.8707	13	14	91.74	94.70
SPSS	Mass + Latitude + Diet6 + Family13	Reg_Log	15	157	56.8445	-28.4223	18	19	94.84	100.39
SPSS	Latitude + Diet6 + Family13	Reg_Log	12	157	59.5946	-29.7973	17	18	95.59	100.55
SPSS	Family13 + Diet7	Reg_Log	19	157	70.9788	-35.4894	13	14	98.98	101.94
SPSS	Mass + Diet7 + Family13	Reg_Log	20	157	70.7479	-35.3740	14	15	100.75	104.15
SPSS	Family13	Reg_Log	4	157	76.2521	-38.1260	12	13	102.25	104.80
SPSS	Mass + Family13	Reg_Log	7	157	75.8589	-37.9295	13	14	103.86	106.82
SPSS	Family13 + Diet6	Reg_Log	10	157	74.1087	-37.0543	16	17	108.11	112.51
SPSS	Mass + Diet6 + Family13	Reg_Log	13	157	73.9141	-36.9570	17	18	109.91	114.87
SPSS	Latitude + Diet6	Reg_Log	8	157	104.9454	-52.4727	5	6	116.95	117.51
SPSS	Mass + Latitude + Diet6	Reg_Log	11	157	104.9304	-52.4652	6	7	118.93	119.68
SPSS	Latitude + Diet7	Reg_Log	18	157	118.1086	-59.0543	2	3	124.11	124.27
SPSS	Mass + Diet7 + Latitude	Reg_Log	22	157	116.3833	-58.1916	3	4	124.38	124.65
SPSS	Mass + Latitude	Reg_Log	5	157	124.9739	-62.4870	2	3	130.97	131.13
SPSS	Latitude	Reg_Log	2	157	127.5971	-63.7986	1	2	131.60	131.68
SPSS	Diet6	Reg_Log	3	157	153.4447	-76.7223	4	5	163.44	163.84
SPSS	Mass + Diet6	Reg_Log	6	157	152.5491	-76.2745	5	6	164.55	165.11
SPSS	Mass	Reg_Log	1	157	167.3407	-83.6704	1	2	171.34	171.42
SPSS	Mass + Diet7	Reg_Log	17	157	166.1296	-83.0648	2	3	172.13	172.29
SPSS	Diet7	Reg_Log	16	157	170.0491	-85.0245	1	2	174.05	174.13

R2_modC	R2_modN	Const	SEC	WaldC	WaldMass	WaldLat	WaldDie6	WaldDie7	WaldFm13
0.5338	0.8034	-12.0629	3.6983	10.6392	3.6281	12.5028		6.4110	13.9706
0.5213	0.7845	-6.1925	1.4781	17.5521		11.6132		6.3289	12.8868
0.5097	0.7671	-10.7334	3.3193	10.4564	3.7348	11.6256			15.5518
0.4965	0.7471	-5.3371	1.3179	16.4001		10.2325			14.3550
0.5181	0.7797	-11.0480	3.6194	9.3173	2.5636	11.4951	2.5102		13.9232
0.5096	0.7669	-6.2401	1.5040	17.2145		11.0595	3.7132		12.7959
0.4727	0.7114	-3.6848	1.1795	9.7602				4.3705	15.0289
0.4735	0.7125	-4.5264	2.1267	4.5299	0.2299			4.2572	15.2400
0.4547	0.6843	-3.2189	1.0198	9.9627					20.7204
0.4560	0.6863	-4.2958	2.0114	4.5614	0.3904				20.9183
0.4621	0.6954	-3.4782	1.0673	10.6207			2.0355		19.5861
0.4627	0.6964	-4.3183	2.2012	3.8486	0.1930		1.8585		19.2831
0.3453	0.5197	-4.9834	0.8016	38.6524		29.6318	15.3993		
0.3454	0.5198	-5.1301	1.4499	12.5199	0.0150	29.3977	13.8107		
0.2881	0.4335	-4.3736	0.6858	40.6670		31.1848		9.3178	
0.2959	0.4452	-5.8827	1.4094	17.4227	1.6627	29.7455		8.5877	
0.2563	0.3857	-5.2485	-5.2485	16.9264	2.5021	29.6682			
0.2437	0.3668	-3.4819	0.5169	9.7086		31.0630			
0.1084	0.1631	-1.7047	0.2905	34.4269			13.9951		
0.1135	0.1707	-2.7062	1.1083	5.9624	0.9000		11.0388		
0.0259	0.0389	-3.1162	1.0001	9.7086	4.0420				
0.0334	0.0502	-3.1813	1.0108	9.9053	3.8585			1.2622	
0.0089	0.0134	-1.2771	0.2100	36.9850				1.4724	

ChiSq	DF	Pmodel	DI30_P0	DI30_P1	DI31_P0	DI31_P1	Prct_C	PL10MASS	P_Lat	P_Diet6	P_Diet7	P_Fam13
119.83	15	0.00000000000000000000	115	5	6	31	92.99	0.0568	0.0004		0.0113	0.3026
115.66	14	0.00000000000000000000	115	5	7	30	92.36		0.0007		0.0119	0.3773
111.90	14	0.00000000000000000000	115	5	8	29	91.72	0.0533	0.0007			0.2126
107.71	13	0.00000000000000000000	115	5	8	29	91.72		0.0014			0.2786
114.61	17	0.00000000000000011102	113	7	7	30	91.08	0.1094	0.0007	0.4735		0.3056
111.86	16	0.00000000000000022204	114	6	7	30	91.72		0.0009	0.2941		0.3841
100.48	13	0.00000000000000133227	103	17	3	34	87.26				0.0366	0.2399
100.71	14	0.00000000000000344169	106	14	5	32	87.90	0.6316			0.0391	0.2286
95.20	12	0.00000000000000477396	103	17	3	34	87.26					0.0546
95.60	13	0.00000000000001176836	107	13	5	32	88.54	0.0000				0.0516
97.35	15	0.00000000000004141132	107	13	7	30	87.26			0.7292		0.0514
97.54	16	0.00000000000009992007	109	11	8	29	87.90	0.6604		0.6023		0.0819
66.51	1	0.00000000050640415756	113	7	14	23	86.62		0.0000	0.0039		
66.52	1	0.00040263721280430000	114	6	14	23	87.26	0.90268	0.0000	0.0079		
53.35	2	0.00000000000260569344	113	7	17	20	84.71		0.0000		0.0023	
55.07	3	0.00000000000662758737	115	5	19	18	84.71	0.19724	0.0000		0.0034	
46.48	2	0.00000000008066980417	116	4	19	18	85.35	0.11369	0.0000			
43.86	1	0.00000000003530642445	114	6	19	18	84.08		0.0000			
18.01	4	0.00000000442566424385	116	4	28	9	79.62			0.0073		
18.91	5	0.00151705981623300000	116	4	28	9	79.62	0.34279		0.0261		
4.11	1	0.04438138041786000000	120	0	37	0	76.43	0.04438				
5.33	2	0.06975202178089000000	120	0	36	1	77.07	0.04950			0.2612	
1.41	1	0.23569556013230000000	120	0	37	0	76.43				0.2250	

$Delta_i$	expdel1	w_i	acc_w_i	ER	Mass	Lat	Diet6	Diet7	Family13
0.00	1.0000	0.6544	0.6544	1	1	1	0	1	1
1.69	0.4306	0.2818	0.9362	2.32228587	0	1	0	1	1
5.45	0.0657	0.0430	0.9792	15.23088788	1	1	0	0	1
7.18	0.0275	0.0180	0.9972	36.30356273	0	1	0	0	1
12.88	0.0016	0.0010	0.9982	625.3558318	1	1	1	0	1
13.04	0.0015	0.0010	0.9992	677.1607304	0	1	1	0	1
14.42	0.0007	0.0005	0.9997	1353.739501	0	0	0	1	1
16.64	0.0002	0.0002	0.9998	4098.689289	1	0	0	1	1
17.28	0.0002	0.0001	1.0000	5659.634786	0	0	0	0	1
19.30	0.0001	0.0000	1.0000	15532.42884	1	0	0	0	1
25.00	0.0000	0.0000	1.0000	267832.9461	0	0	1	0	1
27.36	0.0000	0.0000	1.0000	871215.0579	1	0	1	0	1
29.99	0.0000	0.0000	1.0000	3252887.092	0	1	1	0	0
32.17	0.0000	0.0000	1.0000	9658971.581	1	1	1	0	0
36.75	0.0000	0.0000	1.0000	95540270.52	0	1	0	1	0
37.13	0.0000	0.0000	1.0000	115588089.1	1	1	0	1	0
43.62	0.0000	0.0000	1.0000	2957864612	1	1	0	0	0
44.16	0.0000	0.0000	1.0000	3882956366	0	1	0	0	0
76.33	0.0000	0.0000	1.0000	3.75087E+16	0	0	1	0	0
77.59	0.0000	0.0000	1.0000	7.06764E+16	1	0	1	0	0
83.90	0.0000	0.0000	1.0000	1.6572E+18	1	0	0	0	0
84.77	0.0000	0.0000	1.0000	2.55755E+18	1	0	0	1	0
86.61	0.0000	0.0000	1.0000	6.4193E+18	0	0	0	1	0

WtMass	WtLat	WtDiet6	WtDiet7	WtFam13	SE_Mass	SE_Lat	SE_Diet7
0.6544002954	0.6544002954	0.0000000000	0.6544002954	0.6544002954	0.6712	0.0270	1.6896
0.0000000000	0.2817914469	0.0000000000	0.2817914469	0.2817914469		0.0218	1.6638
0.0429653413	0.0429653413	0.0000000000	0.0000000000	0.0429653413	0.6296	0.0243	
0.0000000000	0.0180257872	0.0000000000	0.0000000000	0.0180257872		0.0199	
0.0010464447	0.0010464447	0.0010464447	0.0000000000	0.0010464447	0.7293	0.0263	
0.0000000000	0.0009663884	0.0009663884	0.0000000000	0.0009663884		0.0223	
0.0000000000	0.0000000000	0.0000000000	0.0004834019	0.0004834019			1.5442
0.0001596609	0.0000000000	0.0000000000	0.0001596609	0.0001596609	0.4586		1.5392
0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0001156259			
0.0000421312	0.0000000000	0.0000000000	0.0000000000	0.0000421312	0.4610		
0.0000000000	0.0000000000	0.0000024433	0.0000000000	0.0000024433			
0.0000007511	0.0000000000	0.0000007511	0.0000000000	0.0000007511	0.5450		
0.0000000000	0.0000002012	0.0000002012	0.0000000000	0.0000000000		0.0177	
0.0000000678	0.0000000678	0.0000000678	0.0000000000	0.0000000000	0.3590	0.0178	
0.0000000000	0.0000000068	0.0000000000	0.0000000068	0.0000000000		0.0175	0.6647
0.0000000057	0.0000000057	0.0000000000	0.0000000057	0.0000000000	0.3238	0.0179	0.6719
0.0000000002	0.0000000002	0.0000000000	0.0000000000	0.0000000000	0.3031	0.0148	
0.0000000000	0.0000000002	0.0000000000	0.0000000000	0.0000000000		0.0143	
0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000			
0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.3590		
0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.2575		
0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.2595		0.4878
0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000			0.4812
0.6986146982	**0.9991959856**	**0.0020162965**	**0.9368348176**	**0.9999997182**			

TABLE 1.2. Conventional logistic regression models with the Firth correction (sorted by independent variables included). Results from the PLogReg Matlab program of Ives and Garland (2010).

Analysis	Ind_Vars	Model	ModelNum	N	Convergd	Boot	Fam13	NIndVars	bo	se_B0
plogreg	Mass*	OLR+Firth	1	157	1	1	0	1	-1.1659	0.18965
plogreg	Latitude*	OLR+Firth	2	157	1	1	0	1	-1.8259	0.27795
plogreg	Diet7*	OLR+Firth	16	157	1	1	0	1	-0.6632	0.38105
plogreg	Diet6	OLR+Firth	3	157	1	1	0	4	-1.4988	0.26659
plogreg	Family13	OLR+Firth	4	157	0	0	1	12	-0.7335	0.14677
plogreg	Mass + Latitude*	OLR+Firth	5	157	1	1	0	2	-1.7781	0.27509
plogreg	Mass + Diet7*	OLR+Firth	17	157	1	1	0	2	0.4410	0.53309
plogreg	Mass + Diet6	OLR+Firth	6	157	1	1	0	5	-1.3270	0.24261
plogreg	Latitude* + Diet7*	OLR+Firth	18	157	1	1	0	2	0.1582	0.55753
plogreg	Latitude* + Diet6	OLR+Firth	8	157	1	1	0	5	-2.3764	0.37159
plogreg	Mass + Family13	OLR+Firth	7	157	1	0	1	13	-0.8322	0.47095
plogreg	Latitude + Family13	OLR+Firth	9	157	1	0	1	13	-1.5540	0.27892
plogreg	Family13 + Diet7	OLR+Firth	19	157	1	0	1	13	1.4485	0.24249
plogreg	Family13 + Diet6	OLR+Firth	10	157	1	0	1	16	-0.3800	0.21399
plogreg	Mass + Latitude* + Diet7*	OLR+Firth	22	157	1	0	0	3	0.0596	0.54550
plogreg	Mass + Latitude* + Diet6	OLR+Firth	11	157	1	0	0	6	-2.3560	0.35253
plogreg	Mass + Latitude + Family13	OLR+Firth	14	157	1	0	1	14	-1.2453	0.27451
plogreg	Mass + Diet7 + Family13	OLR+Firth	20	157	1	0	1	14	0.1350	0.31109
plogreg	Mass + Diet6 + Family13	OLR+Firth	13	157	1	0	1	17	-0.4213	0.09669
plogreg	Latitude + Diet7 + Family13	OLR+Firth	21	157	1	0	1	14	-0.0888	0.38926
plogreg	Latitude + Diet6 + Family13	OLR+Firth	12	157	1	0	1	17	-1.7078	0.24400
plogreg	Mass + Latitude + Diet7 + Family13	OLR+Firth	23	157	1	0	1	15	0.1377	0.18573
plogreg	Mass + Latitude + Diet6 + Family13	OLR+Firth	15	157	1	0	1	18	-1.8956	0.23651

B_Mass	SE_Bmass	B_Lat	SE_Lat	B_Diet7	SE_Diet7	N_boot	b0_1boot	b0_2boot	b0_3boot	bo_pboot
0.4165	0.20792					2000	-1.5626	-1.1666	-0.80108	0
		1.2907	0.23267			2000	-2.4388	-1.8231	-1.3399	0
				-0.60153	0.42429	2000	-0.31044	0.64136	2.037	0.282
						2000	-2.0493	-1.4935	-1.0134	0
0.3711	0.24263	1.2859	0.23804			2000	-2.3735	-1.7761	-1.2923	0
0.2381	0.22056			-1.7818	0.56433	2000	-0.77787	0.4393	1.8719	0.486
0.2387	0.21451					250	-2.0128	-1.3294	-0.80712	0
		1.2760	0.23364	-2.1836	0.59385	2000	-1.371	0.14383	1.5469	0.725
		1.3100	0.23487			2000	-3.2926	-2.3736	-1.6607	0
0.2783	0.30446					250	-2.6292	-1.5085	-0.70325	0
		1.4186	0.24523				-2.6292	-1.5085	-0.70325	0
				-2.5374	0.33556					
0.1549	0.25388	1.2600	0.23330	-2.0323	0.58621					
0.0711	0.24797	1.2921	0.22495							
0.4144	0.26484	1.2506	0.25271							
0.1530	0.16763			-1.3783	0.56315					
-0.0840	0.09970									
		1.2193	0.25803	-1.2913	0.20862					
		1.5174	0.21161							
0.4673	0.10980	1.2035	0.11061	-1.3479	0.18980					
0.2591	0.25650	1.3661	0.24369							

MasbootU	Massboot	MasbootL	pMasboot	LatBootU	LatBoot	LatBootL	p_Lat_b	Die7botU	Die7bot	Die7botL	p_Diet7b
0.000646	0.42335	0.85054	0.048								
								-3.4106	-2.0127	-0.82518	0.002
-0.078903	0.37682	0.90785	0.102	0.83674	1.2813	1.7999	0				
-0.21278	0.24963	0.69528	0.268					-3.2764	-1.776	-0.49034	0.007
-0.21674	0.23773	0.67708	0.296								
				0.84294	1.2727	1.8186	0	-3.8333	-2.1685	-0.70443	0.006
				0.87342	1.3063	1.8475	0				
0.83972	1.4017	2.0071	0								

Table 1.3. Phylogenetic logistic regression models (Ives & Garland 2010; sorted by independent variables included). Results from the PLogReg Matlab program of Ives and Garland (2010). Values of α are listed here with their bootstrapped bounds and significance (H_0: α = -4 (1-tailed test)). Note: α = -4 corresponds to no phylogenetic signal in residual DI.

Analysis	Ind_Vars	ModelNum	Convergd	Boot	Fam13	NIndVars	bo	se_B0
plogreg	Mass*	1	1	1	0	1	-0.8197	0.59896
plogreg	Latitude*	2	1	1	0	1	-0.9229	0.83881
plogreg	Diet7	16	1	1	0	1	0.3831	0.78937
plogreg	Diet6	3	0	1	0	4	-0.5583	0.95316
plogreg	Family13	4	0	0	1	12	-1.9646	1.86110
plogreg	Mass* + Latitude*	5	1	1	0	2	-0.3499	0.74157
plogreg	Mass* + Diet7	17	1	1	0	2	0.1539	0.81000
plogreg	Mass + Diet6	6	1	1	0	5	-0.0794	1.07680
plogreg	Latitude* + Diet7*	18	1	1	0	2	0.1802	0.80015
plogreg	Latitude* + Diet6	8	1	1	0	5	-2.5410	0.85576
plogreg	Mass + Family13	7	0	0	1	13	-9.0110	3.80730
plogreg	Latitude + Family13	9	0	0	1	13	1.0627	1.19210
plogreg	Family13 + Diet7	19	1	0	1	13	-1.0454	1.35540
plogreg	Family13 + Diet6	10	1	0	1	16	-0.3440	0.99501
plogreg	Mass* + Latitude* + Diet7*	22	1	1	0	3	-0.2675	0.72605
plogreg	Mass + Latitude* + Diet6	11	1	1	0	6	-1.4969	0.83340
plogreg	Mass + Latitude + Family13	14	0	0	1	14	-0.4128	0.86946
plogreg	Mass + Diet7 + Family13	20	1	0	1	14	0.4816	1.06700
plogreg	Mass + Diet6 + Family13	13	1	0	1	17	-10.1233	3.91700
plogreg	Latitude + Diet7 + Family13	21	1	0	1	14	-2.6579	2.18640
plogreg	Latitude + Diet6 + Family13	12	0	0	1	17	-2.9919	*Convg failed
plogreg	Mass + Latitude + Diet7 + Family13	23	1	0	1	15	-3.6172	2.61420
plogreg	Mass + Latitude + Diet6 + Family13	15	1	0	1	18	-0.2317	0.99381

B_Mass	SE_Bmass	B_Lat	SE_Lat	B_Diet7	SE_Diet7	a_noBoot	N_boot	b0_1boot	b0_2boot	b0_3boot	bo_pboot
0.5817	0.2458					-0.46351	2000	-1.2084	-0.8140	-0.4536	0.0000
		0.6276	0.1669			1.103	2000	-1.3523	-0.9160	-0.5510	0.0010
				-1.1518	0.7140	0.004012	500	-1.2180	-0.3728	0.4395	0.3760
						0.004859	2000	-4.5333	-3.2206	-2.2828	0.0000
						-0.17017					
0.4620	0.1958	0.5996	0.1518			0.59355	2000	-0.7136	-0.3500	-0.0089	0.0470
0.4659	0.2164			-0.8665	0.6400	0.17553	2000	-1.0088	0.1618	1.6370	0.8330
0.2407	0.1828					0.58882	250	-0.5905	-0.1130	0.4663	0.6320
		0.8798	0.2035	-1.4433	0.4840	0.84781	500	-0.5783	0.1743	1.0910	0.7320
		1.0754	0.2889			-0.40836	1000	-6.9984	-3.9371	-1.7987	0.0220
-10.1350	3.5941					0.23927	100	0.7231	3.6887	10.4797	0.0202
		0.8940	0.3149			0.041554					
				1.0366	0.8097	-0.13632					
						-0.10383					
0.7405	0.3087	0.9887	0.2634	-1.6590	0.6372	0.15492	500	-1.8691	-0.3059	2.3716	0.9880
0.2977	0.3039	0.5086	0.2095			-0.18691					
1.3057	0.3293	1.0481	0.4454			-0.82869					
0.7921	0.3449			0.7358	0.9228	3.9999					
5.3143	2.1265					-0.66332					
		0.7437	0.5407	-4.1878	2.0060	-0.35667					
		-1.3138				-0.44975					
-6.1673	2.5684	2.2484	1.0504	-3.9649	2.6000	0.16982					
4.1378	1.0493	1.7252	0.5572			-0.83482					

MasbootU	Massboot	MasbootL	pMasboot	LatBootU	LatBoot	LatBootL	p_Lat_b	Die7botU	Die7bot	Die7botL	p_Diet7b
0.1853	0.5782	1.0225	0.0070								
				0.2787	0.6262	0.9659	0.0000				
								-1.3284	-0.36143,	0.5559	0.4120
0.0690	0.4556	0.9009	0.0200	0.2571	0.6042	0.9835	0.0000				
0.0351	0.4600	0.9057	0.0400					-2.2772	-0.8711	0.3520	0.1720
-0.1384	0.2664	0.8268	0.2800								
				0.5110	0.8721	1.2730	0.0000	-2.6130	-1.4247	-0.4307	0.0080
				0.8238	1.7767	3.4686	0.0160				
-2.6467	0.1551	1.7983	0.6263								
0.1444	0.7376	1.5439	0.0320	0.3859	1.1557	1.9706	0.0080	-3.7130	-1.6584	-0.0605	0.0480

a_bootU	a_boot	a_bootL	pA__boot
-3.9999	-3.1344	-2.0489	0.1820
-3.9999	-3.1452	-2.0232	0.2030
-3.9999	-3.0862	-2.2668	0.0960
-3.9999	-3.1653	-2.0821	0.1865
-3.9999	-3.1467	-2.1287	0.1725
-4.0000	-3.1197	-2.1483	0.1280
-3.9999	-3.1714	-1.8680	0.2460
-4.0000	-1.7111	4.0000	0.2840
-4.0000	1.0014	4.0000	0.1212
-3.8421	0.4977	4.0000	0.0180

Table 1.4. PGLMM models sorted by AICc (lowest to highest). Output from the PGLMM program of Ives and Helmus (2011, and this paper). Values of S are listed here with their bootstrapped bounds. Lower bounds that do not include zero corresponds to significant phylogenetic signal in residual DI.

Analysis	Ind_Vars	NIndVars	NParam	CnvgFlag	SLConf	S	SUConf	LL
PGLMM	Mass + Diet6	5	7	0	0.0227	0.0880	0.1702	-392.3938
PGLMM	Mass	1	3	1	0.0274	0.0742	0.1647	-403.5697
PGLMM	Diet7	1	3	1	0.0309	0.0778	0.1665	-403.9614
PGLMM	Mass + Diet7	2	4	1	0.031	0.0781	0.1695	-404.0194
PGLMM	Latitude	1	3	1	0.0194	0.0613	0.1525	-416.8564
PGLMM	Diet6	4	6	0	0.0289	0.0777	0.1698	-416.1522
PGLMM	Lat + Diet7	2	4	1	0.0209	0.0655	0.1596	-422.5999
PGLMM	Mass + Latitude	2	4	1	0.0195	0.0615	0.1568	-422.6230
PGLMM	Latitude + Diet6	5	7	0	0.0116	0.0547	0.1506	-425.2689
PGLMM	Mass + Latitude + Diet6	6	8	0	0.0159	0.0577	0.1563	-425.1815
PGLMM	Mass + Latitude + Diet7	3	5	1	0.021	0.066	0.1644	-429.8247
PGLMM	Latitude + Family13	13	15	0	0.0116	0.1222	0.3869	-594.7982
PGLMM	Mass + Latitude + Family13	14	16	0	0.0103	0.1275	0.4300	-609.6703
PGLMM	Mass + Latitude + Diet7 + Family13	15	17	0	0	0	0.1019	-949.3675
PGLMM	Mass + Family13	13	15	0	0.2519	0.2836	2.8326	-1090.8000

AIC	AICc	w_i	*acc*_ w_i	ER	Mass	Lat	Diet6	Diet7	Family13
798.7876	799.5393	0.9980	0.9980	1	1	0	1	0	0
813.1394	813.2963	0.0010	0.9991	971.1612	1	0	0	0	0
813.9228	814.0797	0.0007	0.9998	1436.827	0	0	0	1	0
816.0388	816.3020	0.0002	1.0000	4364.854	1	0	0	1	0
839.7128	839.8697	0.0000	1.0000	5.72E+08	0	1	0	0	0
844.3044	844.8644	0.0000	1.0000	6.95E+09	0	0	1	0	0
853.1998	853.4630	0.0000	1.0000	5.12E+11	0	1	0	1	0
853.2460	853.5092	0.0000	1.0000	5.24E+11	1	1	0	0	0
864.5378	865.2895	0.0000	1.0000	1.89E+14	0	1	1	0	0
866.3630	867.3360	0.0000	1.0000	5.27E+14	1	1	1	0	0
869.6494	870.0468	0.0000	1.0000	2.04E+15	1	1	0	1	0
1219.5964	1223.0007	0.0000	1.0000	8.98E+91	0	1	0	0	1
1251.3406	1255.2263	0.0000	1.0000	8.94E+98	1	1	0	0	1
1932.7350	1937.1379	0.0000	1.0000	1.1E+247	1	1	0	1	1
2211.6000	2215.0043	0.0000	3.0000	2.3E+307	1	0	0	0	1

WtMass	WtLat	WtDiet6	WtDiet7	WtFam13	Intercept	SE_I
0.9980490358	0.0000000000	0.9980490358	0.0000000000	0.0000000000	-4.0872	1.4315
0.0010276863	0.0000000000	0.0000000000	0.0000000000	0.0000000000	-4.4612	1.3663
0.0000000000	0.0000000000	0.0000000000	0.0006946202	0.0000000000	-0.9275	0.7847
0.0002286558	0.0000000000	0.0000000000	0.0002286558	0.0000000000	-3.767	1.4304
0.0000000000	0.0000000017	0.0000000000	0.0000000000	0.0000000000	-3.253	0.7086
0.0000000000	0.0000000000	0.0000000001	0.0000000000	0.0000000000	<1.00E-08	<1.00E-08
0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000	-2.1456	0.7371
0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000	-7.4727	1.5497
0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000	-4.00E-08	1.00E-08
0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000	-7.00E-08	2.00E-08
0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000	-6.3728	2.0318
0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000	-1.00E-08	1.00E-08
0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000	-5.00E-08	2.00E-08
0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000	1.00E+00	2.00E-08
0.0000000000	0.0000000000	0.0000000000	0.0000000000	0.0000000000	<1.00E-08	<1.00E-08
0.9993053779	0.0000000017	0.9980490360	0.0009232760	0.0000000000		

BE_Mass	BSE_Mass	BE_Lat	BSE_Lat	BE_Diet7	BSE_Diet7
0.5969	0.3319				
0.7409	0.3092				
				-0.9133	0.6017
0.7283	0.3052			-0.8848	0.6067
		0.0641	0.0129		
		0.0130		-1.9267	0.6060
1.0565	0.3380	0.0717	0.0133		
		0.0000	0.0000		
1.00E-08	0.0000	0.0000	0.0000		
0.9965	0.3704	0.0712	0.0134	-1.9132	0.7779
		0.0000	0.0000		
1.00E-08	0.0000	0.0000	0.0000		
1.00E+08	0.0000	0.0000	0.0000	-0.0004	0.0001
<1.00E-08	0.0000				

Table 1.5. Phylogenetic ANOVA of body mass and latitude. Output from the Matlab program Regression.v2 (Lavin *et al*., 2010).

Analysis	DepVar	Ind_Vars	Model	N_sp	ML_Like	NIndVars	NParam	AIChand	AIC	AICc	d_REML	d_ML
RegV2	Mass	DI3	OLS	157	-171.9790	1	3	349.96	349.96	350.12		
RegV2	Mass	DI3	OU	157	-88.3497	1	4	184.70	184.70	184.96	0.93	0.91
RegV2	Mass	DI3	PGLS	157	-89.4304	1	3	184.86	184.86	185.02		
RegV2	Mass	Diet6	OLS	157	-166.1640	4	6	344.33	344.33	344.887		
RegV2	Mass	Diet6	OU	157	-88.2659	4	7	190.53	190.53	191.283	0.91	0.88
RegV2	Mass	Diet6	PGLS	157	-89.3646	4	6	190.73	190.73	191.289		
RegV2	Mass	Diet7	OLS	157	-173.8480	1	3	353.70	353.70	353.85		
RegV2	Mass	Diet7	OU	157	-89.5966	1	4	187.19	187.19	187.46	0.92	0.90
RegV2	Mass	Diet7	PGLS	157	-90.3642	1	3	186.73	186.73	186.89		
RegV2	Mass	Lat	OLS	157	-173.8830	1	3	353.77	353.77	353.92		
RegV2	Mass	Lat	OU	157	-89.7937	1	4	187.59	187.59	187.85	0.92	0.90
RegV2	Mass	Lat	PGLS	157	-90.6103	1	3	187.22	187.22	187.38		
RegV2	Latitude	DI3	OLS	157	-646.5700	1	3	1299.14	1299.14	1299.30		
RegV2	Latitude	DI3	OU	157	-640.8270	1	4	1289.65	1289.65	1289.92	0.23	0.22
RegV2	Latitude	DI3	PGLS	157	-676.9180	1	3	1359.84	1359.84	1359.99		
RegV2	Latitude	Diet6	OLS	157	-669.1540	4	6	1350.31	1350.3	1350.87		
RegV2	Latitude	Diet6	OU	157	-656.8800	4	7	1327.76	1327.8	1328.51	0.28	0.26
RegV2	Latitude	Diet6	PGLS	157	-683.1460	4	6	1378.29	1378.3	1378.85		
RegV2	Latitude	Diet7	OLS	157	-668.9140	1	3	1343.83	1343.83	1343.98		
RegV2	Latitude	Diet7	OU	157	-657.5220	1	4	1323.04	1323.04	1323.31	0.40	0.38
RegV2	Latitude	Diet7	PGLS	157	-684.5630	1	3	1375.13	1375.13	1375.28		

Coeff	Se	t_Ho	F	DF	P	T_SS	E_SS	Mod_SS	Mod_MS	R2_model
-0.28	0.14	-2.06	4.22	155	0.04162969	84.4426	82.2025	2.2401	2.2401	0.0265
0.17	0.10	1.70	2.88	155	0.09169312	28.8675	28.3416	0.5259	0.5259	0.0182
0.15	0.10	1.54	2.39	155	0.12415292	21.2174	20.8959	0.3215	0.3215	0.0152
See last two columns				152	0.00385860	84.4426	76.3329	8.1097	2.0274	0.0960
See last two columns				152	0.56736914	28.8680	28.3180	0.5501	0.1375	0.0191
See last two columns				152	0.64892137	29.1624	28.6964	0.4660	0.1165	0.0160
-0.11	0.16	0.16	0.48	155	0.48945906	84.4426	84.1830	0.2596	0.2596	0.0031
0.08	0.12	0.69	0.48	155	0.48945906	28.8841	28.7956	0.0884	0.0884	0.0031
0.08	0.12	0.72	0.52	155	0.47192794	29.1624	29.0642	0.0982	0.0982	0.0034
0.00	0.00	0.64	0.41	155	0.52291493	84.4426	84.2200	0.2226	0.2226	0.0026
0.00	0.00	0.25	0.06	155	0.80681993	28.8785	28.8667	0.0118	0.0118	0.0004
0.00	0.00	0.19	0.04	155	0.84174274	29.1624	29.1554	0.0070	0.0070	0.0002
21.45	2.81	7.62	58.11	155	<0.00000001	47730.3100	34715.0800	13015.2300	13015.2300	0.2727
21.20	3.05	6.95	48.25	155	<0.00000001	42314.3300	32269.2000	10045.1400	10045.1400	0.2374
20.18	4.13	4.88	23.84	155	0.00000258	42895.8000	37177.9600	5717.8440	5717.8440	0.1333
See last two columns				152	0.31991594	47730.3100	46287.5400	1442.7740	360.6935	0.0302
See last two columns				152	0.07078240	41902.1700	39601.6200	2300.5420	575.1354	0.0549
See last two columns				152	0.04488125	58958.2800	55318.6300	3639.6520	909.9130	0.0617
8.83	3.83	2.31	5.32	155	0.02240650	47730.3100	46145.7400	1584.5670	1584.5670	0.0332
11.25	4.05	2.77	7.70	155	0.00620169	41904.9200	39921.7100	1983.2140	1983.2140	0.0473
13.77	5.12	2.69	7.24	155	0.00791195	42895.8000	40980.9300	1914.8770	1914.8770	0.0446

Rate_E	SEE	extraSSR	PartF4df
0.5303	0.7282		
0.1828	0.4276		
0.1348	0.3672		
0.5022	0.7087	8.1097	4.0372
0.1863	0.4316	0.5501	0.7381
0.1888	0.4345	0.4660	0.6171
0.5431	0.7370		
0.1858	0.4310		
0.1875	0.4330		
0.5434	0.7371		
0.1862	0.4316		
0.1881	0.4337		
223.9682	14.9656		
208.1884	14.4287		
239.8578	15.4873		
304.5233	17.4506	1442.7740	1.1845
260.5370	16.1412	2300.5420	2.2075
363.9383	19.0772	3639.6520	2.5002
297.7145	17.2544		
257.5594	16.0487		
264.3931	16.2602		

Chapter 2.

Reproductive delays in mammals: an unexplored avenue for post-copulatory sexual selection

ABSTRACT

Numerous mammalian taxa exhibit reproductive delays, pauses in reproduction that occur between mating and egg fertilization, between fertilization and implantation of the embryo in the female, or after an embryo has implanted. Of the 28 mammalian orders, 9 are known to exhibit delayed implantation, including Diptrotodontia (wallabies), Eulipotyphyta (shrews), Cingulata (armadillos), Carnivora (weasels, seals and bears), Rodentia (gerbils), Chiroptera (bats), Lagomorpha (hares) and Artiodactyla (deer). Most researchers interested in delays have focused on their evolutionary origins. However, the consequences of these delays have not been fully considered. Given the lengthening of the period over which reproduction occurs, it is possible that this unique aspect of reproduction facilitates post-copulatory sexual selection. When considered in the context of sexual selection, delays may allow sperm competition and female manipulation of fertilization (cryptic female choice). We investigate the potential for reproductive delays to facilitate post-copulatory sexual selection and suggest avenues for research that may further our knowledge of sexual selection. We also provide a general review of this phenomenon in mammals.

Keywords: Post-copulatory sexual selection; cryptic female choice; sperm competition; reproductive delays; embryonic diapause; delayed fertilization; delayed implantation; Chiroptera; Carnivora; Mammalia

INTRODUCTION

The study of vertebrate reproductive patterns has been of interest to biologists for centuries. Historically, researchers have approached the investigation of reproduction from an anthropocentric point of view, whereby they assumed the timing of events from fertilization to birth occurred in the same manner as in humans. Despite this common assumption, the diversity of mammalian reproductive physiology has also been recognized for some time (Hamlett, 1935). Indeed, even before the publication of Darwin's On the origin of the species by means of natural selection in 1859, a unique reproductive physiology, reproductive delays, had already been observed in mammals (Ziegler, 1843). Roe deer (*Capreolus capreolus*) were known in 1843 to delay implantation for 4-5 months after fertilization (Hayssen, van Tienhoven & van Tienhoven, 1993). Similar types of delays in fertilization, implantation or development have since been described in many additional species of mammals. Well over 100 mammalian species, including members of the Ursidae (bears), Macropodidae (wallabies), Mustelidae (weasels), Pinnipedia (seals and sea lions), Cingulata (armadillos), Mephitidae (skunks), and Chiroptera (bats), have some form of reproductive delay (Hamlett, 1935; Mead, 1993), and this type of reproductive physiology may be even more widespread but still undocumented in additional species. Delaying fertilization, implantation, or pausing development (delayed development) may in turn alter the timing of subsequent events including parturition. For example, in the long-tailed weasel (*Mustela frenata*), implantation is delayed for 7-9 months (Wright, 1963), while active gestation lasts an additional 9.5 months (Wright, 1942). The California leaf-nosed bat (*Macrotus californicus*) has delayed development for an average of 4.5 months, with active gestation lasting an additional 4 months (Bleier, 1975a). Delayed fertilization is seen in the little brown bat (*Myotis lucifugus*), where females mate with males but store the sperm to be used at a later time. Thus, instead of fertilization occurring within a few days of mating, it occurs months later (Racey, 1982).

What is responsible for this diversity in reproductive timing? Several studies have examined the selective forces involved in the evolution of delays (Mead, 1989; Bernard & Cumming, 1997; Thom, Johnson & Macdonald, 2004), which has led to some understanding of the advantages of delays (see below) and their taxonomic distribution (Mead, 1993). However, the consequences of such delays, once they have evolved, remain to be fully explored. Because reproductive delays lengthen the duration of a reproductive event from copulation to birth, they may provide additional time for post-copulatory sexual selection (Table 2.1) to operate. For example, delayed fertilization increases the time frame during which sperm from various males might interact (sperm competition; Birkhead & Møller, 1993; Parker, 1970) or females could manipulate and select sperm (cryptic female choice; Thornhill, 1983; Eberhard, 1996). During delayed implantation, post-copulatory processes could select among zygotes, allowing some to implant and others to be rejected. Finally, delayed development extends the period of time over which an embryo is in the female's reproductive tract (Bernard & Cumming, 1997), potentially facilitating its re-absorption or rejection (abortion).

The existence of reproductive delays presents an ideal opportunity for asking questions about female versus male control of reproduction. Because specific stages of reproduction are lengthened in species with delays, these stages can be closely scrutinized for what may be evidence of sexual selection. For example, some stages such as fertilization may primarily serve as an arena for male competitive strategies. Other reproductive stages such as development may provide opportunities for females to manipulate events, while implantation might be a period of extreme sexual conflict. We believe delays are an under-appreciated source of natural variation in reproductive physiology, the investigation of which could allow important insights into cryptic female choice or sperm competition. Indeed, Birkhead & Møller (1993) discussed the possible role of sperm storage (delayed fertilization) in facilitating sperm competition. However, the idea that delays at other points during the reproductive cycle might be important in post-copulatory sexual selection (Table 2.1) has remained largely ignored for the last nearly 20 years subsequent to Birkhead & Møller's paper. Meanwhile, our knowledge of post-copulatory sexual selection, reproductive physiology (Heideman & Powell, 1998; Krishna, 1999; Oates *et al*., 2007), and the evolutionary relationships of mammals (Wilson & Reeder, 2005, Bininda-Emonds *et al*., 2007; Miller-Butterworth *et al*., 2007, Meredith *et al.,* 2011; Nyakatura & Bininda-Emonds, 2012) have grown substantially.

In this paper we first describe the distribution of reproductive delays among mammals and briefly review the literature on the evolution of delays and define the types of reproductive delays. Delays are then discussed in greater detail in the order as they might occur in a typical pregnancy; delayed fertilization, implantation and then development. For each type of delay we ask what modes of sexual selection might be operating. After evaluating the likelihood of sexual selection during each delay type (and corresponding time-frame) we review the data consistent with these expectations (Table 2.2). Finally, for each delay type, we suggest experiments to test the hypothesis that delays facilitate post-copulatory sexual selection, and offer potential avenues for future research.

Term	Definition
Active gestation	period of development from egg fertilization until birth that does not include the duration of delays.
Cryptic female choice	post-copulatory ability of polyandrous females to bias paternity, termed 'cryptic' because it is not easily observed in the confines of the reproductive tract (Thornhill, 1983).
Delayed fertilization	an extension of time between copulation and actual use of sperm to fertilize eggs; due to sperm storage and/or delayed ovulation (Vaughan, Ryan & Czaplewski, 2000).
Delayed implantation	period of delay occurring after a blastocyst has formed, when cell division stops and the blastocyst remains unattached for a prolonged period of time; also called 'embryonic diapause' (Daniel, 1970).
Delayed development	the period of suspended or slow growth of the embryo that can occur after implantation has occurred but prior to birth (Bernard & Cumming, 1997).
Delayed ovulation	a type of delayed fertilization whereby ovulation is delayed, resulting in fertilization also being delayed (Hamlett, 1935).
Facultative delays	delays that depend on environmental or female conditions such as low food availability or females nursing other young while pregnant (Daniel, 1970; Mead, 1993).
Genetic inviability avoidance	selection for or against certain genetic combinations after fertilization and subsequent recombination of the maternal and paternal genetic information has occurred; potentially used to avoid the costs and risks associated with prolonged investment in incompatible (poor) offspring genotypes (Zeh & Zeh, 1996, 1997).
Pre-copulatory sexual selection	individual variation in relative ability to gain matings that result in successful fertilizations (Eberhard, 1996).
Post-copulatory sexual selection	sexual selection occurring after mating whereby individuals vary in their relative ability to gain fertilizations (Eberhard, 1996).
Reproductive diapause/ delays	deviation from continuous development in mammalian reproduction, with periods of dormancy in the egg, blastocyst or embryo (Vaughan, Ryan & Czaplewski, 2000).
Sexual conflict	payoffs of a certain event (usually fertilization) deviate drastically between males and females resulting in a competing arms race between the sexes with each sex pushing for control over fertilization (Arnqvist & Rowe, 2005).
Sperm competition	contest for successful fertilization of an ovum within a single female between sperm from several males (Parker, 1970).
Sperm storage (female)	the maintenance of sperm in a female's reproductive tract for extended periods of time, requiring special structures in some animals (Fenton, 1984). We refine this definition to only include cases where sperm are contained in the female's tract for a period of time longer than usual sperm longevity.

Table 2.1. Definitions of terms associated with reproductive delays and modes of sexual selection discussed throughout this paper.

Phenomena consistent with sexual selection	Species	Common name	Delay type	Source
Large percentage of multiple paternity within a single litter	*Capreolus capreolus (15.5 % multiple paternity)*	roe deer	delayed implantation	reviewed Soulsbury, 2010
	Eptesicus fuscus (46.2% multiple paternity)	big brown bat	delayed development	Vonhof *et al*., 2006; reviewed Soulsbury, 2010
	Lasiurus borealis (20.0% multiple paternity)	eastern red bat	delayed fertilization	reviewed Soulsbury, 2010
	Pteropus alecto, Pteropus conspicillatus, Pteropus poliocephalus	flying foxes	-	Fox, Spencer & O'Brien, 2008
	Mus musculus (5.6-42.9% multiple paternity)	house mouse	facultative delayed implantation	reviewed Soulsbury, 2010
	Lepus americanus (25.0% multiple paternity)	snowshoe hare	reviewed Soulsbury, 2010	
	Gulo gulo (12.5% mulptiple paternity)	wolverine	delayed implantation	reviewed Soulsbury, 2010
	Halichoerus grypus	grey seal	delayed implantation	Reviewed Birkhead & Appleton, 1998
	Meles meles (16.1-44.8% multiple paternity)	European badger	delayed implantation	reviewed Soulsbury, 2010
	Mustela erminea (16.7% multiple paternity)	short-tailed weasel, ermine	delayed implantation	Holland & Gleeson, 2005; reviewed Soulsbury, 2010

	Mustela vison (33.3% multiple paternity)	American mink	delayed implantation	Yamaguchi *et al*., 2004; reviewed Soulsbury, 2010
	Ursus americanus 20.0-50.0% multiple paternity)	American black bear	delayed implantation	reviewed Soulsbury, 2010
	Ursus arctos (13.3-28.1% multiple paternity)	brown (grizzly) bear	delayed implantation	reviewed Soulsbury, 2010
	Vulpes vulpes (37.5% multiple paternity)	red fox	facultative delayed implantation	reviewed Soulsbury, 2010
Polytocous with variable litter sizes (This trait is particularly noteworthy for bats which usually have litter sizes of N=1 (Kurta & Kunz, 1987). Many species listed above would also fall into this section).	*Chalinolobus gouldii*	Gould's wattled bat	delayed fertilization	Kitchner, 1975
	Eptesicus fuscus	big brown bat	delayed fertilization	Christian, 1956; Wimsatt, 1942, 1945
	Lasionycteris noctivagans	sliver-haired bat	delayed implantation	Wimsatt, 1945
	Lasiurus borealis	eastern red bat	delayed fertilization	Allen, 1939
	Myotis austroriparius	southeastern myotis	*	Sherman, 1930; Rice, 1957
	Myotis lucifugus	little brown bat	delayed fertilization	Guthrie, 1933
	Parastrellus hesperus	western pipistrelle	delayed fertilization	Krutzsch, 1975
	Perimyotis subflavus	eastern pipistrelle	delayed fertilization	Guthrie, 1933; Wimsatt, 1945
	Scotophilus kuhlii	lesser Asiatic yellow house bat	*	Gopalakrishna, 1949
	Tylonycteris pachypus	lesser bamboo bat	delayed fertilization	Medway, 1972
Abortion and re-absorption of embryos	*Lasiurus borealis, Lasiurus cinereus, Lasiurus ega*	tree bats (Eastern red bat, hoary bat, and southern yellow bat)	delayed fertilization	Shump & Shump, 1982a, 1982b; Kurta & Lahr, 1995

	Saccopteryx bilineata	greater sac-winged bat	delayed development	Bradbury & Vehrencamp 1976, 1977
	Rattus norvegicus, *Mus musculus*	lab rat, house mouse	facultative delayed implantation	Conoway, 1955; Lloyd & Christian, 1969
	Lontra canadensis	American river otter	delayed implantation	
Ovulation during pregnancy (superfetation)	*Macropodidae* (various)	kangaroos and wallabies	delayed implantation	Tyndale-Biscoe & Renfree, 1987
	Carollia perspicillata	Seba's short-tailed bat	delayed development	Roelling *et al.,* 2011
	Rousettus leschenaultia	fulvous fruit bat	-	Roelling *et al.,* 2011
	Lepus europaeus	European hare	delayed fertilization	Roelling *et al.,* 2011
	Meles meles	Eurasian badger	delayed implantation	Roelling *et al.,* 2011
	Mustela vison	American mink	delayed implantation	Roelling *et al.,* 2011
Reproductive tract distinguishes between eggs and moves them differentially within the female's tract suggesting female control	*Glossophaga soricina*	Palla's long-tongued bat	delayed implantation	Rasweiler, 1979; Badwaik & Rasweiler, 2000
	Noctilio albiventris	lesser bulldog bat	delayed fertilization	Rasweiler, 1979; Badwaik & Rasweiler, 2000
	Peropteryx kappleri	greater dog-like bat	-	Rasweiler, 1979; Badwaik & Rasweiler, 2000
Paternity biases, same father across colony despite mating 'on-the-wing'	*Saccopteryx bilineata*	greater sac-winged bat	delayed development	Voigt, Heckel & Mayer, 2005
Multiple eggs ovulated (up to 7) but only one implants	*Perimyotis subflavus*	eastern pipistrelle	*	Wimsatt, 1945
Selective implantation (multiple ovulation, only one fertilized)	*Myotis moluccarum*	large-footed myotis	delayed fertilization (short period)	Lloyd, Hall & Bradley, 1999

Penile elaborations (spines)	*Cynomops planirostris*	southern dog-faced bat	-	Ryan, 1991
	Cynopterus sphinx	greater short-nosed bat	facultative delayed development	Vamburkar, 1958
	Lasiurus borealis, *Lasiurus cinereus,*	tree bats	delayed fertilization	P. M. Cryan, unpublished data
	Mormopterus jugularis	Peter's wrinkle-lipped bat	-	Ryan, 1991
	Nyctinomops laticaudatus	broad-eared bat	-	Ryan, 1991
	Pteropus giganteus	Indian flying fox	-	Vamburkar 1958
	Rhinonicteris aurantius	orange horseshoe bat	*	Armstrong, 2005
	Tadarida brasiliensis	Brazillian free-tailed bat	-	Ryan, 1991
Penile elaborations (scoops)	*Cardioderma cor*	heart-nosed bat	-	Matthews, 1942
	Triaenops afer	triple nose-leaf bat	-	Matthews, 1942
Baccular selection (increased size) authors also noted differences in complexity of shape but did not evaluate statistically)	*Pagophilus (Phoca) groenlandica*	harp seal	delayed implantation	Miller & Burton, 2001

Table 2.2. Evidence suggestive of post-copulatory sexual selection occurring during the reproductive cycle of various species with reproductive delays. If a closely related species has reproductive delays, an * indicates that the listed species may also have delays. Dashes indicate species for which delays are unknown, and it is unknown if related species have delays or not.

THE DISTRIBUTION OF REPRODUCTIVE DELAYS

Over 100 mammalian species exhibit some type of reproductive delay (Mead, 1993; Hayssen, van Tienhoven & van Tienhoven, 1993). The distribution of reproductive delays among mammals is complex. For example, delays are found in ancestral species of the infraclass Metatheria (Marsupials), while bats as a more derived order appear to exhibit multiple origins of all three types of delays (delayed fertilization, implantation, and development) (Mead, 1993). In other groups delays might have been the ancestral state lost in recent species as suggested for the Carnivora (Lindenfors, Dalen & Angerbjoern, 2003). Of the 28 recognized mammalian orders (Wilson & Reeder, 2005), 9 are known to have some type of reproductive delay (Table 2.3). The best studied group with reproductive delays is the Carnivora, particularly the family Mustelidae with 25 species known to have delayed implantation, 19 known to not delay implantation and a remaining 21 species for which data are unavailable (Table 2.3, Orr, Chapter 1). Thus, the Carnivora are a good study group given the prevalence of data both on absence and presence of delays. Another group, the bats (order Chiroptera), are also valuable in the context of delays because they possess all three types of reproductive delays: delayed fertilization, implantation and development (Oxberry, 1979; Racey, 1982; Bernard & Cumming, 1997). For these reasons our review focuses on two taxonomic groups; the Carnivora and Chiroptera.

Order	Representative taxa	Species known to have delays	Species known to not delay	Number of species in Order	Type of delays	Source
Subclass PROTOTHERIA						
Monotremata	platypus, echidna	0	-	6	-	-
Infraclass METATHERIA					-	
Dasyuromorphia	carnivorous marsupials	2	-	~71	fertilization	Marlow, 1961; also see Table 2.5
Didelphimorphia	Opossums	0	1 Virginia opossum *(Didelphis virginiana)*	>60	-	Hartman, 1923
Diprotodontia	kangaroos, wallabies, possums	~23	2 mountain pygmy-possum *(Burramys parvus),* common brushtail possum *(Trichosurus vulpecula)*	~120	implantation, (facultative in some species)	Gilmore, 1969; Renfree & Shaw, 1973 also see Table 2.4 and 2.6
Microbiotheria	monito del monte	0	-	1	-	-
Notoryctemorphia	marsupial moles	0	-	2	-	-
Paucituberculata	shrew opossums	0	-	5	-	-
Peramelemorphia	bandicoots, bilibies	0	1 long-nosed bandicoot (*Perameles nasuta)*	~19	-	Hughes, 1962a
Subclass THERIA						
Infraclass EUTHERIA						
Afrosoricida	tenrecs, golden moles	0	-	~51	-	-
Carnivora	lions, bears, seals	68	132	271	implantation	Orr, Chapter 1; also see Table 2.6
Cetartiodactyla (Artiodactyla and Cetacea)	even-toed ungulates, dolphins, whales	~2	1 reindeer (*Rangifer tarandus*)	310	implantation	Retzius, 1900; also see Table 2.6
Chiroptera	bats	>100	-	>1200	fertilization, implantation, development	see Tables 2.4-2.7
Cingulata	armadillos	2	-	20	implantation	see Table 2.6
Dermoptera	flying lemurs (colugos)	0	-	2	-	-
Eulipotyphla (Lipotyphla)	shrews, moles	2	-	~359	implantation	see Table 2.6
Hyracoidea	hyraxes	0	-	4	-	-
Lagomorpha	rabbits, hares, pika	1	-	80	fertilization	see Table 2.5

Macroscelidea	elephant shrews	0	-	19	-	-
Perissodactyla	odd-toed ungulates	0	-	~19	-	-
Pholidota	pangolins	0	-	7	-	-
Pilosa	sloths, anteaters	0	-	10	-	-
Primates	monkeys, apes	0	-	424	-	-
Proboscidea	elephants	0	-	3	-	-
Rodentia	rats, squirrels, mice	~9	-	2,277	implantation (facultative)	Renfree & Calaby, 1981
Scandentia	tree shrews	0	-	20	-	-
Sirenia	manatees, dugongs	0	-	4	-	-
Tubulidentata	aardvarks	0	-	1	-	-

Table 2.3. Mammalian orders known to exhibit delays (underlined) listed with number of species known to have delays. Type of delays observed are listed; delayed fertilization, delayed implantation, and delayed development together with an estimated total number of species in each order and original citations if not cited in a table below.

SPECIES WITH FACULTATIVE DELAYS

Some mammalian species have delays that only occur under certain environmental conditions. These conditions include changes in photoperiod, food deprivation (Rasweiler & Badwaik, 1997), if a female is nursing another pup (Weichert, 1940; Renfree, 1979) or fluxuations in ambient temperature (Uchida, Inoue & Kimura, 1984). Facultative delays may occur during any of the 3 stages described above, but delayed fertilization and implantation appear to be the most common types of facultative delays (Table 2.4). Additionally, at least one species (*Vulpes vulpes*) is known to be polymorphic for delays, with delays only occuring in some parts of the species' range (Larivière & Pasitschniak-Arts, 1996). Species with facultative delays are particularly useful for addressing the questions outlined in this paper, as they provide an opportunity to contrast circumstances that do and do not co-occur with delays.

Order	Family	Genus Species	Common Name	Delay Type	Cause	Source
Diprotodontia						
	Macropodidae	*Macropus eugenii*	Tammar wallaby	delayed implantation	lactation	Berger, 1966
		Setonix brachyurus	quokka	delayed implantation	lactation	Sharman, 1955b; Shield, 1968
		Macropus rufogriseus	red-necked wallaby	delayed implantation	lactation	Merchant & Calaby, 1981, Fleming, Cinderey & Hearn, 1983
		Macropus rufus	red kangaroo	delayed implantation	lactation	Sharman & Pilton, 1964; Clark, 1966
Chiroptera						
	Emballonuridae	*Taphozous longimanus*	long-winged tomb bat	delayed development	temperature	Krishna & Dominic, 1982
	Pteropodidae	*Ptenochirus jagori*	greater fruit musky bat	delayed development	maternal age	Heidman & Powell, 1998
		Cynopterus sphinx	greater short-nosed bat	delayed development (gastrulation)	fat stores	Meenakumari & Krishna, 2005
	Vespertilionidae	*Miniopterus schreibersii*	Schreiber's long-fingered bat	delayed implantation	temperature	Baker & Bird, 1936
Rodentia						
	Muridae	*Dipodillus simoni*	Simon's dipodil	delayed implantation	lactation	Hamlett, 1935
		Meriones longifrons	Mongolian gerbil	delayed implantation	lactation	Hamlett, 1935
		Meriones shawi	Shaw's jird	delayed implantation	lactation	Hamlett, 1935
		Mus musculus	house mouse	delayed implantation	lactation	McLaren, 1968
		Rattus norvegicus albinus	white lab rat	delayed implantation	lactation	King, 1913
Carnivora						
	Canidae	*Vulpes vulpes*	red fox	delayed implantation	Polymorphic across range	Larivière & Pasitschniak-Arts, 1996

Table 2.4. Species known to exhibit facultative reproductive delays listed together with the presumed driver of the delay and citation.

THE EVOLUTION OF DELAYS

While the focus of this review is to evaluate the consequences of delays once they have already evolved, information regarding their origins is informative. Here we introduce general hypotheses regarding the origins of delays. What might have been the adaptive value of reproductive delays? The origins of reproductive delays have been examined and reviewed by numerous authors (Hamlett, 1935; Sandall, 1990; Mead, 1993; Bernard & Cumming, 1997; Racey & Entwistle, 2000; Ferguson, Higdon & Larivière, 2006). Hamlett (1935) summarized several ideas regarding the origins of delays in mammals, including: 1) delays allow young to be born at an energetically favorable time; 2) delays only occur in old genera that existed during the Pleistocene, because delays assured young were not born during glacial winters, and 3) delays are a byproduct of lower body temperatures of hibernating mammals. Hamlett (1935) also suggested 4) that delays are not adaptations, but instead the ancestral state in some species.

Several additional hypotheses regarding the origin of reproductive delays, many expansions on Hamlett's ideas, have been published in the last sixty years. Indeed, hypotheses fall roughly into Hamlett's four original categories. Consistent with Hamlett's first hypothesis, delays may allow females to match the energetic demands of pregnancy and lactation to food abundances and favorable weather (Ferguson, Higdon & Larivière, 2006) or, more specifically, to wean young when food is available (van der Merwe, 1978). Similarly, females might use delays to coincide estrus with periods of mate availably which may differ seasonally, to elicit maximal male competition, thereby providing a forum for female mate choice (Sandell, 1990). Delays likely enable the synchronization of seasonal breeding, as seen in marine mammals such as pinnipeds (Bartholomew, 1970). Indeed, because pinnipeds are widely dispersed much of the year, the selective advantage of being able to have parturition occurring in a narrow window during which mating also occurs (shortly thereafter) is important (Bartholomew, 1970). Synchronization may allow young to reach independence at roughly the same time and thus saturate predators and increase relative survival rates (Racey, 1982; reviewed Bernard & Cumming, 1997).

In regards to Hamlett's second hypothesis, delays may have been an ancestral state that was lost as species became smaller to counteract other changes in life histories associated with the decrease in body mass due to fecundity selection (i.e. more litters per year and more pups per litter) (Ferguson, Virgl & Larivière, 1996; Lindenfors, Dalen & Angerbjoern, 2003). Recently, it has been argued that because otherwise non-delaying species such as domestic sheep (*Ovis aries*) can be forced to undergo reproductive delays that delays must be conserved across mammals and thus not secondarily lost (Ptak *et al*., 2012). Hamlett's third hypothesis, that lowered body temperatures cause delays, is no longer well accepted because many taxa that have delays live in tropical or very warm habitats (e.g. *Artibeus jamaicensis*; Flemming, 1971; *Macroglossus minimus*; Hood & Smith, 1989; and *Carollia perspicillata*; Rasweiler & Badwaik, 1997; see also Bernard and Cumming, 1997; Racey & Entwistle, 2000). However, consistent with Hamlett's fourth hypothesis, it has been suggested that delays might be a genetic byproduct of changes to other aspects of reproductive physiology (Isakova, 2006). Of the proposed origins of reproductive delays the most commonly accepted view is that delays enable females to negotiate seasonal environments in some way and in doing so they reap energetic, nutritional or mate choice benefits.

CONSEQUENCES OF DELAYS

Life history consequences of delays

The evolution of delays should be considered in relation to the potential costs of delaying. For example, costs of delaying fertilization might include loss of sperm viability and degradation of sperm and associated genetic materials. Ants incur immune costs during sperm storage (Baer, Armitage & Boomsma, 2006) and it is plausible that mammals experience similar costs. Female's delaying implantation should incur minimal costs as she has as of yet not invested in the unimplanted blastocysts. However, it is possible that during delayed implantation females insure costs associated with maintaining the endometerium or depressing immunity to maintain the blastocysts. The immune system is suppressed during pregnancy (Weetman, 1999; Luppi, 2003) and thus, immunological costs may occur during delayed fertilization and delayed development by virtue of extending this period of suppression. During delayed development, females may also experience some costs associated with the metabolic demands of the developing embryo. These costs are expected to be lower if delays occur early in gestation relative to later during the embryo's development.

Postcopulatory sexual selection consequences of delays

The potential fitness advantages to females that utilize post-copulatory sexual selection during reproductive delays may have exciting evolutionary implications. If by lengthening any of the stages of reproduction, females were better equipped to manipulate paternity in a manner that would benefit them, longer durations of delays may be selected. Additionally, it is possible that these processes themselves were responsible for the evolutionary origins of reproductive delays. The potential advantages of post-copulatory sexual selection are discussed to some extent by several authors including; Bernard and Cumming (1997), Heidman (1988), Sandell (1990) and Birkhead and Møller (1993).

At least three main categories of evolutionary relevant benefits or consequences of post-copulatory sexual selection during delays could result in selection for the maintenance and/or evolution of delays. These include bet-hedging, defense against genetically incompatible sperm or offspring, and sexual conflict resolution or intensity which may not be directly beneficial for either sex. Bet-hedging i.e. minimizing risks (variance) may be important in taxa with uncertain mating opportunities, whereby females would benefit from being receptive to high quality mates when they are available (Sandell, 1990). By mating with even poor quality males the female would have sperm available for use in case another male was never available to mate with. Alternatively, if a higher quality male came along she could mate with such additional males and use post-copulatory sexual selection to select between sperm/progeny and thus be at an evolutionary advantage. Another form of bet-hedging might include producing a range of offspring phenotypes (by acquiring matings from multiple males) which may allow females to maintain fecundity without risking reproductive failure due to poor sperm or genetic incompatibility.

Similarly, infertility of some sperm might be an issue for females that do not store sperm for extended periods and have short fertile periods. Again, delays might be selected for under this situation allowing females to secure various sperm during the extended period over which she is receptive, ensuring some will be fertile an obvious benefit. Of course, this assumes males are able to produce long-lived sperm which may not always be true and would complicate this prediction.

Because male quality is not always apparent at the phenotypic level, females may use delays as an extended period within which to prevent themselves from carrying embryos that are genetically incompatible (Zeh & Zeh, 2000) to full term. Indeed, females that have delays may have more time to assess offspring quality and if delaying females gave birth to higher quality young, they would be at a selective advantage. This idea was presented by Heidman (1988) who suggested females might gain fitness advantages by being able to selectively re-absorb some embryos.

In species where females use delay as a means of controlling paternity, one should not assume males, or their sperm, are passive bystanders. Rather one might expect males to aggressively counteract female measures by many of the processes described below (copulatory plugs, penis spikes, etc.). In response, females may evolve additional tactics or lengthen delays to secure increased opportunities for control of post-copluatory events. Furthermore, if females change the timing of events like sperm storage, males must 'follow' or be left in the preverbal dust and thus they are expected to counter-adapt. Females may thus become increasingly dependant on the period of delay for sorting out genetically incompatible sperm, blastocysts or embryos. Males may meanwhile be selected to curtail her efforts and as a result, pressure for females to select paternity using delays or other methods may be selected further. Delays may as a result become un-escapable like Ritchie's Scotsman "immobilized between a chip shop and a pub" (Ritchie, 2007). The resolution of the resulting conflict remains unclear but presents an interesting direction for future studies.

SEXUAL SELECTION AND DELAYS

Regardless of their evolutionary origin, once they are present delays could provide an opportunity for sexual selection to act after copulation. Sexual selection has long been known to occur prior to copulation but more recently it has been shown to also operate after mating via post-copulatory sexual selection, which as the name suggests is sexual selection that occurs after mating. Post-copulatory sexual selection is frequently divided into two categories: sperm competition (the rivalry between sperm from different males for the fertilization of an egg) and cryptic female choice (biases in paternity due to female driven processes after mating) (Table 2.1). Reproductive delays are likely to facilitate both processes.

Outlined below are the different delays as they occur sequentially in a typical pregnancy. We focus our review on delayed fertilization and to a lesser degree delayed implantation. This bias is representative of the number of species known to have these delays. Furthermore, the potential for post-copulatory sexual selection may be greater during delayed fertilization and then delayed implantation relative to delays later in pregnancies (delayed development). In some situations, expectations for both male and female based post-copulatory sexual selection will be identical or barely distinguishable.

(1) Delays between copulation and fertilization

After mating there may be a delay of variable length between insemination and fertilization (Table 2.5). Termed delayed fertilization, this phenomenon occurs when females store sperm. In most cases, they also delay ovulation for up to an entire winter (Wimsatt, 1979). However, female mammals, unlike insects, birds or squamates, do not generally store sperm for long periods of time, with the exception of some bats (Racey, 1979; Birkhead, 2000). Sperm storage may be more uncommon in mammals because they lack special sperm storage structures like the spermatotheca of insects, or sperm-storage tubules of birds (Birkhead, 2000; Orr & Zuk, 2012).

However, some mammals co-opt pre-existing structures, namely the lining of the reproductive tract, to store sperm (Racey & Potts, 1970; Racey, 1979; Table 2.5).

Delayed fertilization can offer an opportunity for cryptic female choice when little opportunity exists for pre-copulatory mate selection. For example, females of many temperate bat species such as *Myotis lucifugus* (Thomas, Fenton & Barclay, 1979; Wai-ping & Fenton, 1988), *Nyctalus nyctula* (Racey, 1973a) and *Corynorhinus townsendii* (Pearson, Koford & Pearson, 1952) mate while in torpor, cryptic female choice would give them their only opportunity for exerting mate choice. Similarly, mating occurs 'on the wing' in some species (i.e. *Lasiurus borealis* (Murphy & Nichols, 1913)), possibly limiting female pre-copulatory mate assessment. Many of these species are temperate bats that have minimal pre-copulatory sexual selection opportunities because of separate migration routes between the sexes and hence uncertain mating opportunities, or because as mentioned above females are mated while they are torpid (Wai-Peng & Fenton, 1988). Could the details of mate choice be worked out in the confines of the female's reproductive tract in species with delayed fertilization? Most bats only have one or two young on average per litter (Kurta & Kunz, 1987) so females probably receive enough sperm from a single copulation for successful fertilization. For this reason, the potential for post-copulatory sexual selection in these taxa seems particularly worthy of investigation. Delayed fertilization could facilitate three forms of post-copulatory sexual selection: sperm competition, cryptic female choice (including female sperm selection and selective ovulation), and genetic incompatibility avoidance.

In addition to facilitating cryptic female choice, the lengthened period of sperm co-occurrence due to delayed fertilization greatly increases the opportunity for sperm competition. If females store sperm from several males, Parker's (1970) requisites for sperm competition are satisfied; namely, the co-occurrence of sperm from more than one male inside the female's reproductive tract. Indeed, females of many species with delayed fertilization mate multiple times (Miller & Burton, 2001; Vonhof *et al*., 2006; Table 2.2).

Where might we look for evidence of sperm competition? Morphological indications of sperm competition may be noted in both reproductive anatomy and sperm cell morphology (Gage, 1998; Pitnick, Hosken & Birkhead, 2009) including; relatively larger testes (e.g., Harcourt *et al*., 1981), elaboration in female internal reproductive morphology (Eberhard, 1996; Brennan *et al*., 2007), and penile (bone or cartilaginous) structures (Eberhard, 1985; Dixson, 1987; Hogg, 1988; Edwards, 1993). Furthermore, sperm performance itself (i.e. swimming speed) may be altered in the context of sperm competition and may in some cases evolve independently of sperm length or other morphological variables (Kleven *et al*., 2009). These possibilities are discussed in detail below.

Order	Family	Genus Species	Common Name	Delay length (days)	Source
Carnivora	Canidae	*Canis familiaris*	domestic dog	11	Birkhead & Møller, 1993
Chiroptera	Molossidae	*Molossus alter*	black mastiff bat	50	Rasweiler, 1987
	Noctilionidae	*Noctilio albiventris*	lesser bulldog bat	-	Rasweiler, 1977
	Pteropodidae	*Macroglossus minimus*	long-tongued bat	60-90	Hood & Smith, 1989
	Rhinolophidae	*Rhinolophus ferrumequinum*	greater horseshoe bat	150	Matthews, 1937; Racey, 1975
	Vespertilionidae	*Chalinolobus gouldii*	Gould's wattled bat	-	Kitchner, 1975
		Eptesicus furinalis	Argentine brown bat	-	Myers, 1977
		Eptesicus fuscus	big brown bat	156	Gates, 1936; Wimsatt, 1944
		Lasiurus borealis	eastern red bat	-	P. M. Cryan, unpublished data
		Lasiurus cinereus	hoary bat	-	P. M. Cryan, unpublished data
		Lasiurus ega	southern yellow bat	-	Myers, 1977
		Myotis dasycneme	pond myotis	-	Strelkov, 1962
		Myotis daubentonii	Daubenton's bat	-	Strelkov, 1962
		Myotis lucifugus	little brown bat	~100	Wimsatt, 1944
		Myotis mystacinus	whiskered bat	-	Strelkov, 1962
		Myotis ricketti	Rickett's big-footed bat	225	Wang *et al.,* 2008
		Myotis sodalis	Indiana bat	68	Gates, 1936
		Myotis velifer	cave myotis	-	Krutzsch, Crichton & Nagle, 1982

		Nyctalus noctula	common noctule	198	Racey, 1973a, 1973b
		Nyctophilus geoffroyi	lesser long-earred bat	~90	Hosken, 1998
		Perimyotis subflavus	eastern pipistrelle	-	Guthrie, 1933
		Pipistrellus abramus	Japanese house bat	175	Hiraiwa & Uchida, 1956
		Pipistrellus ceylonicus	Kelaart's pipistrelle	30	Racey, 1979
		Pipistrellus nanus	banana pipistrelle	75	Bernard & Cumming, 1997
		Pipistrellus pipistrellus	common pipistrelle	151	Pagenstecher, 1859; Racey, 1973b
		Plecotus auritus	brown long-eared bat	-	Strelkov, 1962
		Scotophilus heathi	greater Asiatic yellow bat	60	Krishna & Dominic, 1982
		Tylonycteris pachypus	lesser bamboo bat	<30	Medway, 1972
		Tylonycteris robustula	greater bamboo bat	<30	Medway, 1972
Dasyuromorphia	Dasyuridae	*Antechinus stuartii*	brown antechinus	16	Birkhead & Møller, 1993
		Dasyurus viverrinus	Eastern quoll	14	Birkhead & Møller, 1993
Lagomorpha	Leporidae	*Lepus europaeus*	European hare	30	Birkhead & Møller, 1993

Table 2.5. Species known to exhibit delayed fertilization (sperm storage, delayed ovulation) are listed with their common names and associated citations. Dashes indicate that the length of the delay is unclear.

(a) Characteristics indicative of sexual selection during delayed fertilization

(i). Spermatozoa morphology/performance and delayed fertilization

Sperm cell performance and structure may bear witness to the presence of post-copulatory sexual selection and thus could be used to infer sperm competition. Features of sperm that might aid a male's competitive ability include number, motility, mid-piece volume, mitochondrial arrangement, amount of ATP (Jeulin & Soufir, 1992; Perchec *et al*., 1995; Travis *et al*., 2001) and length (Pitnick, Hosken & Birkhead, 2009). In particular, mid-piece volumes may indicate the quantity of mitochondria that a sperm possesses (with more mitochondria resulting in more motility) (Anderson & Dixson, 2002). Mitochondrial arrangement within the mid-piece may also play a role in sperm longevity and sperm storage capabilities (Wimsatt, Krutzsch & Napolitano, 1966) as mitochondrial respiration capabilities are correlated with successful fertilization (Windsor, 1997).

(ii). Sperm lifespan and delayed fertilization

Increased sperm longevity also extends the time over which sperm would compete for fertilization. Sperm lifespan is known to correlate with the length of female estrus cycles (Chang & Rowson, 1965; Miyamoto & Change, 1972) and it is reasonable to expect that co-evolution occurs between female ability to store sperm and a male's ability to produce long-lived sperm (Parker, 1984). If sperm from several males compete among each other for longevity an evolutionary arms race may result.

(iii). Testes size and delayed fertilization

Larger testes are correlated with increased sperm competition (Harcourt *et al*., 1981). More sperm competition due to delays during delayed fertilization may place selective pressure on males to make more sperm. A relationship between testes size and sperm competition risk has been demonstrated in many mammalian taxa, including bats (Hosken, 1997; Wilkinson & McCracken, 2003), primates (Harcourt *et al*., 1981), zebras (Ginsberg & Rubenstein, 1990), and other mammals (Kenagy & Trombulak, 1986). This is presumably because mammals with larger testes produce more sperm (Willet & Ohms, 1957; Amann, 1970), and sperm are costly to produce (Dewsbury, 1982; Olsson, Madsen & Shine, 1997). Indeed, bats with delayed fertilization might have larger residual testes volume relative to bats without delayed fertilization if they are experiencing more sperm competition during this delay type (P7 below).

(iv). Seminal fluids and delayed fertilization

Seminal fluids may also have diversified in a manner consistent with a function in sperm competition (Ramm *et al.,* 2008; Lemaître *et al*., 2011). These compounds are likely indicators of sexual selection operating and may thus be unique in species with delayed fertilization. Research aimed at understanding how the features of seminal accessory organs as well as their products are associated with degree of sperm competition would be valuable.

(v). Penile elaborations and delayed fertilization

Penile elaborations (defined by the presence of fleshy/cartilaginous or ossified extrusions) have been implicated in post-copulatory sexual selection in several species of mammals possess (Eberhard, 1985; Stockley, 2002; Hosken & Stockley, 2004; Ramm, Parker & Stockley, 2005). The use of male genital features to remove sperm has been demonstrated in damselflies (Waage, 1979), implicated in other insects (Eberhard, 1985), and inferred in mammals (Dixson, 1987; Verrell, 1992; Stockley, 2002; Ferguson & Larivière, 2004; but see Hosken *et al*., 2001). Delaying species might use spines to remove sperm from stores or to elicit cryptic female choice.

(b) Cryptic female choice and delayed fertilization

Delayed fertilization could facilitate cryptic female choice if, during the period of delay, females initiate processes that enable them to later selectively fertilize eggs. In species with variable delay lengths, the duration of delay might be expected to correspond to the quality of a male, with near-immediate fertilization when a female mates with a high-quality male and a lengthier delay when the mate is less than optimal. This strategy could allow a female to hedge her bets and use sperm from lower-quality mates only if no better options become available. Female processes relating to cryptic female choice that may occur during delayed fertilization include multiple egg release, female ejection of sperm or copulatory plug removal (see below). Indeed, differential ovulation, i.e. ovulation only after mating with particular males (Larivière & Ferguson, 2003), may be used during delayed fertilization in females of some Carnivora after mating with a high quality male.

In a few species of bats (Table 2.2) the female's reproductive tract distinguishes dead eggs from living ones and selectively moves the former out of the oviducts (Rasweiler, 1979; Badwaik & Rasweiler, 2000). Thus, some complex processes such as only allowing particular sperm to fertilize eggs; removing genetically incompatible blastocysts or those from poor-quality fathers from the reproductive tract may be mechanistically possible. Similarly, in bat species with delayed fertilization and sperm storage, specialized cells in the female's reproductive tract appear to regulate sperm attachment and release prior to fertilization (Krutzsch, Crichton & Nagle, 1982; Krishna, 1997; Scott, 2000). Unused sperm cells are destroyed by phagocytosis in the spring (Krutzsch, Crichton & Nagle, 1982; Krishna, 1997).

Female sensitivity to sperm quality suggests potential for differential use of sperm from different males. In addition to navigating elaborated female reproductive morphologies (Brennan *et al*., 2007; Higginson *et al.,* 2012), variation in male genitalia (as discussed above) may be required for female stimulation and subsequent cryptic female choice. Many species with delays are polytocous, meaning that more than one egg is released per ovulation, including the Roe deer (Vanpé *et al.,* 2009) and members of Chiroptera, Rodentia and Carnivora (Table 2.2) (Badwaik & Rasweiler, 2000; Stockley, 2003). In species with post-partum estrus, some reproductive stages can overlap, allowing for the accumulation of eggs from successive ovulations (Stockley, 2003). For example, in *Myotis moluccarum*, only one of several eggs released at ovulation is actually fertilized (Lloyd, Hall & Bradley, 1999). In the Eastern pipistrelle (*Perimyotis subflavus)*, a species with delayed fertilization (Guthrie, 1933), only one embryo develops despite the ovulation of multiple eggs (Wimsatt, 1945).

Even in species that only ovulate a single ovum at a time, eggs could overlap within the reproductive tract if a developing blastocyst is present while ovulation occurs (Scrimshaw, 1944; Roelling *et al.,* 2011). Marsupials are particularly noteworthy in their ability to have various reproductive stages occurring simultaneously. Reproductive delays in this group are unique in that they are highly controlled in relation to what is happening with co-occurring pregnancies (Tyndale-Briscoe & Renfree, 1987). This level of reproductive control could be due to the extended period of time over-which females are able to evaluate sperm, zygotes and implanted embryos.

(c) Sexual conflict and delayed fertilization

Males interests may be subverted by females during delays. As a result, delays may present increased opportunity for sexual conflict, which may manifest in several ways. For example, species with delayed fertilization may have a greater use for copulatory plugs, an organic vaginal seal formed after mating by substances produced by either sex (Baumgardner *et al*., 1982; Tideman, 1993; Keeley & Keeley, 2004) if sexual selection occurs during the delay. In some mammalian species, these plugs can serve as a form of 'mate guarding' and consequently elicit intra-sexual competition (Devine, 1977; Voss, 1979). The role of copulatory plugs can be interpreted in the context of their origin: male or female. Plugs produced by females (i.e. from vaginal secretions; Voss, 1979) may be employed to keep sperm sequestered in the reproductive tract; by blocking subsequent ejaculates from reaching the egg, females could control the fate of sperm from lower-quality males. However, if the vaginal plug is male-derived (i.e. from seminal fluid) it may play a role in male-male competition (intra-sexual selection) by preventing other males from mating with the female.

Alternatively, the plug may be used to prevent the female from immediate sperm dumping (inter-sexual selection). Female bats have been observed removing copulatory plugs and dumping the sperm from a previous mating (*Rhinolophus ferrumequinum* (Fenton, 1984), *Nyctophilus gouldi* (Phillips & Inwards, 1985) and *Corynorhinus townsendii* (Pearson, Koford & Pearson, 1952). Penile elaborations such as spines may be used to remove copulatory plugs (Armstrong, 2005).

(d) Predictions- delayed fertilization

Below we make predictions about the opportunity for post-copulatory sexual selection in species with delayed fertilization. We also suggest experiments or other work that could be used to test these predictions.

P1. We expect species with delays may have unique or exaggerated sperm features such as larger mid-pieces, and additional mitochondria.

P2. Because delayed fertilization requires long-lived sperm, features of sperm morphology that increase longevity should correlate with presence or absence of delayed fertilization and even the duration of the delay.

P3. If seminal products aid sperm longevity, we might expect them to be particular to delaying species either in quantity or composition. All of these predictions (P1-P3) could be investigated by performing a comparative study of sperm from species both with and without delays.

P4. We expect penile morphology of species with delays to be more elaborate if it functions in pre-fertilization paternity biases specifically for sperm competition (sperm removal, placement or displacement). Comparing data from artificially inseminated females to data from naturally mating females with known mating orders of males with different degrees of penile (bacular) elaboration would allow an assessment of this prediction. Cryptic female choice due to stimulation via male genital elaborations would be extremely difficult to test but is worth further investigation. Perhaps a better test would be to perform a comparative analysis of penis size and elaboration as dependant variables across numerous taxa with delay (0,1) as the independent variable. Another fruitful study would reanalyze bacular size or elaborations in relation to delays (presence or absence) using degree of multiple mating as a covariate to determine if there is any morphological evidence of sexual selection operating on primary sexual characteristics.

P5. The female genital tract may be more elaborate or convoluted (Gomendio & Roldan, 1993), described by Eberhard (1996) as "a torturous route to the egg," when delays are present.

P6. Species such as marsupials that have delays and simultaneously occurring pregnancies are expected to use delays to fine-tune reproduction and thus have a lower failure of pregnancies (see also P9).

P7. Species with delayed fertilization should have a greater degree of sperm competition than species without delayed fertilization. In species with delayed fertilization, stored sperm, potentially from several males, remains in the female's reproductive tract for a longer time than in species without delays. Furthermore, this length of time likely increases the probability of female remating . Thus, delayed fertilization is expected to enhance the potential for sperm competition. This prediction can be tested by comparing the relative testis sizes of species with and without delays. Species with delayed fertilization are expected to produce more sperm if there is an increased occurrence of sperm competition due to the extended period over which fertilization occurs and thus, will have larger relative testes sizes.

Instead of indirect tests to examine if delays facilitated sperm-competition, experiments could identify the presence of sperm competition during delayed fertilization. By artificially inseminating females with a mix of sperm from different males (of known amounts or numbers), (following methods suggested and outlined in Parker, 1990) the effects of both sperm number and order would be removed. If paternity biases were observed in resulting offspring, such as paternity being dominated by the same male, this could be evidence of differential male competitive abilities via sperm competition. Additionally, sperm could be labeled with chemical markers to inform us of the degree of mixing between ejaculates of different males within the female's reproductive tract in species with delayed fertilization. A particularly good example of this method is presented by King *et al.* (2002) who artificially inseminated female fowl (hens and turkeys) with stained and unstained sperm. A similar experimental design would allow researchers to detect a) where sperm from different males are stored and b) if there is sperm order fertilization precedence. Mixing may be greater in species with delayed fertilization if sperm move within the reproductive tract across time or fill in gaps as added to storage sites; alternatively sperm from the same male may be stored in groups.

P8. Species with delayed fertilization are expected to have sperm morphologies that facilitate their competitive abilities, i.e., increase sperm longevity or swimming speed. Because some aspects of sperm structure are associated with sperm competition (Gage, 1998; Anderson & Dixson, 2002; Immler *et al*., 2007), these morphologies are expected to be found more often in species with delays than in those without. For example, sperm size (length) and mid-piece volume are expected to be positively correlated with the presence of delayed fertilization.

P9. Species with delays will exhibit greater paternity biases relative to those species without delays. In species with delayed fertilization there is more time for females to bias success of sperm from different males relative to species with fertilization occurring within hours of mating. Time provided by delaying may allow two main things to occur: genes for sperm longevity might be selected and alternatively, female's may accumulate sperm from several putative fathers from which she may select for the best. To test the role of delays in facilitating this second option i.e. female-driven fertilization biases, it might be possible to demonstrate changes in paternity of fertilized eggs at different intervals across the term of a pregnancy. Field-based studies of female social groups for which paternity could be assayed would be informative, but lab-based studies allowing controlled manipulation of mating opportunities would be better. Paternity analysis of blastocysts is the best way to determine the sperm fate from individual males and the resulting zygotes.

Currently, studies of paternity in species with delays are only available for a few mammalian species with delays and are of offspring resulting from a completed pregnancy thus other selective forces after implantation may have biased paternity results. Nevertheless, Soulsbury lists data for percent of multiple paternities for around 67 species of mammals including approximately 15 species with reproductive delays (Soulsbury, 2010). Studies that have examined multiple paternity in species with delays include; *Mustela vison* (Yamaguchi *et al.,* 2004), *Eptesicus fuscus* (Vonhof *et al*., 2006; Metheny *et al*., 2008), *Rhinolophus ferrumequinum* (Rossiter *et al*., 2000) among others (see Table 2.2). Additional population-level paternity studies at earlier stage (i.e. pre-implantation) are necissary to disentangle the patterns of paternity due to pre and post-copulatory processes.

P10. If males are using copulatory plugs as a form of behavioral intra-sexual competition, such plugs are expected to be male-derived, and found in females that delay fertilization. Copulatory plugs are expected to be particularly important (and thus may be more common) in species with delayed fertilization. This is because males of many species will mate with torpid females (Wai-Peng & Fenton, 1988), thus male-derived plugs could be used as a form of passive mate guarding. Copulatory plugs are typically interpreted as a way for male's to prevent additional copulations or at least provide additional time for their sperm to be stored in good locations by the time the next male has tried to mate with the female (i.e. a precedence effect). Thus, if mating plugs serve a role in mate guarding, females with intact versus experimentally removed plugs would be expected to show different patterns of paternity. It would likely be advantageous for plugs to be removed by subsequent males (or the female) resulting in sexual conflict. In species with copulatory plugs males may be observed physically removing plugs and in species with mating plugs males may have spikes etc. on their genitalia that serve to remove plugs from previous mating females.

(2) Delays between fertilization and implantation

After fertilization, the zygote begins to develop. Cell division continues until the conceptus becomes a blastocyst. In many species (Table 2.6), the blastocyst remains unattached but is maintained in the female's reproductive tract. This delay in implantation can last from 20 days (*Miniopterus minor,* the least long-fingered bat; Mori & Uchida, 1980) to over 2 months (*Rhinolophus landeri*, Lander's horseshoe bat; Racey, 1982) or almost a year (*Martes pennanti,* fisher; Wright & Coulter, 1967). Several modes of post-copulatory sexual selection would be facilitated by a greater time frame between fertilization and implantation.

(a) Cryptic female choice via selective implantation, zygote comparison, and re-absorption

By delaying implantation, females may allow for the accumulation and comparison of zygotes. The female might also fail to prepare the uterus for an embryo by foregoing a luteal cycle (Eberhard, 1996) and as a consequence rejecting embryos form particular males.

During this stage the selective re-absorption of some embryos may occur. In some mammals, including some carnivores, females re-absorb embryos (Larivière & Ferguson, 2003), and re-absorption may be aimed at specific embryos or blastocysts. In the bat *Pipistrellus rusticus,* about four blastocysts co-occur in the tract, but only 1-2 implant (van der Merwe & Rauthenbach, 1990), while in *Perimyotis subflavus,* up to seven eggs are fertilized but only one implants (Wimsatt, 1945; Table 2.2). A useful strategy in species that have small litters, including those bats that typically only have 1-2 offspring per litter (Barclay & Harder, 2003), could be to allow multiple fertilization events to occur, facilitating the comparison of blastocysts and their genotypes. A similar concept of overproduction of young as a strategy to maintain female reproductive success has been discussed in reference to the production of eggs and asynchronous hatching in birds (Konarzewski, 1993).

Order	Family	Genus species	Common Name	Delay Length (days)	Source
Artiodactyla (Cetartiodactyla)					
	Cervidae	*Capreolus capreolus*	European roe deer	135	Ziegler, 1843; Bischoff, 1854
		Elaphurus davidianus	Père David's deer	*implicated	Brinklow & Loudon, 1993
Carnivora					
	Ailuridae	*Ailurus fulgens*	red panda	*implicated	Roberts & Gittleman, 1984
	Mephitidae	*Arctonyx collaris*	hog badger	-	Parker, 1979
		Conepatus mesoleucus	western hog-nosed skunk	60	Macdonald, 1984
		Enhydra lutris	sea otter	130	Novikov, 1956; Sinha, Conaway & Kenyon, 1966
		Gulo gulo	wolverine	210 *may be facultative?	Wright & Rausch, 1955; Rausch & Pearson, 1972
		Lontra longicaudis	Neotropical river otter	*may be facultative?	Cubas *et al.,* 1993; Jacome & Paera, 1995
		Lutra canadensis	American river otter	285	Hamilton & Eadie, 1964
		Martes americana	American marten	225	Ashbrook & Hansson, 1930; Pearson & Enders, 1944; Wright, 1963
		Martes flavigula	yellow-throated marten	105	Roberts, 1977
		Martes foina	beech marten	240	Prell, 1927; Canivenc *et al*
		Martes gwatkinsii	Nilgiri marten	-	Macdonald, 1984

	Martes martes	pine marten	240	Prell, 1927; Stubbe, 1968 ; Canivenc *et al*., 1981
	Martes melampus	Japanese marten	195	Macdonald, 1984
	Martes pennanti	fisher	315	Enders & Pearson, 1943; Eadie &
	Martes zibellina	sable	247	Novikov, 1956; Bernatskii *et al*., 1976
	Meles meles	Eurasian badger	255	Fries, 1880; Neal & Harrison, 1958; Canivenc, 1966; Canivenc & Bonnin, 1981
	Mellivora capensis	honey badger	*implicated	Rosevear, 1974
	Mephitis macroura	hooded skunk	30	Macdonald, 1984
	Mephitis mephitis	striped skunk	30	Wade-Smith & Richmond, 1975, 1978; Wade-Smith *et al.,* 1980
	Mustela erminea	short-tailed weasel, ermine	270	Watzka,1940; Deanesly, 1943; Enders, 1952
	Mustela frenata	long-tailed weasel	240	Wright, 1942
	Mustela lutreola	European mink	8.5	Nowak & Paradiso, 1983
	Mustela vison	American mink	27.5	Hansson, 1947; Enders, 1952
	Spilogale gracilis	western spotted skunk	150	Mead, 1968; Greensides & Mead, 1973; Mead, 1981
	Taxidea taxus	American badger	195	Hamlett, 1932b; Wright, 1966
	Vormela peregusna	marbled polecat	223	Mendelssohn *et al*., 1988
Odobenidae	*Odobenus rosmarus*	walrus	135	Fay, 1981; 1982
Otariidae	*Arctocephalus australis*	South American fur seal	120	Riedman, 1990

Arctocephalus philippii	Juan Fernández fur seal	*implicated	Riedman, 1990
Arctocephalus galapagoensis	Galápagos fur seal	*implicated	Riedman, 1990
Arctocephalus gazella	Antarctic fur seal	126	Riedman, 1990
Arctocephalus pusillus	cape fur seal, South African fur seal	~120	Riedman, 1990
Arctocephalus townsendi	Guadalupe fur Seal	120	Riedman, 1990
Arctocephalus tropicalis	Subantarctic fur seal	129	Riedman, 1990
Callorhinus ursinus	northern fur seal	120	Craig, 1964; Daniel, 1981
Eumetopias jubatus	stellar sea lion	105	Harrison, 1969; Schusterman, 1981
Neophoca cinerea	Australian sea lion	*implicated	Riedman, 1990
Otaria byronia	southern sea lion, or South American sea lion	105	Hamilton, 1939; Daniel, 1981
Phocarctos hookeri	Hooker's sea lion	-	Riedman, 1990
Zalophus californianus	California sea lion	97.5	Odell, 1981
Cystophora cristata	hooded seal	105	Ørtisland, 1964
Erignathus barbatus	bearded seal	75	McLaren, 1958; Harrison 1969
Halichoerus grypus	grey seal	105	Hewer & Backhouse, 1968; Harrison, 1969
Hydrurga leptonyx	leopard seal	48	Sinha & Erickson, 1972; Riedman,
Leptonychotes weddellii	Weddell seal	75	Mansfield, 1958; Stirling, 1969;
Lobodon carcinophagus	crabeater seal	60	Harrison, 1969; Daniel, 1981
Mirounga angustirostris	northern elephant seal	75	Steward & Huber, 1993
Mirounga leonina	southern elephant seal	105	Harrison, Matthews & Roberts, 1952; Laws, 1956

Phocidae	*Monachus monachus*	Mediterranean monk seal	*implicated	Riedman, 1990
	Monachus schauinslandi	Hawaiian monk seal	*implicated	Riedman, 1990
	Ommatophoca rossii	Ross seal	~75	Reeves & Ling, 1981; Kovacs & Lavigne, 1986
	Pagophilus groenlandicus	harp seal	105	Harrison, 1969; Harrison, 1963
	Pusa (Phoca) caspica	Caspian seal	*implicated	Riedman, 1990
	Pusa (Histriophoca) fasciata	ribbon seal	*implicated	Riedman, 1990
	Pusa (Phoca) hispida	ringed seal	105	McLaren, 1958; Frost & Lowry, 1981; Harrison, 1969
	Pusa (Phoca) largha	larghe, spotted seal	75	Bigg, 1981; Harrison 1969
	Pusa(Phoca) sibirica	Baikal seal	-	Kozhov, 1947; Riedman, 1990
	Pusa (Phoca) vitulina	harbor seal	~60	Fisher 1954, Harrison, 1963; 1969; Bigg & Fisher, 1974
Ursidae	*Helarctos malayanus*	sun bear	*implicated	Dathe, 1963; McCusker, 1975
	Melursus ursinus	sloth bear	-	Laurie & Seidensticker, 1977; Puschman, Schuppel & Kronberger, 1977
	Ursus americanus	American black bear	165	Hamlett, 1935; Wimsatt, 1963

		Ursus arctos	brown (grizzly) bear	150	Dittrich & Kronenberger, 1963; Craighead, Hornocker & Craighead, 1969
		Ursus maritimus	polar bear	240	Dittrich, 1961; Volf, 1963
	Viverridae	*Civettictis civetta*	African civet	~20 *implicated	Ewer & Wemner, 1974
Chiroptera					
	Pteropodidae	*Cynopterus brachyotis*	lesser short-nosed fruit bat	-	Kofron, 1997
		Cynopterus minutus	minute fruit bat	-	Kofron, 1997
	Rhinolophidae	*Rhinolophus landeri*	Lander's horseshoe bat	60	Racey, 1982
		Rhinolophus rouxi	rufous horseshoe bat	43	Ramakrishna & Rao, 1977; Richardson, 1977 Racey, 1982
	Vespertilionidae	*Corynorhinus townsendii*	Townsend's big-eared bat	*implicated	Pearson, Koford & Pearson, 1952
		Lasionycteris noctivagans	silver-haired bat	10	Druecker, 1972
		Miniopterus australis	little long-fingered bat	30	Racey, 1982
		Miniopterus fraterculus	lesser long-fingered bat	~135	Racey, 1982
		Miniopterus minor	least long-fingered bat	21	Mori & Uchida, 1980
Cingulata					
	Dasypodidae	*Dasypus hybridus*	Mulita armadillo	60	Hamlett 1932a, 1935; Enders 1966
		Dasypus novemcinctus	nine-banded armaddio	120	Hamlett, 1932, 1935; Enders, 1966
Diprotodontia					

Acrobatidae	*Acrobates pygmaeus*	feathertail glider	-	Ward & Renfree, 1988
	Distoechurus pennatus	feathertailed possum	-	Ward & Renfree, 1988; Ward, 1990
Burramyidae	*Cercartetus concinnus*	western pygmy-possum	-	Ward, 1990
	Cercartetus lepidus	little pygmy-possum	-	Ward, 1990
	Cercartetus nanus	eastern pygmy-possum	-	Ward, 1990
Macropodidae				
	Lagostrophus fasciatus	banded hare-wallaby	-	Sharman (unpublished data) cited in Sharman.
	Macropus agilis	agile wallaby	-	Merchant, 1976
	Macropus eugenii	tammar wallaby	<330	Renfree, 1994
	Macropus giganteus	eastern grey kangaroo	-	Kirkpatrick, 1965; Poole & Catling, 1974; Poole, 1975
	Macropus parma	Parma wallaby	~31	Maynes, 1973
	Macropus parryi	pretty-faced (whiptail) wallaby	-	Maynes, 1973
	Macropus robustus	common wallaroo, eastern wallaroo	35	Ealey, 1963
	Macropus rufogriseus	red-necked wallaby	facultative?	Buchanan, 1963
	Macropus rufus	red kangaroo	facultative?	Merchant & Calaby, 1981
	Protemnodon eugenii	kangaroo island wallaby	-	Sharman, 1955a
	Protemnodon ruficollis	Bennett's wallaby	-	Sharman, 1955a
	Setonix brachyurus	quokka	facultative?	Sharman, 1955b; Shield 1968
	Thylogale billardierii	Tasmanian pademelon, red-bellied pademelon	-	Sharman (unpublished data), cited in Sharman, 1963; Rose and

					McCartney, 1982
		Thylogale thetis	red-necked pademelon	-	Sharman (unpublished data), cited in Sharman 1963
		Wallabia bicolor	swamp wallaby	-	Calaby & Poole, 1971
	Potoroidae	*Aepyprymnus rufescens*	rufous bettong	-	Flynn, 1930
		Bettongia cuniculus	pygmy possum	-	
		Bettongia gaimardi	Tasmanian (eastern) bettong	-	Rose, 1978
		Bettongia lesueur	boodie, burrowing bettong, coccyx	-	Tyndale-Biscoe, 1968
		Bettongia penicillata	Woylie, brush-tailed bettong	-	Parker, 1977
		Potorous tridactylus	long-nosed potoroo	-	Hughes, 1962b; Shaw and Rose, 1979
	Tarsipedidae	*Tarsipes rostratus*	honey possum	*slow	Hughes, 1962b; Shaw and Rose, 1979 Renfree, 1980
Eulipotophyla					
		Sorex araneus	*Common shrew*	-	Brambell, 1935
		Talpa altaica	*Altai mole*	-	Baevskii, 1967; Judin, 1974

Table 2.6. Species known to exhibit delayed implantation listed by family with length of delay (if known) and citation. According to Tyndale-Biscoe and Renfree (1987) all macropods except *Macropus fuligincsus* have delayed implantation.

(*b*) *Genetic incompatibility avoidance and delayed implantation*

Between fertilization and implantation, zygotes that present incompatible combinations of genes could fail to implant and this process could be refined if the period over which implant occurs was extended as in delayed implantation. Thus, during delays post-copulatory sexual selection may also operate via genetic incompatibility avoidance (Tregenza & Wedell, 2000). Zeh and Zeh (1996, 1997, 2000) suggested that females may passively reject embryos that have undesirable genetic patterns. This process is dictated by genetic compatibility of the female and male genomes when combined in the new zygote, and may be due to several processes, including immune system interactions, intra and intergenomic conflict, dominance, over-dominance and embryo-maternal interactions (Zeh & Zeh, 1996, 1997; Tregenza & Wedell, 2000; Stockley, 2003). Thus, genetic incompatibility avoidance differs from cryptic female choice focused on paternity determination because it is the zygote's genome (i.e. the combination of both maternal and paternal chromosomes) that is the target of selection.

Delays might serve as 'check points' for a pregnancy to continue to the next stage of development, or as a point of assessment during which genetic incompatibility is assessed. For example, if a zygote's genetic makeup can only be detected in mature blastocysts, we might expect females of species with delayed implantation to be evaluating the genetic compatibility of their blastocysts during the delay. Support for this idea comes from a comparative study of polytocous mammals (those with litters rather than a single offspring), which revealed that species in which females mate multiply (and have numerous eggs available for fertilization) have lower rates of reproductive failure than do monogamous species (Stockley, 2003). This result supports the role of genetic incompatibility avoidance, presumably through the comparison of fertilized eggs (Stockley, 2003).

(c) Predictions- delayed implantation

P11. Females of species with delayed implantation will exhibit a higher frequency of post-copulatory choice among embryos than females of species without such delays. Thus we also expect delays to facilitate this same process by offering females a longer window in which to evaluate offspring and thus for females with delayed implantation to have lower reproductive failure than those without delays. Evidence for multiple paternity within litters of twinning bats and other mammals is accumulating (Table 2.2), consistent with several blastocysts of different origins co-occurring in the female's reproductive tract, even in species that usually only give birth to twins or a single offspring (Vonhof *et al*., 2006; Fox, Spencer & O'Brien, 2008). Reproductive delays would allow females more time to evaluate blastocysts and compare different paternity combinations. Alternatively, if females exhibit delays of variable lengths they may allow some blastocysts to immediately implant after mating with a high quality male while blastocysts from low quality males may remain unimplanted until future mating events or seasonal changes 'force' the female to continue the pregnancy (allow implantation) or forego the reproductive event. Implanted blastocysts could then be evaluated at a later stage of development (see delayed development below). Embryos of different sizes that may be a result of different implantation times have been noted in numerous taxa and are often mistaken for superfetation (Roelling *et al.,* 2011).

(3) Delays between implantation and birth (delayed development)

In a few species of bats (and possibly other taxa), development may be slowed or stop completely after implantation (Table 2.7). The primary mode of sexual selection during embryonic diapause would be differential re-absorption of certain embryos which is discussed above in reference to re-absorption of blastocysts. If the reabsorbed embryos are fathered by particular males this may be an example of cryptic female choice. Some bats are able to re-absorb or abort embryos in response to stressful environmental conditions (Wimsatt, 1945; Bleier, 1975b; Bouchard, Zigouris & Fenton, 2001) and it is possible they could/can do so under other conditions.

Order	Family	Genus Species	Common Name	Delay Length (days)	Source
Chiroptera					
	Emballonuridae	*Saccopteryx bilineata*	greater sac-winged bat	-	Bradbury & Vehrencamp, 1976, 1977
	Molossidae	*Otopteropus cartilagonodus*	Luzon fruit bat	*implicated	Heidman, Cummings & Heaney, 1993
	Natalidae	*Natalus stramineus*	Mexican funnel-eared bat	*implicated	Mitchell, 1965
	Phyllostomidae	*Artibeus jamaicensis*	Jamaican fruit bat	75	Fleming, 1971
		Carollia perspicillata	Seba’s short-tailed fruit bat	50	Rasweiler & Badwaik, 1997
		Macrotus californicus	California leaf-nosed bat	135	Bleier, 1975a
	Pteropodidae	*Haplonycteris fischeri*	Philippine pygmy fruit bat	135	Heidman, 1989
	Rhinolophidae	*Rhinolophus hipposideros*	lesser horseshoe bat	-	Kolb, 1950
	Vespertilionidae	*Myotis myotis*	mouse-eared bat	-	Petri *et al*., 1997
		Pipistrellus pipistrellus	common pipistrelle	-	Pagenstecher, 1859; Racey & Potts, 1970

Table 2.7. Species known to exhibit delayed development. Species with delayed development are listed with length of delay and citations.

(b) Predictions and experiments- delayed development

P12. If delayed development facilitates post-copulatory sexual selection, species with delayed development should exhibit lower degrees of late-stage reproductive failure. This is because if females with delays are able to accumulate and compare developing embryos, they may have more opportunities to successfully carry a high quality pup to full term. During delays females may assess the combined maternal and paternal genetic make-up of the embryo as well as the resulting phenotype. Poorly developing embryos could be reabsorbed with the remaining embryos being of higher-quality and thus surviving better upon birth. Similar logic was discussed by Stockley (2003) who suggested that multiply-mating females are less likely to experience reproductive failure because they will have higher potential for genetically diverse and thus compatible offspring. We suggest that in delaying species the process operates by allowing embryo re-absorption. Tests of this prediction could include comparing percent of successfully weaned pups from delaying versus non-delaying species. Another important study would be one that could demonstrate that absorption of specific low-quality embryos is possible.

(b). Predictions- post-copulatory sexual selection and the potential evolution of delays

P13. The length of delays in species with extreme sexual conflict will be longer than those with lower degrees of conflict. For example if males exhibit baccular or penile elaborations and increased testes sizes we may expect females to have longer delays than those species whereby males have only just started to ‘respond’ by evolving counter adaptations.

P14. If delays have important evolutionary significance via post-copulatory sexual selection we might expect them to serve as a post zygotic isolation mechanism relevant for speciation. This prediction would be best examined in recently diverged species or in species with facultative delays that vary by a fixed extrinsic factor like latitude. Currently, gaps in our knowledge of species with and without delays make a prerequisite to first determine the presence and/or absence of delays across taxonomic groups. For this reason, the Carnivora may serve as a good starting point (see Tables 2.5-2.7). If cycles between individuals become mis-matched they might be an important mechanism for speciation reinforcement. Gametic isolation could also be examined using artificial insemination of females with sperm from males of diverging taxa following the methods of Ludlow and Magurran (2006). Specifically, by artificially inseminating females experiments could control both for pre-copulatory mate choice and sperm numbers. Paternity analysis both of resulting offspring and aborted embryos aimed at determining if paternity biases occur would allow researchers to determine if gametic isolating is occurring. Assume delays are used by females as a means of post-copulatory sexual selection (scrutiny of male sperm or offspring genotypes). Furthermore, imagine two recently diverged incipient species coming back into contact, with subsequent gene flow. Females with delay may have greater opportunity to prevent 'foreign sperm' from fertilizing their precious eggs or have more elaborate mechanisms in place to prevent investment in hybrid offspring (genetic incompatibility avoidance). Thus delay may contribute to a self-reinforcing post-zygotic isolating mechanism such that species with delay evolve reproductive isolating barriers quicker than species lacking delays.

CONCLUSIONS

Reproductive delays offer an unconsidered avenue for the operation of post-copulatory sexual selection in mammals because they lengthen the reproductive periods over which post-copulatory sexual selection operates. Delayed fertilization (female sperm storage or delayed ovulation) may increase the opportunity for sperm competition and sperm selection, and in species with delayed implantation, cryptic female choice can operate more freely. Delayed implantation may allow females to bet-hedge by continuing to mate before committing to a pregnancy. Meanwhile, species that have delayed development are presented with an extended period over which the embryo may be evaluated via cryptic female choice and genetic incompatibility processes whereby patterns of maternal and paternal gene expression are revealed in the offspring.

Delays may prove an important factor in explaining phenomena such as bacular size variation (Hosken *et al*., 2001; Lüpold, McElligott & Hosken, 2004) or the use of penile elaborations. Including delays as a covariate may explain previously confusing results in studies of mammalian post-copulatory sexual selection. Because females with delays have a longer time frame in which processes such as sperm selection or embryo re-absorption can occur, reproductive delays may provide a new and interesting way to look for cryptic female choice. Several species already commonly studied possess delays. These may make excellent study species for researchers interested in the potential for post-copulatory sexual selection. Additionally, species with facultative delays may provide researchers with the opportunity to conduct controlled experiments by manipulating presence or absence of delays.

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Chapter 3.

Reproductive delays facilitate post-copulatory sexual selection in bats: evidence from museum specimens

ABSTRACT

Reproductive delays between mating and birth may provide a previously unconsidered avenue for post-copulatory sexual selection. In particular, delayed fertilization could provide an enhanced opportunity for sperm competition by extending the time for ejaculates to interact in the female reproductive tract. We tested the prediction that species with delays exhibit greater degrees of sperm competition than those without delays by examining testis volume (a proxy for sperm competition) in 40 species of bats. Examination of fluid-preserved museum specimens of bat species with and without delays revealed that species with delays had significantly larger testes than species without them. Thus, we suggest that once they evolve reproductive delays may facilitate post-copulatory sexual selection.

Keywords: delayed fertilization; sperm competition; sexual selection; testis size

INTRODUCTION

Reproductive delays occur when the sequence of events from insemination to birth is interrupted, either 1) after copulation but before fertilization (delayed fertilization), 2) after fertilization but before the blastocyst implants (delayed implantation), or 3) during gestation (delayed development) (Daniel, 1970; Sandell, 1990). Such delays are quite common, but while their evolutionary origins have been studied (Sandell, 1990; Bernard & Cumming, 1997; Lindenfors, Dalen & Angerbjoern, 2003; Ferguson, Higdon & Larivière, 2006), their role in enhancing the opportunity for post-copulatory sexual selection has not been examined. Because some reproductive delays provide a longer time window for processes leading up to fertilization, they may pose an avenue for both sperm competition and cryptic female choice (Birkhead & Møller, 1993; Orr, Chapter 2). Nearly half of all mammal species that possess delays are bats, and bats are the only group exhibiting all three types of delay (Bernard & Cumming, 1997). Given that some female bats can store sperm from 16-200 days (Hood & Smith, 1989), the opportunity for sperm competition is expected to be greater in bat species with this type of delayed fertilization than in those without (Birkhead & Møller, 1993; Orr, Chapter 2).

Sperm are costly to produce (Dewsbury, 1982), but because the probability of fertilizing an egg is correlated with sperm production (Martin *et al*., 1974), males are expected to invest more in sperm only when necessary for successful fertilization. In cases where females mate multiply, males have a decreased certainty of fertilizing eggs and thus more investment in sperm may help males increase their chances of a successful fertilization. The result is sperm competition, a battle between sperm from more than one male for fertilization (Parker, 1970). Evidence of sperm competition can be seen in larger relative testes size, specialized sperm morphology, elaboration of the female reproductive tract (Hosken, 1997; Pitnick, Hosken & Birkhead, 2009), and penile morphology (Eberhard, 1985). The most commonly used estimate for sperm competition is testis size. Larger testes produce more sperm (Amann, 1970; Parker, 1970) and species with increased risk of sperm competition have larger testes (Kenagy & Trombulak, 1986; Wilkinson & McCracken, 2003; Dixson & Anderson, 2004). We used testis size as a proxy for sperm competition in bat species with and without reproductive delays to test the hypothesis that species with delays experience a greater risk of sperm competition than species without delays. Species with different types of delays should experience different degrees of sperm competition; for example, sperm competition would occur prior to the period of delay associated with delayed development, when a female already has an embryo implanted. If females with delayed development were mating multiply they could still experience sperm competition but the period over which it might occur would be shorter relative to species with delayed fertilization. We therefore predicted that species with delayed fertilization would have the highest degree of sperm competition.

METHODS

Data collection

To find reports of bats with reproductive delays we performed a literature search in SciSearch using the key words reproductive delay, diapause and Chiroptera, and supplemented this with data from species accounts and Hayssen, van Tienhoven & van Tienhoven (1993) (Table 3.1). Only species confirmed to have one of the three types of delays or for which it was evident from the literature (often detailed physiological data) that delays were absent, were included in our sampling. Delays were coded two ways; delayed fertilization (presence or absence) and by delay type (4 categories; delayed fertilization, implantation, development and no delays). Because published data on testis size were limited, we measured fluid specimens of bats with intact and preserved testes.

Most bats for which we know the mating system (less than 6% of all species) are polygynadrous with females mating multiply (McCracken & Wilkinson, 2003). Currently, there are not good data on how many times females mate in the field for more than a few species (Hosken, 1998; Heckel, 2002). Although we don't know the mating systems of all bats, it is likely that most species mate multiply. Indeed in the few species for which there are data on both reproductive physiology and mating system this appears to be the case (Ortega *et al*., 2003; also see McCracken & Wilkinson, 2003). Additionally, multiple paternity has now been documented in some of the few twinning species (two males father offspring in one litter) (Vonhof *et* al., 2006; Fox *et al.*, 2008). However, genetic evidence of multiple mating will remain elusive because generally only one pup is born per reproductive event. For this reason although we could not use mating system as a covariate there is sufficient evidence to suggest most species mate multiply.

We examined testis volume of 285 individuals from 40 species in relation to reproductive delays, testing the prediction that testes are larger in species with delayed fertilization compared to species with delays occurring later in a pregnancy (delayed implantation or development). Because related species may resemble each other due to evolutionary relationships (Garland *et al*., 2005) we incorporated phylogenetic comparative methods (Garland *et al*., 2008).

We dissected male bats from the Los Angeles County Museum, Museum of Vertebrate Zoology, and American Museum of Natural History. We sampled approximately 6 (range: 1-17, mean: 5) reproductive adults from the same population and date (when possible). Male reproductive condition was approximated from known seasonal trends of spermatogenesis i.e.

during the spring with disjunctive mating in winter for temperate species, bimodal and simultaneous spermatogenesis and mating in tropical species (Racey & Entwistle, 2000). Condition was then assigned based on examination of individual specimens (Racey, 2009).

We measured testis linear dimensions to the nearest 0.03 mm with digital calipers. Body mass was recorded for dry patted specimens to the nearest 0.1 g. We used the formula for a prolate spheroid volume to examine testis size (Myers, 1977).

$$TV=(4/3)\quad r^2 \text{ x } l/2= 0.5236 \text{ x } l \text{ x } w^2$$

Testis volume (TV) can thus be calculated from linear measurements of testis length (l) and width (w). The resulting number was multiplied by two in cases where only one testis was measured to yield total testis volume. Testis volume can be used as an unbiased proxy for testis mass (Wilkinson & McCracken, 2003).

Body mass was used as a measure of body size. However, because mass was measured on wet and potentially altered bat carcasses; our values may have been higher than if we had measured animals pre-preservation. Hosken found no significant differences in integrity of similar tissues before or after extended preservation (Hosken, 1998). However, to evaluate this possible bias in our data we compared data from museum records pre-preservation (field notes and museum tags) to our measurements. We found no significant difference ($p>0.53$, *n*=28, t-test). Additionally, because all of our data on body mass come from fluid-preserved specimens any effects of preservation should be consistent among individuals.

Species	common name	n	TV	BM	FA	delay type	citation (delay)
Vespertilionidae							
Antrozous pallidus	pallid bat	1	0.04 (NA)	20 (NA)	49.46 (NA)	DF	Orr, 1954
Corynorhinus townsendii	Townsend's big-eared bat	8	0.06 (0.01)	11.74 (1.58)	43.82 (1.24)	DI	Racey, 1982
Eptesicus furnalis	Argentine brown bat	2	0.047 (0.02)	11.90 (1.27)	35.15 (2.53)	DF	Racey, 1982
Eptesicus fuscus	big brown bat	8	0.19 (0.11)	21.51 (3.09)	47.06 (1.26)	DF	Gates, 1936
Lasiurus borealis	Western red bat	5	0.04 (0.01)	10.82 (1.28)	39.27 (1.78)	DF	For all lasiurines: Racey, 1982; Hayssen, van Tienhoven & van Tienhoven, 1993; Cryan *et al.*, *unpublished data*
Lasiurus cinereus	hoary bat	2	0.02 (0.01)	23.40 (3.41)	50.53 (0.08)	DF	
Lasiurus ega	Southern yellow bat	13	0.02 (0.01)	13.11 (0.94)	45.15 (1.39)	DF	
Lasiurus xanthinus	Western yellow bat	5	0.02 (0.01)	7.18 (2.21)	44.47 (0.475)	DF	
Lasionycteris noctivagans	silver-haired bat	6	0.04 (0.01)	13.57 (2.93)	40.60 (1.59)	DI	Hayssen, van Tienhoven & van Tienhoven, 1993
Miniopterus australis	little long-fingered bat	5	0.02 (0.01)	9.78 (0.72)	39.53 (0.47)	DI	Racey, 1982; Hayssen, van Tienhoven & van Tienhoven, 1993
Miniopterus schreibersi	Schreiber's long-fingered bat	1	0.03 (NA)	19.50 (NA)	48.53 (NA)	DI	Racey, 1982; Hayssen, van Tienhoven & van Tienhoven, 1993
Myotis albescens	silver-tipped myotis	10	0.16 (0.05)	7.82 (0.57)	35.10 (0.86)	DF	Racey, 1982
Myotis californicus	California myotis	2	0.029 (0.01)	6.5 (0.71)	33.83 (0.59)	DF	Krutzsch, 1954
Myotis daubentonii	Daubenton's bat	2	0.03 (0.02)	37.39 (1.17)	10.3 (0.14)	DF	Racey, 1982
Myotis lucifugus	little brown bat	5	0.13 (0.04)	7.72 (0.56)	36.27 (1.59)	DF	Racey, 1982
Myotis nigricans	black myotis	1	0.02 (NA)	5.9 (NA)	34.61	DF	Myers, 1977
Myotis velifer	cave myotis	11	0.09 (0.07)	10.14 (1.02)	42.51 (0.78)	DF	Krutzsch, Crichton & Nagle, 1982
Nyctalus noctula	common noctule	1	0.03 (NA)	29.0 (NA)	52.09 (NA)	DF	Racey, 1982
Parastrellus Hesperus	Western pipistrelle	3	0.01 (0.00)	4.13 (0.12)	30.06 (0.79)	DF	Krutzsch, 1975
Pipistrellus nanus	banana pipistrelle	4	0.02 (0.01)	4.65 (0.19)	27.06 (0.79)	DF	Bernard & Cumming, 1997
Pipistrellus pipistrellus	common pipistrelle	6	0.037 (0.01)	5.45 (0.64)	31.31 (0.94)	DF	Racey, 1982
Perimyotis subflavus	Eastern pipistrelle	1	0.01 (NA)	6 (NA)	24.16 (NA)	DF	Guthrie, 1933

Scotophilus dinganii	African yellow bat	3	0.03 (0.01)	21.17 (0.29)	51.26 (1.83)	DI	Okia, 1987
Scotophilus heathi	greater Asiatic yellow bat	4	0.03 (0.01)	21.60 (11.23)	50.19 (6.97)	DF	Racey, 1982
Tylonycteris robustula	greater bamboo bat	4	0.02 (0.07)	8.23 (0.17)	30.03 (0.48)	DF	Racey, 1982
Natalidae							
Natalus stramineus	Mexican funnel-eared bat	1	0.01 (NA)	7 (NA)	36.81 (0.37)	DD	Racey,1982
Phyllostomidae							
Artibeus jamaicensis	Jamaican fruit-bat	8	0.14 (0.05)	44.31 (9.11)	59.30 (2.48)	DD	Racey, 1982; Hayssen, van Tienhoven & van Tienhoven, 1993
Carollia perspicillata	Seba's short-tailed fruit bat	17	0.12 (0.05)	24 (4.26)	42.26 (2.03)	DD	Rasweiler & Badwaik, 1997
Desmodus rotundus	common vampire bat	9	0.12 (0.07)	34.97 (7.19)	59.94 (2.41)	N	Fleming, Hooper & Wilson, 1972
Glossophaga soricina	Palla's long-tongued bat	15	0.047 (0.24)	11.94 (1.05)	35.45 (0.92)	DI	Hayssen, Tienhoven & Tienhoven, 1993
Macrotus californicus	California leaf-nosed bat	5	0.01 (0.01)	15.56 (1.56)	50.13 (1.10)	DD	Racey, 1982; Hayssen, van Tienhoven & van Tienhoven, 1993
Uroderma bilobatum	tent-making bat	3	0.11 (0.05)	23 (1.14)	42.85 (0.87)	N	Baker & Clark, 1987
Mormoopidae							
Pteronotus parnellii	Parnell's mustached bat	6	0.05 (0.02)	2.50 (2.50)	63.09 (0.80)	DF	Herd, 1983
Rhinolophidae							
Hipposideros caffer	Sundevall's bat	6	0.01 (0.01)	11 (2)	47.72 (2.75)	DI	Racey, 1982; Hayssen, van Tienhoven & van Tienhoven, 1993
Rhinolophus ferrumequinum	greater horseshoe bat	2	0.03 (0.03)	26.15 (4.45)	57.19 (0.58)	DF	Racey, 1982
Emballonuridae							
Saccopteryx bilineata	greater sac-winged bat	5	0.03 (0.01)	13.0 (1.17)	46.14 (2.31)	DD	Bradbury, 1979
Pteropodidae							
Cynopterus sphinx	greater short-nosed fruit bat	5	0.35 (0.19)	59 (6.56)	69.62 (1.81)	DD	Racey, 1982
Eidolon helvum	straw-colored fruit bat	3	12.96 (17.65)	280 (0)	124.77 (3.12)	DF°	DeFrees & Wilson, 1988

Haplonycteris fischeri	Philippine pygmy fruit bat	1	0.07 (NA)	23.00 (NA)	49.08 (NA)	DD	Hayssen, van Tienhoven & van Tienhoven, 1993
Macroglossus minimus	long-tongued nectar bat	2	0.10 (0)	17 (0)	38.3 (0)	DF	Hood & Smith, 1989

Table 3.1. Data from museum dissections and literature review. Data used in analyses shown here including; sample size, testis volume (cm^3) (TV), body mass (BM) (g), forearm length (FA) (mm). Standard errors are in parentheses and delay type listed with abbreviations DF, delayed fertilization; DI, delayed implantation; DD, delayed development; N, no delay). ˚*Eidolon helvum* is categorized as having DF rather than DI or DD because of the extended length of time between mating and blastocyst formation (DeFrees & Wilson, 1988).

Data analysis

We evaluated the relationship of testis size to delay type (scored two different ways, see above) and clade membership (Megachiroptera vs. Microchiroptera), with body mass as a covariate, using both conventional and phylogenetic analyses performed using the Matlab Regressionv2.m program (Lavin *et al*., 2008; Gartner *et al*., 2010). Data for body mass and testis volume were $\log_{10}$-transformed.

We used ordinary least squares (OLS) to fit analysis of covariance (ANCOVA) models, which effectively assume a "star" phylogeny (Garland *et al*., 2005; Lavin *et al*., 2008). For phylogenetic models, we used the Binninda-Emonds supertree (Bininda-Emonds *et al*., 2007, Figure 3.1) with estimates of branch lengths in Million years. We used phylogenetic generalized least squares (PGLS, Lavin *et al*., 2008; Gartner *et al*., 2010) ANCOVA models and also ANCOVA models in which the residual trait variation is modeled using an Ornstein-Uhlenbeck (OU) process (Lavin *et al*., 2008). We used maximum ln-likelihoods and corrected Akaike information criterion (AICc) to compare model fits (Burnham & Anderson, 2002), and we report model coefficients of determination (r^2), the OU parameter (d), and F statistics from models fitted by Restricted Maxiumum Likelihood (REML) (Lavin *et al*., 2008).

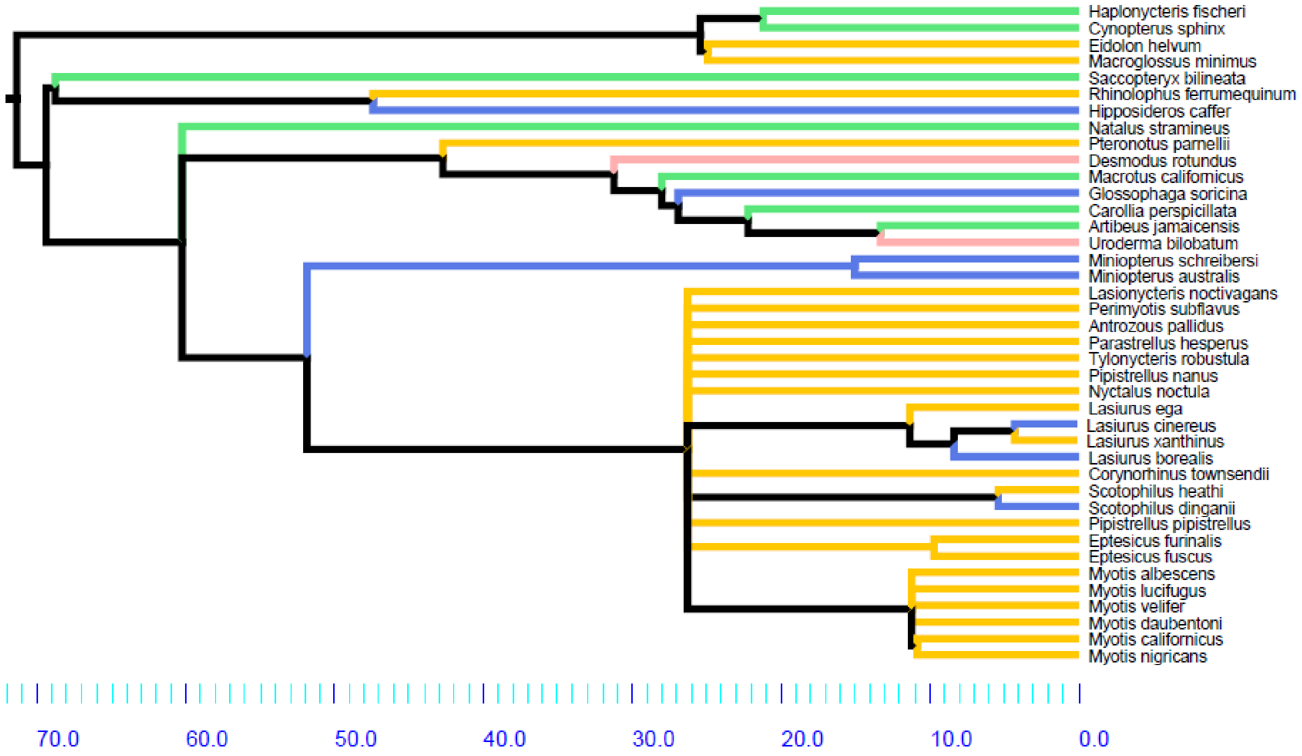

Figure 3.1. The phylogenetic tree used in our testes and delay analyses. The tree (shown above) used in our analyses was a smaller version of the Bininda-Emonds tree (Bininda-Emonds *et al.*., 2007). Species with delayed fertilization are in orange, delayed implantation in blue, delayed development in green and those with no delay in pink. Numbers illustrated below the tree indicate Million year divergence times.

RESULTS

As expected, log testis volume was strongly correlated with log body mass (Fig. 3.2, Table 3.2: $p < 0.0001$ in all models). Based on AICc, the best fit was obtained by an OLS model that included delay as a binary independent variable (indicating delayed fertilization or not) and clade (Megachiroptera vs. Microchiroptera), as shown in Figure 3.2.

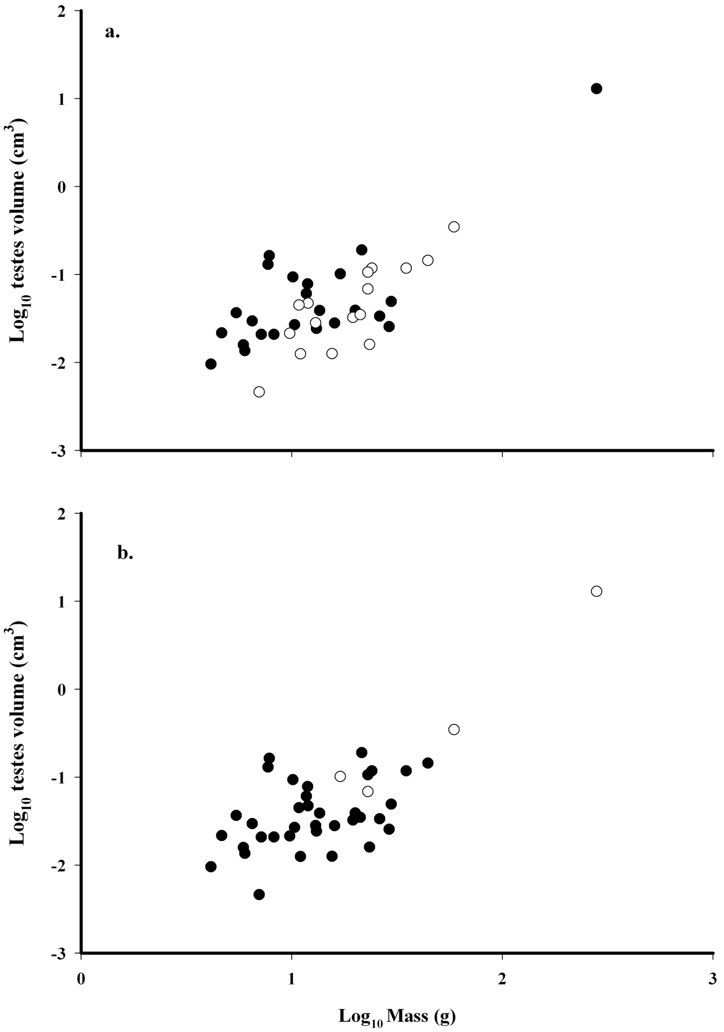

Figure 3.2. Testes mass and mass relative to (a) delay type and (b) clade membership. Plots of $\log_{10}$ testes mass versus $\log_{10}$ body mass in relation to (a) delay type (delayed fertilization (open circles) and no delayed fertilization (solid circles), and (b) in relation to clade (Megachiroptera (open circles) vs. Microchiroptera (solid circles)).

Model	ln ML	AICc	r^2	predictors	F for predictors	p for predictors	DF
mass (simple allometry)							
conventional (OLS)	-18.082	42.8	0.5316	mass	43.13	<0.0001	1, 38
phylogeny (PGLS)	-20.727	48.1	0.3617	mass	21.53	<0.0001	1, 38
phylogeny OU transform (d=0.204)	-16.473	42.1	0.5099	mass	39.53	<0.0001	1, 38
mass + delay (2 categories)							
conventional (OLS)	-14.789	38.7	0.6027	mass	55.69	<0.0001	1, 37
				delay	6.62	0.0142	1, 37
phylogeny (PGLS)	-20.509	50.2	0.3769	mass	21.53	<0.0001	1, 37
				delay	0.41	0.5259	1, 37
phylogeny OU transform (d=0.106)	-14.669	41.1	0.5866	mass	52.13	<0.0001	1, 37
				delay	3.70	0.0621	1, 37
mass + delay (2 categories) + clade (2 categories)							
conventional (OLS)	**-12.671**	**37.1**	**0.6426**	**mass**	**30.41**	**<0.0001**	**1, 36**
				delay	**6.24**	**0.0171**	**1, 36**
				clade	**4.02**	**0.0525**	**1, 36**
phylogeny (PGLS)	-19.885	51.5	0.3880	mass	18.79	0.0001	1, 36
				delay	0.33	0.5692	1, 36
				clade	1.14	0.2928	1, 36
phylogeny OU transform (d=0.119)	-12.536	39.6	0.6256	mass	29.46	<0.0001	1, 36
				delay	3.24	0.0802	1, 36
				clade	4.05	0.0517	1, 36
mass + delay (4 categories)							
conventional (OLS)	-14.418	43.4	0.6100	mass	49.57	<0.0001	1, 35
				delay	2.35	0.0892	3, 35
phylogeny (PGLS)	-19.17	53.8	0.3962	mass	20.69	<0.0001	1, 35
				delay	0.67	0.5762	3, 35
phylogeny OU transform (d = 0.128)	-14.408	46.3	0.5871	mass	46.89	<0.0001	1, 35
				delay	1.26	0.3031	3, 35
mass + delay (4 categories) + clade (2 categories)							
conventional (OLS)	-11.706	40.9	0.6595	mass	25.79	<0.0001	1, 34
				delay	2.62	0.0667	3, 34
				clade	4.94	0.0329	1, 34
phylogeny (PGLS)	-18.855	55.2	0.4187	mass	17.93	<0.0001	1, 34
				delay	0.709	0.5533	3, 34
				clade	1.32	0.2499	1, 34
phylogeny OU transform (d=0.128)	-11.584	43.8	0.6413	mass	25.95	<0.0001	1, 34
				delay	1.59	0.2098	3, 34
				clade	5.14	0.0299	1, 34

Table 3.2. Testes size results from Regressionv2.m analyses. Results from analysis of covariance models implemented in Regressionv2.m Matlab program (Lavin *et al.*, 2008). Model with lowest AICc (indicating best fit) is in bold. Note: r^2 is not comparable between conventional and phylogenetic models.

DISCUSSION

Species with reproductive delays also have statistically larger testes, suggesting that delays facilitate sperm competition, perhaps by providing more time for post-copulatory sexual selection to occur. In addition, Megachiropterans tended to have larger testes than Microchriopterans. Larger testis volume in bats with delayed fertilization is consistent with competition between sperm of different males for fertilization or preferential storage by the female. The percentage of sperm stored relative to sperm received is small in mammals, and females generally receive sufficient sperm for fertilization from a single copulation (Neubaum & Wolfner, 1999). Thus, selection for larger testes is likely due to sperm competition rather than longer storage time.

Many temperate species have sperm storage (i.e. delayed fertilization) (Racey & Entwistle, 2000). Many of these species are temperate bats that have minimal pre-copulatory sexual selection opportunities because of separate migration routes for the sexes and hence uncertain mating opportunities, or because females are mated while they are torpid (Wai-Peng & Fenton, 1988). Thus, these species may benefit from storing sperm and relying on sperm competition as their main mode of mate choice. Thereby females would have a longer period of time prior to fertilization and can allow sperm competition. This may result in higher offspring quality or at the very least fertilization by highly-competitive sperm which would ensure that her sons might also inherit this trait and be good sperm competitors. Mating with many males (even if inactively) would increase a female's likelihood of receiving good sperm from at least one male.

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Chapter 4.

Are frugivorous bats only eating fruit?

ABSTRACT

Bats display an impressive diversity of dietary niches, including frugivory. Fruit, however, is a nitrogen poor food source and, especially during reproduction, frugivorous bats are expected to be nitrogen-limited. Perhaps for this reason, many otherwise frugivorous bats supplement their diet facultatively with insects, which provide a good source of protein. It is unclear if this incorporation is seasonally or physiologically determined. One possible explanation for the use of insects is that it varies in relation to seasonality (and thus both sexes might use insects when they are available, independent of reproduction). Alternatively, we expected females to supplement their frugivorous diets with insects during the nitrogen-demanding periods of late stage pregnancy and lactation. To test if insect use was driven by season or physiological state, we measured naturally occurring stable isotopes of nitrogen ($\delta^{15}N$‰) to examine the roles of fruit vs. insects in the diets of the Jamaican fruit bat, *Artibeus jamaicensis*, a neotropical bat. Finally, we wanted to know how diet breadth varied with season, sex and by reproductive state (namely if bats were truly specialists). Seasonal variation in diet was evident, as were dietary shifts according to reproductive state.
The highest $\delta^{15}N$ values and consequently largest portion of insect usages was found in pregnant females. Surprisingly, lactating females did not consistently supplement their diets with insects and also exhibited narrow diet breadths focused on fruits. Males exhibited the narrowest dietary breadth and the greatest diet breadths were seen in non-reproductive females. Diet breath also varied by month. Our isotopic data combined with dietary (seed and fecal) samples from under roosts in Mexico indicate that, while fruits remain an important part of their diet, insects may be an extremely valuable source of nitrogen during reproduction of these bats.

Keywords.—*Artibeus jamaicensis*; insectivory; diet; $\delta^{15}N$; stable isotopes; trophic level

INTRODUCTION

Members of the Order Chiroptera exhibit dietary diversity that far surpasses that seen in other mammalian orders (Dumont, 2003). Most bat species are carnivorous, consuming blood, insects, and fish, while others exhibit various types of herbivory, including nectivory, frugivory and folivory. Even species thought to be specialists are now known to eat a greater variety of food items than previously expected. For example, bats long considered insectivorous, such as the pallid bat (*Antrozous pallidus*), will supplement their diets with nectar (Frick *et al.,* 2009). It has been known for some time that frugivorous bats will supplement their diets with insects (Dinnerstein, 1986; Herrera *et al.,* 2001a, 2001b, 2002). This is not surprising because exclusive frugivory is relatively rare in nature (Mack, 1990). Several hypotheses to explain the rarity of strict frugivory have been advanced. First, fruit may be nutritionally deficient (Dinnerstein, 1986; Mack, 1990). Second, fruit is not always available and thus may be an unreliable food source (Mack, 1990; Dumont, 2003). Third, unripe fruit may contain toxic or indigestible secondary compounds (Rosenthal & Janzen, 1979).

Given the low protein content of many fruits and the high nutritional demands of mammalian reproduction (Kunz & Orrell, 2004), frugivory may be particularly nutritionally insufficient during pregnancy and lactation (Dinnerstein, 1986). Therefore, it is reasonable to expect deviations from strict frugivory to include protein-rich resources like insects during reproduction. Herrera *et al.* (2002) demonstrated that some frugivorous bats supplement their diets with insects, but when and why this occurs is not clear. Because most bat reproductive cycles, particularly those of females, are highly correlated with food availability (Racey & Entwistle, 2000), we suspect that females are more likely than males to supplement their diets with insects.

Furthermore, we expect such supplementation to occur most often during periods of nutritional stress, in particular lactation or pregnancy. We tested three hypotheses regarding why bats might supplement their diets with insects.

H1) The Seasonal Insectivore Hypothesis.—Bats shift to eating insects independent of reproduction but in relation to seasonality either because insects are easier to get, or because fruit are scarce. If this were the case we would predict (P1) that all bats (both sexes and all reproductive categories) supplement their diets with insects whenever insects are more available (or fruit less available). If the Seasonality Hypothesis is correct, (P1) all bats will eat more insects at certain times of the year.

H2) The Nutritionally Wise Female Hypothesis.— insect supplementation is more common in female bats during lactation and pregnancy relative to other reproductive states. This would be the case if bats use insects during times of high energetic demand as experienced during reproduction. Specifically, we expected (P2) only pregnant and lactating bats to eat insects. H2 is a special case of what has been called the Nutritional Wisdom Hypothesis that posits that animals should seek foods that contain specific nutrients versus specializing on specific foods (Tracey *et al*., 2006). Thus if H2 were true diets would not be random as that would indicate that bats are eating foods based solely on their abundances (P3).

H3) **The Evenness and Diet Width Hypothesis.—***Artibeus jamaicensis* is generally considered a frugivore. However, it does occasionally consume insects (Herrera *et al*., 2001a, 2001b, 2002). Given previous quantifications of this shift we hypothesized that *A. jamaicensis* will exhibit an isotopically narrow diet breadth (i.e. a highly even diet in the ecological sense) either as a ‘true specialist’, a ‘synchronized specialist’ or a ‘specialized individuals’. This third hypothesis is not mutually exclusive from H1 and H2 but instead focuses on **how** these bats might change to insectivory and to what degree. This shift should be because of one of options illustrated in Figure 4.1. We did not expect to observe strict frugivory (Figure 4.1, top left-hand illustration (P4)). However, dietary differences could fall into several different types. Specifically, switching between fruits and insects could be synchronous (Figure 4.1, top right illustration (P5)) consistent with H1 the '**seasonal insectivore hypothesis**' whereby all bats switch diets toward insectivory simultaneously). Synchronous switching would be observed as lower isotopic variance (diet breadth). Diet changes could be asynchronous (Figure 4.1, bottom right-hand illustration (P6)) which would inconsistent with either H1 or H2. Alternatively, only specific bats may switch to eating insects (Figure 4.1 bottom left-hand illustration (P7)). This population-level asynchronous switching would result in increased variance of diets (Bearhop, 2005). If this asynchrony is associated with reproductive state it would be consistent with H2, **The Nutritionally Wise Female Hypothesis**.

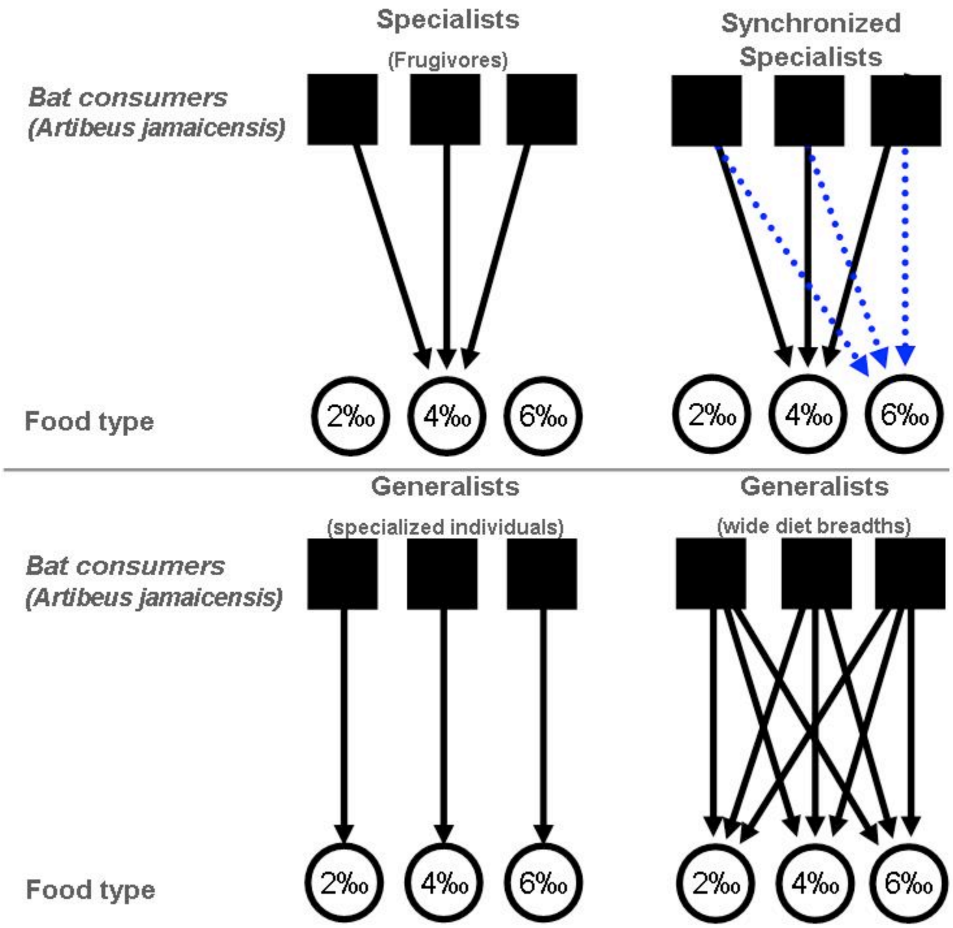

Figure 4.1.—Predictions associated the Evenness and Diet Width Hypothesis. Modified from Bearhop (2003) this figure illustrates the main isotopic outcomes for the hypotheses above. Boxes indicate bat consumers. Each box is an individual and can be considered as males, non-reproductive females and lactating females or any other subsets of the population. Their utilization of food sources (indicated by arrows) would be evident given the different isotopic signatures of their foods (in circles listed in increasing trophic level from left to right). Fruits would have lower isotopic values relative to insects. Specialists (P4), Synchronized specialists (blue dashed arrows indicate a synchronized shift in diet (P5)), Generalists with specialized individuals (P7) and Generalists with wide diet breadths are illustrated (P6).

We examined these three hypotheses regarding the diet of *Artibeus jamaicensis* (the Jamaican fruit bat) to determine if insect supplementation is seasonally (H1) or nutritionally (i.e. physiologically) (H2) driven. However, it is possible that the best explanation of bat insect use includes both variables simultaneously. Furthermore, we were interested in evenness and wanted to determine how these patterns were realized by the members of the population (H3).

Artibeus jamaicensis is abundant throughout its range, which extends from North-Central Mexico to Northern Argentina (Ortega & Castro-Arellano, 2001). The reproductive cycle of *A. jamaicensis* appears to be highly correlated with fruit availability (Fleming, 1971, Fleming *et al.*, 1972; Handley *et al.*, 1991). *A. jamaicensis* is a known *Ficus* specialist, with roughly 80% of its diet derived solely from this food source (Heithaus, 1975; Morrison, 1978, 1980; Ortega & Castro-Arellano, 2001; Handley *et al.,* 1991).

Figs are relatively high in energy content (6.4-8.3 kJ / fruit) and are primarily composed of sugar (14.4% wet mass) and water (50.59% wet mass). *Ficus* pulp is about 1/3 digestible water soluble carbohydrates (Wedeln *et al.*, 2001) with a small amount of fat (1.1%) and contains only very small amounts of protein (0.35%: (Morrison, 1978, 1980)). Figs are therefore often considered to be of low nutritional value (Wendelen, Runkle & Kalko, 2000). Figs also contain indigestible fiber, tannins and complex carbohydrates that may pose digestive problems. Another preferred food of *A. jamaicensis, Piper*, contains even less nitrogen than *Ficus* (Dinerstein, 1986). When ingesting figs, *A. jamaicensis* is able to successfully digest 25-35% of fig materials by weight. Thus they would require 66 grams of figs to maintain body condition (i.e. maintain themselves in a non-reproductive state), which requires approximately 12 kcals per night (Morrison, 1980). However, this estimate is for non-reproductive adults of either sex. Energy demands increase between 66-133% during lactation (Dinerstein, 1986).

Demands of reproduction

In bats, relative size at weaning is much greater than in other mammals (Kurta & Kunz, 1987) and reflects a higher investment in young compared to other mammals. Given the large size of offspring both at birth and upon weaning, pregnant and lactating bats are expected to consume a great deal of energy. For example, females may reach masses of 25-40% more than non-reproductive states during pregnancy (Charles-Dominique, 1991). In *A. jamaicensis* neonates constitute about 26% of maternal mass at birth (Taft & Handley, 1991). This is higher than the average for bats (22.3% ± 0.8 SE), and small (< 500g) non-volant mammals (7.8% ± 0.5 SE) (Kurta & Kunz, 1987). Because weaning coincides with the ability to fly, it occurs when bat pups are nearly adult size (Voigt, 2003). In *A. jamaicensis,* lactation lasts 15-60 days and pups weigh around 90% of their mother's mass at weaning (Kwiecinski *et al.,* 2003). Food consumption increases in some lactating bats by between 45-200% over the non-reproductive intake (McLean & Speakman, 1999, Fleming, 1988) and energy usage may increase by up to 85% (McLean & Speakman, 1999; Voigt, 2003). To meet energetic demands, foraging duration increases (McLean & Speakman, 1999).

The increase in energetic demands during lactation may have direct influence on a female's somatic condition in some cases resulting in depletion of fat stores (Speakman & McQuinny, 1996). Energy budgets in other bats reveal that because food intake jumps substantially during lactation and the fat stores are insufficient for increased energetic demands of lactation (McLean & Speakman, 1999). Incorporating data on *A. jamaicensis* estimated costs to females during lactation this corresponds to an equivalent of 1.5-2 times the estimated basal metabolic rate (Table 4.1).

Therefore, lactating fruit bats are faced with three problems. First, the energetic cost of lactation is high. Second, this energetic cost is compounded by the fact that mother bats must forage more to meet that demand, thereby increasing the daily energy demand even more.

Finally, the protein content of the available fruit is relatively low. Here we asked whether lactating bats are more likely than other classes of individuals to supplement their diets with insects. Our approach relies on the observation that the δN is enriched with trophic level (DeNiro, 1978; Peterson, 1987; Kelly, 2000). Nitrogen enrichment between trophic levels averages 3.2‰ and ranges from 1.4 - 5.0‰ (Kelly, 2000). Thus, measurements of the δ^{15}N of plant and animal tissues can provide insight into the trophic structure of an ecosystem including how it varies temporally and spatially (Post, 2002). We used this natural tendency of nitrogen isotopes to enrich with increasing trophic levels (Bearhop *et al.*, 2004) within a single species of bats to estimate the relative contribution of insects relative to fruits to the diets of these bats. Each 3.4% increase in ^{15}N indicates an increase in one trophic level from the fruits themselves (0-3.4 ‰) to frugivores (6.8 – 10.2 ‰), bats eating some insects (10.2-13.6 ‰) and just insects being consumed (13.6 ‰ or higher).

If female frugivorous bats eat insects to obtain additional nitrogen during periods of nutritional stress, females should eat more insects than males, and lactating females should consume the most insects, followed by pregnant females. However, another way to consider the same issue is diet variability. If females are using insects because of reproductive demands we might expect their diets to be more variable than males. Alternatively, diets may be more or less variable by different reproductive stages if individuals are specializing on particular food items pending their physiological demands (Bearhop *et al.*, 2004).

Type of Activity	Metabolic Rate	Citation	Comments
Basal metabolic rate (BMR)	36.8 kJ / day	McNab, 1969	
Resting metabolic rate (RMR)	0.03 kJ / min	McNab, 1969	Converted to kJ by Morrison, 1978
Horizontal flight	0.30 kJ / min	Morrison, 1978	
Field metabolic rate (FMR)	39.7 kJ / day		
Male commuting costs	0.46 kJ / night		
Female commuting costs	1.09 kJ / night		
Energy from a single fig	6.4 kJ - 8.3 kJ after nutrient extraction		Derived from fig values of Hladik *et al*., 1971
Estimated energetic costs of females when lactating	55.58 - 71.46 kJ/day	Extrapolated from Voigt, 2003	This value was calculated using mass specific values determined experimentally for lactating *Glossophaga soricina. These values were* multiplied by average mass of *A. jamaicensis* (33.5 g).

Table 4.1.—Published energetic data for *Artibeus jamaicensis* and the energy content of figs from various sources.

METHODS

Bats were captured during both pregnancies, associated lactational periods and during periods of reproductive inactivity from November 2009 until May 2011 at Las Vegas Cave in Puebla, Mexico (20º08.910’N, 97º24.650’W). The ecosystem surrounding this large cave is categorized as tropical dry forest, experiences two wet seasons per year (Leopold, 1950) and is in a pastoral semi-disturbed area. On average, 80-90% of the precipitation occurs between May and October, with the average annual precipitation in dry forests at 1650 mm (Vargas *et al.,* 2008). The wet season at our field site spans June-October transitioning in November to the dry season December - May (Vargas *et al.,* 2008). Among the most dominant plant species are fig trees in the genus *Ficus*. The plant community also includes *Cecropia sp., Piper sp., Mangifera sp. (Mango)*, *Soleneum sp*, *Melastomidae*, and native bamboo (Poaceae: *Bambusoideae*).

Bats were hand-netted using bucket traps. Each field session bats were assessed for reproductive stage, a blood sample collected for isotopic analyses, and released. Bats were weighed in holding bags using an electronic or mechanical Pesola scale. To age individuals, their tooth-wear was scored and their forearms held over a flashlight to determine degree of epiphyseal-diaphyseal fusion in hand (wing) bones (indicating adulthood) (Anthony, 1988). Bats were assigned to one of three age categories: juvenile, adult, or sub-adult. Isotopic data reported here are for adult bats unless otherwise noted. Field methods complied with the UCR Institutional Animal Care and Use Committee (20080011) and samples were collected under USDA / APHIS import permits for blood and milk (107181).

Using stable isotopes to trace food resources through the food web

Stable isotope values are reported using delta notation (δ) on a per milliliter basis (‰) compared to an international standard, which for $\delta\,^{15}N$ is air (AIR). Isotopic ratios are expressed in standard delta notation in parts per thousand (‰):

$$\delta \text{ Sample} = ((R_{sample}-R_{standard})/(R_{standard})) \text{ X } 1000$$

where R_{sample} and $R_{standard}$ are the ratios of heavy ($\delta^{15}N$) to light ($\delta^{14}N$) isotopes (Craig, 1964).

Sampling bats

Reproductive state was assessed following Racey (2009). Pregnancy was assigned using palpation (Handley *et al*., 1991; Heideman & Powell, 1998) and lactation if milk was expressed with gentle pressure on the nipples. Because nipple morphology is a useful predictor of pregnancy stage, including early stages (Racey, 1969), this information was recorded using a key available for

A. jamaicensis (Handley *et al*., 1991). Males were assigned to 3 reproductive categories based on the appearance of the scrotum: non-scrotal, slightly scrotal, and scrotal, following Racey (2009). Additionally, linear dimensions of both testes (length and width) and embryo (crown-rump length) were measured with digital calipers to verify reproductive categories.

All individuals were measured and sampled for blood and released within an hour of capture. Blood was collected from the interfemoral or cardiac veins using a heparinized 50 µL capillary tube via a slight puncture from a beveled syringe following methods in Wimsatt *et al.* (2005, and Ellison *et al*. (2006). Between 25 and 100 µl of blood were obtained from most individuals and spun for three minutes in a centrifuge (IEC MB Centrifuge- Micro hematocrit) to separate erythrocytes from plasma within 24 hours of collection. Hematocrit (erythrocytes/total blood) was measured to the nearest mm using a ruler. Plasma samples were used for stable isotope analysis because they typically exhibit high carbon turnover rates (T½=3-7 days) (Hobson & Clark, 1993) and thus provide insight into the resources the animal has been using within a week or two previous to capture (Tieszen *et al*., 1983). Tubes were flame-sealed and stored on ice in a cooler until processed further in the lab at which point they were transferred to an electronic refrigerator.

Plasma samples of 15 µl were pipetted into pre-cleaned tin capsules (5 x 3.5 mm, Alpha Resources Inc.) and allowed to dry overnight in a covered container with a thin base layer of Drierite (anhydrous calcium sulfate, W.A. Hammond) before folding for isotopic analysis. Sample carbon and nitrogen isotope ratios were measured using a continuous flow mass spectrometer (Thermo-Finnigan IRMS Delta Plus; Thermo-Finnigan, Waltham, Mass.) and samples were combusted in a Costech ECS 4010 Elemental Analyzer (Costech Analytical Technologies, Valencia, Calif.) at the University of California, Santa Barbara Analytical Lab at the Marine Science

Institute. These analyses were precise to the ±0.1‰ VPDB SD for $\delta^{13}C$. Lab values were calibrated against Acetanilide and USGS 40 glutamic acid standards every run of plasma samples to correct for instrument drift.

Plant phenology determined by transects

We conducted walking surveys during each of our visits to the site (sampling periods) to describe the phenology of the plant community and estimate fruit availability. During each field session 2-3 225 m transects (each separated by about 250 m) were sampled. Reproductive stages and counts of fruit on each tree were made at 10 m intervals on a 100 m long transect. All fruit-bearing plants within a 15 m line of the transect line were included. Species were identified to Family or Genus and height estimated. Additionally, photographs were taken of these plant genera each month so as to further verify fruit counts. Given the known mutualism between *Artibeus* and figs (Morrison, 1978; Kalko, Herre & Handley, 1996), we focused on figs as a food resource. However, other fruits in transects were noted and compared to the extensive list of plants eaten by *A. jamaicensis* from Ortega and Castro-Arellano (2001). Any fruits in the transect were also compared to those exhibiting 'the bat-syndrome,' i.e. fruits that are eaten by bats and that share several traits that differ from fruits that are eaten by frugivorous birds (Kalko, Herre & Handley, 1996; Dumont, 2003). 'Bat fruits' exhibit variable sizes on the same tree, remain green, ripen synchronously and have strong scents (Kalko, Herre & Handley, 1996). Few fruits were noted that fit these criteria but were not listed by Ortega and Castro-Arellano (2001).

Plant material was collected from six or more individuals from each plant species at the site for isotopic analysis of nitrogen ($\delta^{15}N$ values) following Wolf, Martinez-del-Rio & Babson (2002) to facilitate comparisons to plasma $\delta^{15}N$ content. A seed and leaf reference library and photo-bank was maintained for comparison and identification of seeds from traps and bat feces.

Drop-cloths and dietary assessment

Feces and processed plant materials were collected from drop-cloths placed under day roosts. The night before each netting period, after bats had left the roost for their nightly foraging, linen cloths were placed on a plastic tubing ring raised on wooden spikes to elevate the trap from the ground. Drop-cloths were installed under each harem group following Flores Martinez (1999). These seed and feces traps were left in place for 3 days and collected mid-day on the 3rd day. Seed samples were further sorted by seed type and weighed to estimate relative representations of the various fruits.

Statistical Analysis

Statistical analyses were performed in SPSS and consisted of general linear models and post-hoc Fisher's least significant difference (***LSD***) tests. Statistical significance was considered at the 0.05 level but p-values of 0.1 and below are reported. Using a factorial ANOVA design both sex and reproductive state were treated as categorical variables (reproduction-4 categories: females lactating, females pregnant, or males scrotal; non-reproductive-2 categories: male or female). Sex was examined first followed by reproductive state. Below mean values are reported with ± standard errors and coefficients of variation (CV).

We calculated individual specialization using IndSpec4.0, a program based on Roughgarden’s (1974) concept that the niche of an animal can be broken into two components:

within individual component (***WIC***) and between individual component (***BIC***) of variation. The program, IndSpec4.0 (Bolnick *et al*., 2002) reports *WIC:TNW*, where ***TNW*** is the ratio of the total niche breadth of the population and ***WIC*** (the within-individual niche breadth) (Roughgarden, 1974; Araújo *et al*., 2007). With larger *WIC* values relative to *TNW* less variation is observed between individuals. Thus, *WIC:TNC* ranges from 0 to 1. This value is based on a Shannon-Weaver like measure of total niche width (Bolnick *et al*., 2002). Values near 0 indicate that a subgroup of the population restricts their diet to a small range of foods (narrow diet breadth, i.e. 'strong individual specialization' (Bolnick *et al*., 2002)). Values close to 1 indicate that all individuals in the population eat similar foods (i.e. minimal differences in diet breadth or 'individual generalists' (Bolnick *et al*., 2002; consistent with Figure 4.1 bottom right-hand example). Also using the Indspec program we were able to conduct bootstrapping using Monte Carlo simulations (resampling) to determine log-likelihoods as well as calculate **Petraitis' W_i** (see Bolnick *et al*., 2002 for details). Ultimately, this program allowed us to use the likelihoods of any particular observed diet to calculate a p-value and test the significance of diet specialization using a chi-square distribution. For this method diets had to be binned. We chose three bins: pure frugivory, some insectivory and extensive insectivory. Finally pair-wise diet overlap was calculated for a sample size of *N* individuals using an *N* * *N* matrix and calculating the proportional similarity between.

RESULTS

We sampled blood from 98 individuals: 70 females, (26 non-reproductive, 25 pregnant, and 19 lactating) and 28 males (14 scrotal ‘reproductive’ and 14 non-reproductive). The mean mass of non-reproductive *A. jamaicensis* females was 41.12 g (± 1.05, N=54), mid to late stage pregnant females 52.56 (± 1.01g, N=45) and lactating females 46.50 (± 0.67g, N=52). Food source isotopic values were taken from published data (insects; δ^{15}N 4.67±3.68‰ AIR and fruit δ^{15}N 1.22 ±2.57‰ AIR) (Herrera *et al.,* 2001). Bat plasma δN values averaged: δ^{15}N 6.21‰ AIR with a maximum δ^{15}N 11.98‰ AIR, and a minimum δ^{15}N 2.90‰ AIR. Juvenile bats were excluded from all analyses.

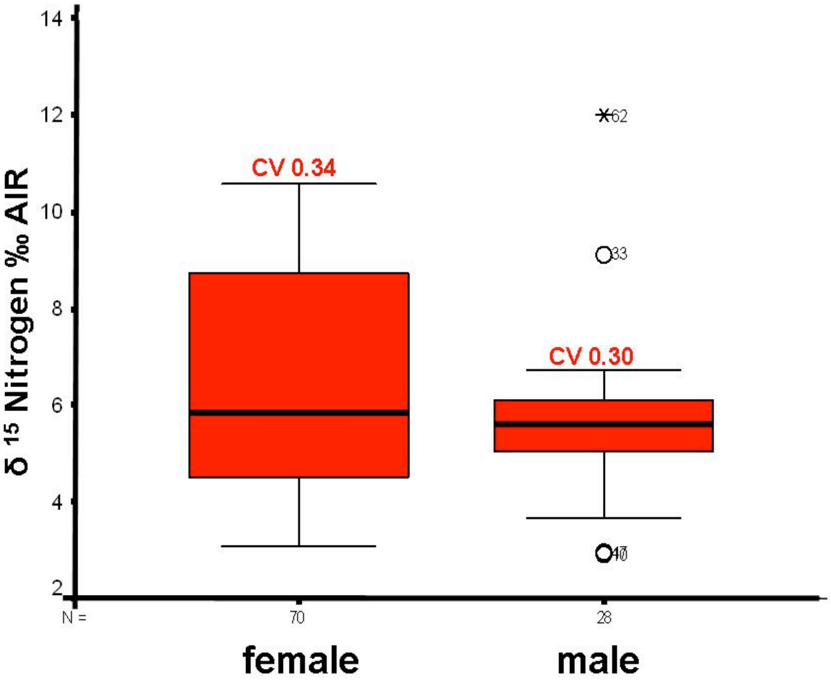

Figure 4.2.—Nitrogen isotope values by sex. δ^{15}N‰ AIR values for both sexes. Mean values are shown by the line. Outliers are illustrated based on the inter-quartile range (*IQR*), which utilizes probability distribution. Using this system if Q_1 and Q_3 are lower and upper quartiles, an outlier is any value outside of: $[Q_1 = k(Q_3-Q_1), Q_3+k(Q_3-Q_1)]$ where k is some constant (ASTM, 2008). Outliers are further categorized into ‘mild’ outliers (open dots) and ‘extreme’ outliers

(denoted by an asterisk and are values greater than 1.5 inter-quartile ranges away from the 'outlier fence' designated by the equation above). All outliers are listed together with identification numbers for the individual bat who is the outlier. Sample sizes are listed on the x-axis under each associated box plot and coefficients of variation (CV) above each plot.

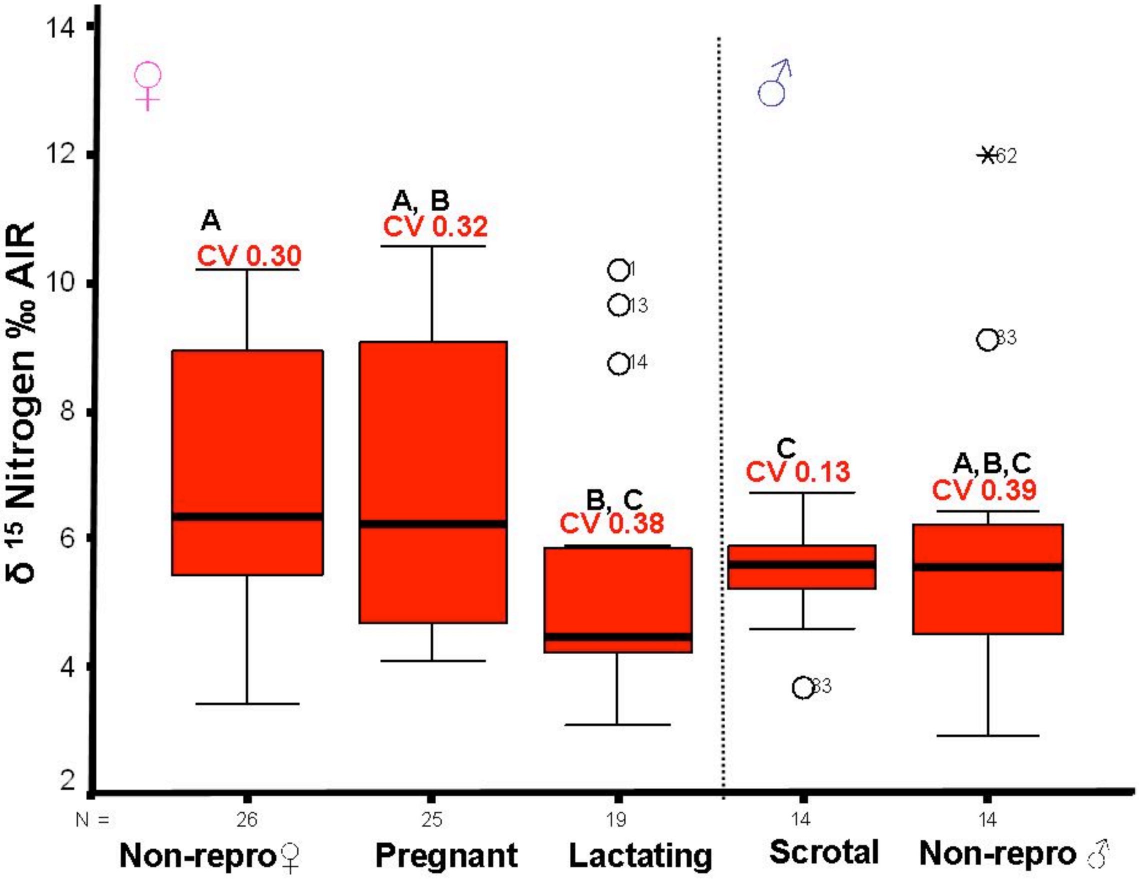

Figure 4.3.—Nitrogen isotope values by reproductive state for both sexes. δ^{15}N‰ AIR values illustrated by the various reproductive categories (females on left of dotted line, males on the right). Mean values are shown by the lines on the box plots. CV values are shown above each plot and significance is shown by letters (A, B, C) in the plot (LSD, $p<0.05$). Shared letters indicate no significant differences between groups. Outliers are illustrated based on the *IQR* (see above) with 'mild' outliers illustrated by open dots and 'extreme' outliers denoted by an asterisk. Sample sizes are listed on the x-axis under each associated box plot.

Independent variable	WIC/TNW	Percent with lower values
Sex	**0.8782**	4.5% had lower *WIC/TNW* (a Shannon-Weaver index of niche width provided by IndSpec) was less than observed
Reproductive state	**0.7163**	0% had lower WIC/TNW
Month	**0.5894**	0% had lower WIC/TNW

Table 4.2.—Niche parameters for independent variables. δ^{15}N‰ AIR values compared by groups using IndSpec (Bolnick *et al*., 2002) and Roughgarden's *WIC/TNW* calculations (Roughgarden, 1974). Percentages were calculated using bootstrapping (N=2000 bootstraps).

Differences between the sexes

Female plasma values of δN averaged δ^{15}N 6.43±0.26‰ AIR (N=70) and males averaged δ^{15}N 5.67±0.32‰ AIR (N=28), indicating that females feed 1/4 of a trophic level higher than males. Mean δ^{15}N values were not significantly different between the sexes (p=0.100, R^2 0.028, $F_{1,97}$ 2.755, from a general linear model in SPSS). However, females exhibited greater diet breadth than males (Table 4.3 W_i 0.98 vs. 0.86) and the *WIC/TNW* (the Shannon-Weaver based measure of total niche width) of sex (Figure 4.3) was 0.878 i.e. similar to population diet breadth width. The most specialized of the sexes were males. Examining Figure 4.2 it is apparent that this small degree of dietary specialization (large W_i) is on fruit (i.e. males are shifted to lower δ^{15}N values indicating a more frugivorous diet). Sex with a *WIC/TNW* value of 0.878 is close to 1 (relative to the other models) and thus males and females do not differ substantially in diet breath from the population mean (Table 4.2).

Category	Likelihood	Petraitis's W_i	p-value	Overlap
Female	0.241	0.980	0.417	
Male	0.016	0.863	0.041	
				0.657

Table 4.3.—Indspec values for Petraitis' W_i, a measure of niche width relative to a specified distribution by sex. Values of W_i=1 indicate a generalist. This value decreases as individuals become more specialized. The two sexes have a dietary overlap of 0.657.

Differences among reproductive stages

Nitrogen (δ^{15}N) values differed significantly among the reproductive categories (p=0.039, R^2 0.102, $F_{4,97}$ 2.635, Table 4.4). Diet breadth also varied among reproductive stages (Table 4.2). Statistically significant differences were observed between groups as illustrated by letters (Table 4.2). Non-reproductive females utilized more insects and differed from lactating females and scrotal males while scrotal males differed from non-reproductive and pregnant females by relying more on fruit (Figure 4.3).

Consistent with these differences between groups, the WIC/TNW value for reproductive state (0.716) is closer to 0 than the other hypothesized predictors of insect supplementation indicating more diet breadth differences between groups (reproductive groups) relative to the population. Petraitis' W decreases as individuals become more specialized. Here the most specialized group was lactating females and non-reproductive females. However, it is apparent (Figure 4.3) that this specialization was on fruit rather than insects. Both lactating females and non-reproductive males had diets that were significantly different (at a 0.05 level) from the mean dietary distribution observed at our site. Because both of these groups had W_i values nearing 0 (vs. 1) they can be described as having more specialized diets relative to other reproductive categories of bats. Dietary overlap among the different reproductive categories is 0.634 (see Table 4.4 for log-likelihood results). The greatest diet breadth we observed was in non-reproductive females followed by scrotal males (Figure 4.32). However, in neither case was this difference significant. The narrowest diet breadths, even with outliers included in the dataset, were seen in lactating and non-reproductive males and in both cases this was significant (Table 4.4).

Category	δ^{15}N values	Likelihood	Petraitis's W_i	p-value	Overlap
Lactating (N=19)	5.35±0.46‰ AIR	0.004	0.747	0.011	
Pregnant (N=25)	6.84 ±0.44‰ AIR	0.172	0.935	0.318	
Non-reproductive females(N=26)	6.84 ±0.41‰ AIR	0.840	0.988	0.950	
Scrotal males (N=14)	5.45±0.21‰ AIR	0.380	0.962	0.586	
Non-reproductive Males(N=14)	5.85±0.59‰ AIR	0.001	0.611	0.003	
					0.634

Table 4.4.—Nitrogen values and Indspec results for Petraitis' W, a measure of niche width relative to reproductive state. Values of W_i=1 indicate a generalist.

Diet across time

Insect supplementation of *A. jamaicensis* diets also varied significantly during the sampling period ($p<0.0001$, R^2 0.281, $F_{6,97}$ 5.934, Figure 4.4). The month with the highest observed $\delta^{15}N$ values was December 2009, when 50% of the females were pregnant. In September 2009, July 2010 and May 2011, high proportions of bats were lactating (Figure 4.4), but bats during these times exhibited very different diet compositions. Specifically they were eating more insects in September 2009 and May 2011 relative to July 2010. An even starker contrast is between the months of December 2010 and March 2011 when bats were pregnant yet had entirely different diets.

In December 2009 bats were supplementing their diets extensively with insects whereas in March (and July) 2009 they were primarily eating fruit. Month had the smallest *WIC/TNW* value (0.589) of all hypothesized predictors (Table 4.2) indicating high levels of specialization. Of all the hypothesized predictors of diet, month (vs. sex or reproductive state) exhibited the most variation in dietary niche breadth relative to the entire population of bats. The most dietary specialization was seen in September 2009. Bats had significantly different (i.e. specialized niches with a small W_i) diets in July of 2010 (at a 0.05 level) relative to the mean dietary distribution. Dietary overlap among the different months is 0.588.

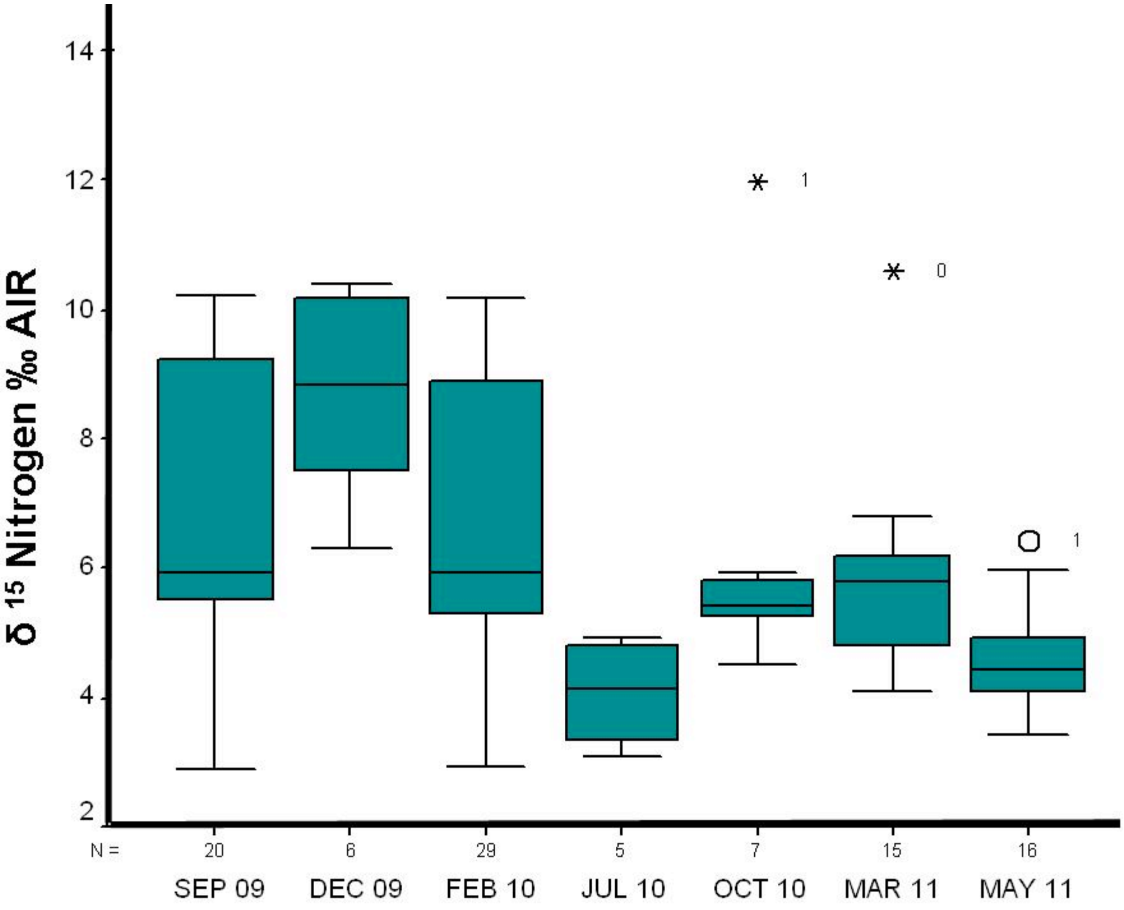

Figure 4.4.—Nitrogen isotope values for both sexes of bats by month. δ^{15}N‰ AIR values illustrated by date (both sexes and all reproductive categories lumped). Mean values are shown by the line, outliers are indicated by an asterisk. Outliers are illustrated based on the *IQR* (see above) with ‘mild’ outliers illustrated by open dots and ‘extreme’ outliers denoted by an asterisk. Sample sizes are listed on the x-axis under each associated box plot.

Category	Likelihood	Petraitis's W_i	p-value	Overlap
Sep 2009	0.043	0.591	0.098	
Dec 2009	0.044	0.898	0.100	
Feb 2010	0.111	0.644	0.221	
Oct 2010	0.136	0.875	0.262	
Jul 2010	0.002	0.670	0.005	
Mar 2011	0.076	0.692	0.161	
May 2011	0.132	0.904	0.256	
				0.588

Table 4.5.—Indspec values for Petraitis' W_i by month. Values of W_i closer to 1 indicate more of a generalist. This value decreases (approaches 0) as individuals become more specialized.

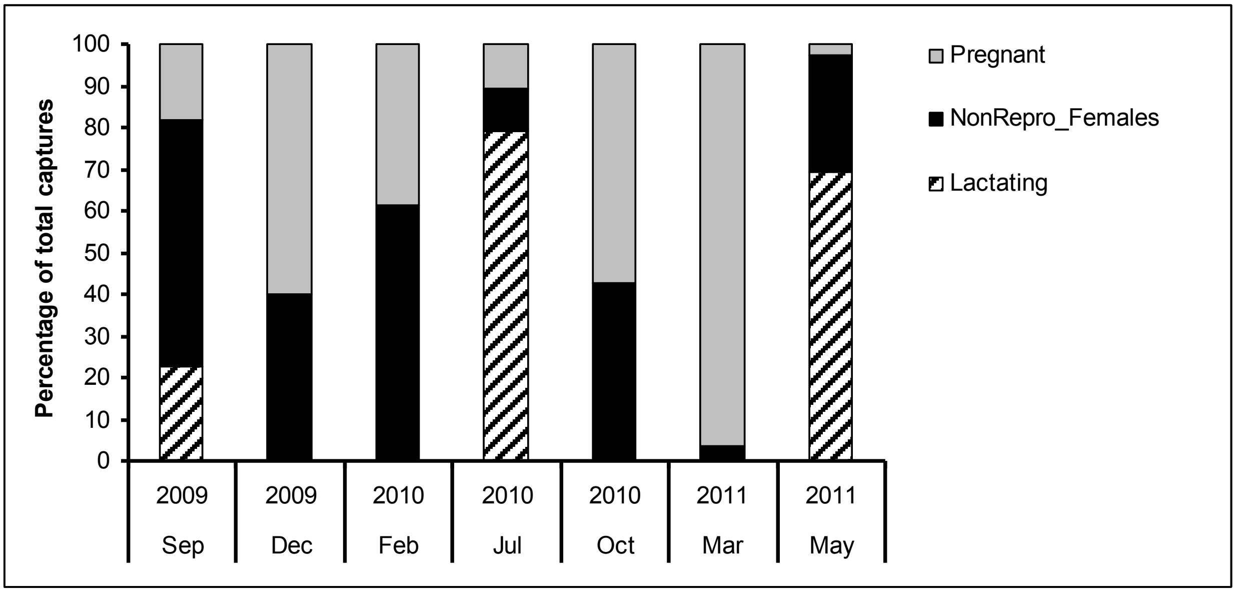

Figure 4.5.—Reproductive state of adult female captures illustrated by month. The percentage make up of each reproductive state by each month is illustrated.

Fruit availability varied between months with the exception of the main food resource of this bat, *Ficus*. Indeed, *Ficus* were found in our fruit transects during all months, but fewer ripe fruit were present in November (2009) and February (2010). *Piper* fruits were primarily available late summer to fall (July and October 2010) and in the spring (May and March 2011). Periods of high food abundances (all fruits) coincided with drier months: July 2010, March 2011. Periods of particularly low food abundances were during winter months: November 2009- February 2010. Drop-cloth analyses indicate *Ficus* fruits were important during all months and did not vary significantly in abundance nor percent representation across months (p=0.349, R^2 0.21, $F_{1,5}$ 1.12, Figure 4.6).

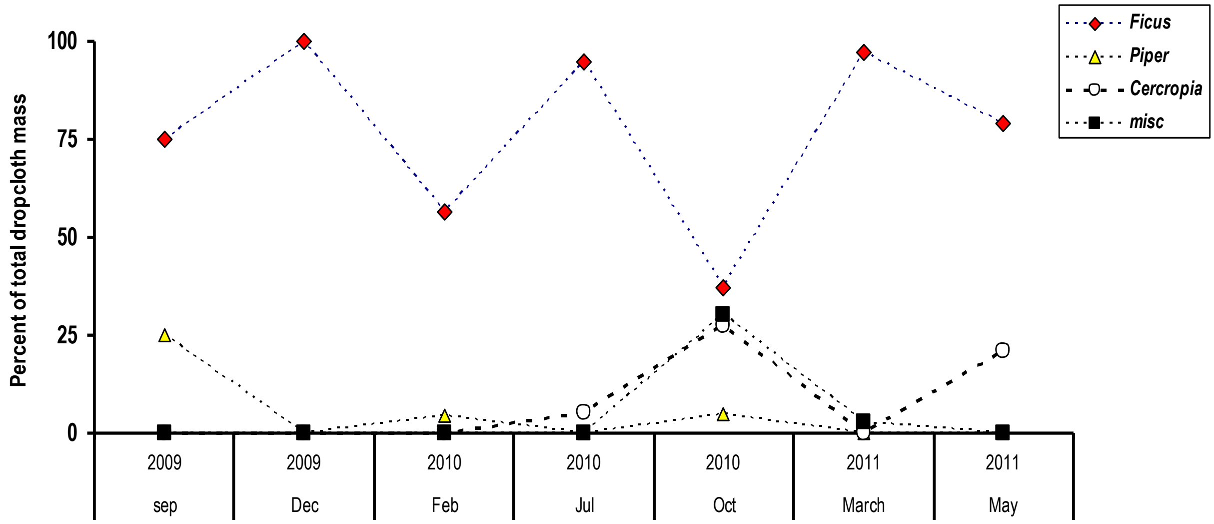

Figure 4.6.—Plant materials from drop-cloths at Las Vegas Cave in Puebla, Mexico. Values on the y-axis indicate percent of total sample of the various plant types. Plants illustrated include: *Ficus, Cecropia, Piper* and miscellaneous fruits (seeds we were unable to identify to genus).

DISCUSSION

Artibeus jamaicensis is generally considered a fruit specialist (Heithaus, 1975; Handley *et al*., 1991). However, these bats shift their diets to some degree of insectivory and this may be due to changes in resource abundances or because fruits are unable to suffice for certain physiological demands (Dinnerstein, 1986). We examined the hypothesis that bats supplement based on seasonal availability (H1- the Seasonal Insectivore Hypothesis) or physiological demands (H2-the Nutritionally Wise Female Hypothesis).

We found that *A. jamaicensis* shift their frugivorous diets to include more insects to some degree under both of conditions. Support for the Seasonal Insectivore Hypothesis includes (P1) that the sexes did not differ significantly in insect consumption (i.e. all bats will eat insects) and that there were periodic and statistically significant shifts to greater or lesser degrees of insect use during some months (Figure 4.4). Our results for the Nutritionally Wise Female Hypothesis were somewhat more surprising. Bats did indeed differ significantly in insect use by reproductive state. However, our results do not entirely support the prediction (P2) that pregnant and lactating females would be the reproductive categories to exploit insects. Pregnant bats but not lactating ones shifted toward insectivory. Furthermore, reproductive males also eat more insects than bats in other reproductive stages (Figure 4.3).

Why didn’t lactating females eat more insects relative to other reproductive groups? Although both sexes supplemented their frugivorous diet with insects, females used insects to a qualitatively greater (but not significant) degree than males. Pregnant and non-reproductive females consumed the most insects, which does not support our prediction (P2) that reproductive (lactating and pregnant) females would eat more insects than the other reproductive categories. We offer several explanations for this observation. (1) Because of seasonal changes in diets it is reasonable to suspect that the entire population (both sexes, all reproductive stages) are exhibiting similar behaviors either because of limited fruit availability or because of peaks in insect abundance (i.e. H1 is a confounding factor). Lactation occurred during two different seasons (see

Figure 4.5). Because diet differs by season it is not unreasonable to expect a large variation in diet relating to the particular season during which females lactate. While we have no data to evaluate insect abundances we did note that fruits are always available to bats (Figure 4.6). (2) Females may also eat figs as a source of water when water is scarce. Lactation may thus require females maintain water balance in addition to other nutritional aspects of their physiologies. Finally, (3) dietary changes might have something to do with demands of spermatogenesis and embryogenesis rather than lactation. Any of these options might explain the relatively low use of insects we observed by lactating females. Of further interest is the relatively narrow diet breadth observed in lactating females relative to other reproductive states.

We also investigated a third hypothesis (H3- the Evenness and Diet Width Hypothesis). We noted interesting trends in diet niche breadth whereby this population was best described as utilizing a dual strategy of both synchronized specialists (Figure 4.1upper right-hand illustration consistent with H1) and specialized individuals (Figure 4.1 lower left hand illustration consistent with H2). In any case *A. jamaicensis* exhibits diverse dietary breadth and is not a strict frugivore.

Because of the correlated nature of reproduction and seasonality, both are important predictors of when these bats supplement their otherwise frugivorous diets. Our results are consistent with other studies (Herrera *et al.,* 2001a, 2001b, 2002; Frick *et al.,* 2009) that serve as reminders that diet may not be fixed, and that many taxa will alter their diets during certain periods of their life histories or when nutritionally or energetically stressed. These results would be best evaluated in the context of insect abundances which we did not collect data on. Indeed, future work would do well to quantify insect abundances in addition to fruit production.

It is likely that our results could be extended to other neotropical bat species especially closely related species of Carolinae, that have similar reproductive patterns (Racey & Entwistle, 2000). Understanding what these bats are eating and when as well as how flexible their diets are is informative both in terms of understanding their evolutionary ecology but also for conservation efforts. The role of diet has been suggested for the evolution of reproductive strategies in bats in general (Racey & Entwistle, 2000) and *Artibeus jamaicensis* in particular (Fleming, 1971). Our data indicate that insects may be a valuable resource if not for lactating females for pregnant females. This importance could present additional complexity to previous hypotheses about the reproductive evolutionary ecology of this bat but simultaneously lends indirect support for preexisting hypotheses that bats time parturition and lactation with their primary food resource (in this case figs).

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CONCLUSION

Predictable and reoccurring environmental stressors can lead to selective pressures for organisms to develop highly-timed reproduction (Badyaev, 2005). If through climate change, environmental cues that previously served as predictors for animals to time reproduction become unreliable, species may mis-time reproduction (Visser, Both & Lambrechts, 2004). Thus, knowledge of how and why organisms time reproduction is imperative for conservation efforts during this time of drastic environmental change we currently inhabit (Greyner, *et al*., 2006). Knowledge of these types of reproductive dynamics in both the Carnivora as well as Chiroptera is particularly valuable. Because bats provide ecological services ranging from pollination to predation they are important members of numerous ecosystems from temperate to tropical. Despite this importance, bats remain elusive and poorly understood. The Carnivora on the other hand while well studied, remain one of the clades most heavily impacted by climate change (Greyner, *et al*., 2006; Davies *et al*., 2007). Knowledge of reproductive ecology of both groups is imperative for their conservation and reproductive delays remain an understudied integral part of the biology of many species (Chiroptera about 100, Carnivora about 70).

My dissertation investigates the presumed adaptive nature of reproductive delays from several different avenues including; large scale ecological predictors of delays (chapter 1), sexual selection (chapters 2 and 3), and reproductive ecology (chapter 4). I analyzed the significance of reproductive delays in an integrative manner.

In my dissertation I have 1). shown that several hypothesized predictors of the occurrence delays in the Carnivora are indeed statistically relevant. Specifically, body size, diet, latitude and clade membership are all important predictors for the presence of delayed implantation. This result is consistent with delays facilitating the negotiation of energetic and other demands associated with living in highly seasonal environments, and fecundity selection based on body size changes as the Carnivora have become smaller. This analysis included the largest data-set of reproductive delays to be examined thus far (all mammals). Furthermore, I present analyses with the most current phylogenetic statistical models (Lavin *et al.,* 2008; Ives & Garland, 2010; Ives & Helmus, 2012) as well as the most current phylogenetic hypothesis for the Carnivora (Nyakatura & Bininda-Emonds, 2012).

2a). Additionally, I evaluated the potential for delays, after they have evolved- to facilitate sexual selection. Reproductive delays offer an unconsidered avenue for the operation of post-copulatory sexual selection in mammals because they lengthen the reproductive periods over which post-copulatory sexual selection operates. I synthesized the literature to postulate the various areas we might expect to see post-copulatory sexual selection operating in species with the three types of delays. Delayed fertilization may increase the opportunity for sperm competition and sperm selection. Meanwhile, delayed implantation may allow females to bet-hedge by continuing to mate before committing to a pregnancy. Finally, species with delayed development experience an extended period over which the embryo may be evaluated via cryptic female choice and genetic incompatibility processes whereby patterns of maternal and paternal gene expression are revealed in the offspring. This is because females with delays have a longer time frame in which processes such as sperm selection or embryo re-absorption can occur, reproductive delays. Thus, delays may provide new and interesting ways to look for cryptic female choice. Delays may also prove an important factor in explaining phenomena such as bacular size variation (Hosken *et al*., 2001; Lüpold, McElligott & Hosken, 2004) or the use of penile elaborations. In chapter 2 I conclude that species with facultative delays may provide researchers with the opportunity to conduct controlled experiments by manipulating presence or absence of delays.

2b). To test my hypothesis (chapter 2) that species with delays specifically delayed fertilization experience a greater risk of sperm competition than species without delays, I investigated museum specimens of fluid-preserved male bats from species with and without delays. By measuring testes size in these specimens I was able to evaluate the prediction that if delays facilitate post-copulatory sexual selection, species with delays up to implantation will have larger testes sizes indicative of more sperm competition (chapter 3). I found that log testis volume was strongly correlated with log body mass. Species with reproductive delays also have larger testes, suggesting that delays facilitate sperm competition, perhaps by providing more time for post-copulatory sexual selection (specifically sperm competition) to occur. In addition, Megachiropterans tended to have larger testes than Microchriopterans. Larger testis volume in bats with delayed fertilization is consistent with competition between sperm of different males for fertilization or preferential storage by the female. The best model was obtained by an OLS model (star phylogeny) that included delay as a binary independent variable (indicating delayed fertilization or not) and clade (Megachiroptera vs. Microchiroptera).

4). Finally, in a field setting I evaluated the natural history and physiological ecology of *Aritbeus jamaicensis* to determine what if any evidence for delays serving as a way to match the expensive stages of reproduction (late pregnancy and lactation) with periods of food abundance. I found that females supplement their diets with insects due to seasonal changes more so than due to physiological state i.e. reproductive stage. This result is consistent with Flemings's hypothesis (Fleming, 1971) that delays in *A. jamaicensis* are timed to periods of fruit availability but paints a more complex picture of the ecological nuances of reproduction in this bat. Future work would surely benefit from sampling insect blooms in relation to the reproductive cycle of *A. jamaicensis*.

By investigating diet relative to periods of delays in one well understood bat, I determined how food availability impacts reproduction and whether females are more physiologically strained during either of their 2 annual pregnancies. Because I tested the assumption that delays are beneficial in a field setting, my dissertation provides much-needed data to those interested in the evolution of delays. Understanding how the environment impacts reproductive delays, a trait *A. jamaicensis* shares with over 200 other species of mammals (including 7 bats and 9 carnivores listed as at risk for extinction) is imperative for informing conservation efforts. This is particularly true for Mexico, where my field work was conducted.

Mexico is home to immense biodiversity, and houses 5.5% of the world's bio-reserves (Medellín, 1998). Although Mexico is among the top 3 countries in total number of species of mammals, it has fewer than 40 PhD level biologists who study mammalian ecology (Medellín, 1998). As part of my collaboration with Mexican labs (Dr. Rodrigo Medellín and Dr. Jorge Ortega) for this research I have been teaching students new field and laboratory methods and helping them refine their research skills, contributing to the training of future scientists.

The existing hypotheses regarding the origins of delays REVISITED

Subsequent to Hamlett's 1935 review paper (Hamlett, 1935) numerous additional hypotheses regarding the origins of delays in mammals have been presented. Hamlett's original list which included 1) the advantage of being born at a favorable time for both parent and young, 2) delays only occur in old genera that existed during the Pleistocene, because delays assured young were not born during glacial winters, and finally 3) that delays are a byproduct of lower body temperatures of hibernating mammals. And 4) delays are not advantageous, but instead fixed inherited phenotypes present in some species. Modern hypotheses have been expanded to include and focus on the importance of delays in timing reproductive stages within the context of seasonal environments. For example delays may allow females to match the energetic demands of pregnancy and lactation to food abundances and favorable weather (Ferguson, Higdon, & Lariviere, 2006), for females to wean young when food is available (van der Merwe, 978) or allow females to be receptive to mating (independent of reproductive state) during periods of mate availably to elicit maximal male competition (Sandell, 1990) or to synchronize breeding, as seen in marine mammals such as pinnipeds that have a narrow window during which mating occurs (Bartholomew, 1970). Additional hypotheses have focused on life history changes such as an something lost as species became smaller to counteract other changes n the life history associated with the decrease in body mass due to fecundity selection (Ferguson Higdon, & Lariviere ,1996; Lindenfors, Dalen, & Angerbjoern, 2003). Finally, it has been suggested that delays might be a genetic byproduct resulting from changes to other aspects of reproductive physiology and thus are not the result of selective pressures for this particular phenotypic trait at all (Isakova,2006).

My dissertation instigated individual and seasonal differences of how energy is divided among the different functions; body maintenance, growth or reproduction as well as species level patterns and provides an assessment of these pre-existing hypotheses as well as re-thinking of them to apply in new contexts (Table 5.1). I found substantial evidence for delays evolving to ameliorate living in seasonal environments (measured by latitude) in the Carnivora (chapter 1). This is consistent with H1 (figure 1) and I also found some relationship with body size (H4). I also determined the exciting potential for delays to facilitate sexual selection after copulation which is a twist on H3. Thus, I maintain that reproductive delays serve as a life-history strategy and can be explored as such. Field data suggest female *A. jamaicensis* do utilize consistently fig based diets (Fleming, 1971) across reproduction even during lactation and that *A. jamaicensis* may be timing reproduction with food abundances but not necessarily to just the abundance of a single resource i.e. *Ficus* fruits. Both conclusions are consistent with H1 that delays provide benefits by allowing the birth of young to coincide with periods of food availability.

Hypothesis	Variables	Citation	Chapters	Support
H_o- Selectively neutral	**NA**		chapters 1-4	+
H1- Timing of expensive stages with food	**Seasonality (latitude)**	Hamlett, 1970	chapters 1 and 4	+
H2- Synchronization of reproduction	**Breeding sites (physical characteristics)**	Bartholomew, 1970	-	NA
H3- Receptivity timed with mate abundance	**Seasonal mate abundance**	Sandall, 1990	Chapters 2 and 3	(+)
H4- Lost as mammals (Order: Carnivora) decreased in body size	**Fecundity selection**	Lindenfors, Dalen, & Angerbjoern, 2003	Chapter 1	+
H5- Chromosomal rearrangement alters timing	**Genomic byproduct**	Isakova, 2006	-	NA

Table 5.1. The common hypotheses regarding the origins of reproductive delays and their original citations as related to my chapters. The support demonstrated in my chapters for each of these hypotheses is indicated in the last column by + for support, - for no support, (+) for marginal support and NA (not applicable) if the hypothesis was not tested.

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Appendix 1.1.—Data for Chapter 1 including presence and absence of delayed implantation. DSC_ord is order in tree (Figure 1. 1).

DSC_Ord	name	Common	Fam13	Diet6all	Diet7
1	aj_Acinonyx_jubatus	Cheetah	1	4	1
2	zq_Puma_concolor	Puma	1	4	1
3	hy_Puma_yagouaroundi	jaguarundi	1	4	1
4	l8_Lynx_canadensis	Canadian lynx	1	4	1
5	l9_Lynx_lynx	Eurasian lynx	1	4	1
6	la_Lynx_pardinus	Iberian lynx, Spanish lynx	1	4	1
7	lb_Lynx_rufus	Bobcat	1	4	1
8	FB_Felis_bieti	Chinese mountain cat	1	4	1
9	fs_Felis_silvestris	Wild cat	1	4	1
10	fm_Felis_margarita	Sand cat	1	4	1
11	fc_Felis_chaus	Jungle cat	1	4	1
12	fn_Felis_nigripes	Black-footed cat	1	4	1
13	p7_Prionailurus_bengalensis	Leopard cat	1	4	1
14	p8_Prionailurus_viverrinus	Fishing cat	1	4	1
15	PQ_Prionailurus_planiceps	Flat-headed cat	1	0	1
16	pu_Prionailurus_rubiginosus	Rusty-spotted cat	1	4	1
17	om_Felis_manul	Palla's cat	1	4	1
18	oc_Leopardus_colocolo	Pampas cat	1	1	1
19	og_Leopardus_geoffroyi	Geoffry's cat	1	4	1
20	O2_Leopardus_guigna	Kodkod	1	4	1
21	lt_Leopardus_tigrinus	Little spotted cat	1	4	1
22	lo_Leopardus_pardalis	Ocelot	1	4	1
23	lw_Leopardus_wiedii	Margay	1	4	1
24	OJ_Leopardus_jacobitus	Andean Mountain cat	1	4	1
25	CB_Catopuma_badia	Bay cat	1	4	1
26	ct_Catopuma_temminckii	Asiatic golden cat	1	4	1
27	pm_Pardofelis_marmorata	Marbled cat	1	4	1

DSC_Ord	Diet_Cite	lmass
1	Munoz-Garcia & Williams, 2005	4.769007871
2	Munoz-Garcia & Williams, 2005	4.681241237
3	Munoz-Garcia & Williams, 2005	3.829303773
4	Iwaniuk, Pellis & Whishaw, 2000	3.949390007
5	Gittleman, 1985	4.255915509
6	Hogue, 2003	3.973127854
7	Munoz-Garcia & Williams, 2005	3.934498451
8	Hogue, 2003	3.755874856
9	Munoz-Garcia & Williams, 2005	3.618048097
10	Munoz-Garcia & Williams, 2005	3.371067862
11	Gittleman, 1985	4
12	Hogue, 2003	3.327358934
13	Hogue, 2003	3.618048097
14	Gittleman, 1985	4.035429738
15	Gittleman, 1985	3.243038049
16	Iwaniuk, Pellis & Whishaw, 2000	3.130333768
17	Hogue, 2003	3.544068044
18	Gittleman, 1985	3.469822016
19	Hogue, 2003	3.602059991
20	Hogue, 2003	3.290034611
21	Gittleman, 1985	3.352182518
22	Munoz-Garcia & Williams, 2005	3.944482672
23	Munoz-Garcia & Williams, 2005	3.564271825
24	Yensen & Seymour, 2000	3.602059991
25	Hogue, 2003	3.341434525
26	Hogue, 2003	4.06069784
27	Gittleman, 1985	3.544068044

DSC_Ord	Mass Cite	abslat	Lat cite.
1	Gittleman,1986; Iossa et al., 2008	2.7860	Davies et al., 2007
2	Gittleman,1986; Iossa et al., 2008	3.2800	Davies et al., 2007
3	Munoz-Garcia & Williams, 2005	3.2024	Davies et al., 2007
4	Ferguson, S. H. & S.Lariviere. 2002	53.8287	Davies et al., 2007
5	Gittleman,1986; Iossa et al., 2008	48.9570	Davies et al., 2007
6	Carbone, Teacher & Rowcliffe, 2007	38.3732	Davies et al., 2007
7	Gittleman,1986; Iossa et al., 2008	37.7557	Davies et al., 2007
8	Hogue, 2003	37.0860	Davies et al., 2007
9	Gittleman,1986; Iossa et al., 2008	8.5921	Davies et al., 2007
10	Munoz-Garcia & Williams, 2005	24.3805	Davies et al., 2007
11	Gittleman,1986	26.7873	Davies et al., 2007
12	Iwaniuk, Pellis & Whishaw, 2000	24.6578	Davies et al., 2007
13	Gittleman,1986	20.7547	Davies et al., 2007
14	Gittleman,1986	18.2911	Davies et al., 2007
15	Hogue, 2003	0.1443	Davies et al., 2007
16	Gittleman,1986	14.6420	Davies et al., 2007
17	Hogue, 2003	40.5689	Davies et al., 2007
18	Lindenfors et al., 2007	26.4121	Davies et al., 2007
19	Gittleman,1986	33.2137	Davies et al., 2007
20	Hogue, 2003	39.4031	Davies et al., 2007
21	Lindenfors et al., 2007	9.3563	Davies et al., 2007
22	Gittleman,1986; Munoz-Garcia & Williams, 2005	0.4151	Davies et al., 2007
23	Munoz-Garcia & Williams, 2005	3.3875	Davies et al., 2007
24	Yensen & Seymour, 2000	20.5670	Davies et al., 2007
25	Hogue, 2003	2.8426	Davies et al., 2007
26	Lindenfors et al., 2007	15.4856	Davies et al., 2007
27	Hogue, 2003	14.9240	Davies et al., 2007

DSC_Ord	DI3	DI Cite
1	0	Lindenfors, Dalen & Angerbjoern, 2003
2	0	Ferguson, Virgl & Larivière, 1996
3	0	Lindenfors, Dalen & Angerbjoern, 2003
4	0	Lindenfors, Dalen & Angerbjoern, 2003
5	0	Lindenfors, Dalen & Angerbjoern, 2003
6	0	Lindenfors, Dalen & Angerbjoern, 2003
7	0	Ferguson, Virgl & Larivière, 1996
8	0	Lindenfors, Dalen & Angerbjoern, 2003
9	0	Lindenfors, Dalen & Angerbjoern, 2003
10	0	Lindenfors, Dalen & Angerbjoern, 2003
11	0	Lindenfors, Dalen & Angerbjoern, 2003
12	0	Lindenfors, Dalen & Angerbjoern, 2003
13	0	Lindenfors, Dalen & Angerbjoern, 2003
14	0	Lindenfors, Dalen & Angerbjoern, 2003
15	0	Lindenfors, Dalen & Angerbjoern, 2003
16	0	Lindenfors, Dalen & Angerbjoern, 2003
17	0	Lindenfors, Dalen & Angerbjoern, 2003
18	0	Lindenfors, Dalen & Angerbjoern, 2003
19	0	Lindenfors, Dalen & Angerbjoern, 2003
20	0	Lindenfors, Dalen & Angerbjoern, 2003
21	0	Lindenfors, Dalen & Angerbjoern, 2003
22	0	Lindenfors, Dalen & Angerbjoern, 2003
23	0	Lindenfors, Dalen & Angerbjoern, 2003
24	0	Lindenfors, Dalen & Angerbjoern, 2003
25	0	Lindenfors, Dalen & Angerbjoern, 2003
26	0	Lindenfors, Dalen & Angerbjoern, 2003
27	0	Lindenfors, Dalen & Angerbjoern, 2003

DSC_Ord	name	Common	Fam13	Diet6all	Diet7
28	cc_Caracal_caracal	Caracal	1	1	1
29	py_Profelis_aurata	African golden cat	1	4	1
30	ls_Leptailurus_serval	Serval	1	4	1
31	n2_Neofelis_nebulosa	Clouded leopard	1	4	1
32	p1_Panthera_leo	Lion	1	4	1
33	pp_Panthera_pardus	Leopard	1	4	1
34	po_Panthera_onca	Jaguar	1	4	1
35	pt_Panthera_tigris	Tiger	1	4	1
36	uu_Uncia_uncia	Snow leopard	1	4	1
37	pd_Prionodon_pardicolor	linsang	2	4	1
38	ab_Arctictis_binturong	Binturong	3	1	0
39	ph_Paradoxurus_hermaphroditus	Asian palm civet	3	1	0
40	pl_Paguma_larvata	Masked palm civit	3	1	0
41	hd_Hemigalus_derbyanus	Banded palm civet	3	1	0
42	cn_Cynogale_bennettii	Otter civet	3	1	0
43	c5_Civettictis_civetta	African Civet	3	1	0
44	vz_Viverra_zibetha	civet	3	4	1
45	vi_Viverricula_indica	indian civet	3	4	1
46	gm_Genetta_maculata	Panther genet	3	4	1
47	g5_Genetta_tigrina	Large-spotted civet	3	4	1
48	g4_Genetta_genetta	spotted genet	3	4	1
49	ad_Atilax_paludinosus	MarshMongoose	4	1	0
50	ck_Cynictis_penicillata	Yellow mongoose	4	2	1
51	ps_Paracynictis_selousi	Selous' mongoose	4	2	1
52	ia_Ichneumia_albicauda	White-tailed mongoose	4	2	1
53	go_Galerella_sanguinea	Slender mongoose	4	4	1
54	hi_Herpestes_ichneumon	Egyptian mongoose	4	4	1

DSC_Ord	Diet_Cite	lmass
28	Gittleman, 1985	4.146128036
29	Gittleman, 1985	4.027349608
30	Munoz-Garcia & Williams, 2005	4.125481266
31	Hogue, 2003	4.290034611
32	Munoz-Garcia & Williams, 2005	5.144574208
33	Gittleman, 1985	4.626596967
34	Munoz-Garcia & Williams, 2005	4.909288524
35	Munoz-Garcia & Williams, 2005	5.07809415
36	Gittleman, 1985	4.659202877
37	Hogue, 2003	3.653212514
38	Munoz-Garcia & Williams, 2005	4.088136089
39	Munoz-Garcia & Williams, 2005	3.491361694
40	Iwaniuk, Pellis & Whishaw, 2000	3.633468456
41	Hogue, 2003	3.375663614
42	Hogue, 2003	3.602059991
43	Ray, 1995	4.135662708
44	Hogue, 2003	4
45	Hogue, 2003	3.477121255
46	Iwaniuk, Pellis & Whishaw, 2000	3.347330015
47	Munoz-Garcia & Williams, 2005	3.260071388
48	Larivière & Calzada, 2001	3.271067548
49	Gittleman, 1985; Baker, 1992	3.51851394
50	Gittleman, 1985	2.777064155
51	Gittleman, 1985	3.255272505
52	Gittleman, 1985	3.498310554
53	Hogue, 2003	2.651278014
54	Hogue, 2003	3.465382851

DSC_Ord	Mass Cite	abslat	Lat cite.
28	Gittleman,1986	5.5095	Davies et al., 2007
29	Hogue, 2003	2.4843	Davies et al., 2007
30	Gittleman,1986; Munoz-Garcia & Williams, 2005	7.6657	Davies et al., 2007
31	Lindenfors et al., 2007	11.5755	Davies et al., 2007
32	Gittleman,1986; Iossa et al., 2008	3.4156	Davies et al., 2007
33	Gittleman,1986	5.6945	Davies et al., 2007
34	Munoz-Garcia & Williams, 2005	1.7609	Davies et al., 2007
35	Gittleman,1986; Munoz-Garcia & Williams, 2005	19.2228	Davies et al., 2007
36	Gittleman,1986	35.4204	Davies et al., 2007
37	Ernest, 2003	21.4105	Davies et al., 2007
38	Munoz-Garcia & Williams, 2005	14.8614	Davies et al., 2007
39	Munoz-Garcia & Williams, 2005	12.1201	Davies et al., 2007
40	Iossa et al., 2008	18.9098	Davies et al., 2007
41	Gittleman,1986	0.1443	Davies et al., 2007
42	Ernest, 2003	2.9045	Davies et al., 2007
43	Ray, 1995	6.0264	Davies et al., 2007
44	Gittleman,1986	16.6442	Davies et al., 2007
45	Gittleman,1986	17.0045	Davies et al., 2007
46	Hogue, 2003	5.9011	Davies et al., 2007
47	Munoz-Garcia & Williams, 2005	28.4832	Davies et al., 2007
48	Larivière & Calzada, 2001	7.5315	Davies et al., 2007
49	Baker, 1992	8.8855	Davies et al., 2007
50	Iossa et al., 2008	25.7108	Davies et al., 2007
51	Gittleman,1986	19.0488	Davies et al., 2007
52	Gittleman,1986	5.0973	Davies et al., 2007
53	Lindenfors et al., 2007	7.0648	Davies et al., 2007
54	Lindenfors et al., 2007	0.6158	Davies et al., 2007

DSC_Ord	DI3	DI Cite
28	0	Lindenfors, Dalen & Angerbjoern, 2003
29	0	Lindenfors, Dalen & Angerbjoern, 2003
30	0	Lindenfors, Dalen & Angerbjoern, 2003
31	0	Lindenfors, Dalen & Angerbjoern, 2003
32	0	Lindenfors, Dalen & Angerbjoern, 2003
33	0	Lindenfors, Dalen & Angerbjoern, 2003
34	0	Lindenfors, Dalen & Angerbjoern, 2003
35	0	Lindenfors, Dalen & Angerbjoern, 2003
36	0	Lindenfors, Dalen & Angerbjoern, 2003
37	0	Lindenfors, Dalen & Angerbjoern, 2003
38	0	Lindenfors, Dalen & Angerbjoern, 2003
39	0	Lindenfors, Dalen & Angerbjoern, 2003
40	0	Lindenfors, Dalen & Angerbjoern, 2003
41	0	Lindenfors, Dalen & Angerbjoern, 2003
42	0	Lindenfors, Dalen & Angerbjoern, 2003
43	1	Ewer & Wemner, 1974
44	0	Lindenfors, Dalen & Angerbjoern, 2003
45	0	Lindenfors, Dalen & Angerbjoern, 2003
46	0	Lindenfors, Dalen & Angerbjoern, 2003
47	0	Lindenfors, Dalen & Angerbjoern, 2003
48	0	Larivière & Calzada, 2001
49	1	Baker, 1992; Lindenfors, Dalen & Angerbjoern, 2003
50	0	Lindenfors, Dalen & Angerbjoern, 2003
51	0	Lindenfors, Dalen & Angerbjoern, 2003
52	0	Lindenfors, Dalen & Angerbjoern, 2003
53	0	Lindenfors, Dalen & Angerbjoern, 2003
54	0	Lindenfors, Dalen & Angerbjoern, 2003

DSC_Ord	name	Common	Fam13	Diet6all	Diet7
55	he_Herpestes_edwardsi	Indian gray mongoose	4	4	1
56	hj_Herpestes_javanicus	Javan mongoose	4	4	1
57	cg_Crossarchus_obscurus	Long-nosed Cusimanse	4	2	1
58	ho_Helogale_parvula	Dwarf mongoose	4	1	0
59	mb_Mungos_mungo	Banded mongoose	4	2	1
60	ss_Suricata_suricatta	Meerkat	4	2	1
61	ci_Cryptoprocta_ferox	Fossa	4	4	1
62	eg_Eupleres_goudotii	Falanouc	4	2	1
63	ff_Fossa_fossana	Malagasy civet	4	4	1
64	ge_Galidia_elegans	mongoose	4	4	1
65	g2_Galidictis_fasciata	mongoose	4	4	1
66	md_Mungotictis_decemlineata	mongoose	4	2	1
67	c8_Crocuta_crocuta	Spotted Hyena	5	4	1
68	pb_Hyaena_brunnea	Brown hyena	5	4	1
69	hh_Hyaena_hyaena	Striped hyena	5	1	1
70	zj_Proteles_cristata	Aardwolf	5	2	1
71	n3_Nandinia_binotata	African palm civet	6	1	0
72	hm_Helarctos_malayanus	Sun bear	7	1	0
73	ua_Ursus_americanus	American black bear	7	1	0
74	u1_Ursus_arctos	Brown (grizzly) bear	7	1	0
75	um_Ursus_maritimus	Polar bear	7	4	1
76	mu_Melursus_ursinus	Sloth bear	7	1	0
77	a9_Ailurus_fulgens	Red panda	8	3	0
78	a1_Aonyx_capensis	African clawless otter	9	4	1
79	a2_Aonyx_congicus	Congo Clawless otter	9	1	1
80	ac_Aonyx_cinerea	Asian small-clawed otter	9	1	1
81	l5_Lutrogale_perspicillata	Indian smooth-coated otter	9	4	1

DSC_Ord	Diet_Cite	Imass
55	Hogue, 2003	3.003460532
56	Hogue, 2003	2.903089987
57	Goldman, 1987	2.861534411
58	Iwaniuk, Pellis & Whishaw, 2000	2.439016728
59	Gittleman, 1985	3.124341171
60	Munoz-Garcia & Williams, 2005	2.889861721
61	Köhncke & Leonhardt, 1986	3.977723605
62	Hogue, 2003	3.477121255
63	Munoz-Garcia & Williams, 2005	3.187520721
64	Hogue, 2003	2.903089987
65	Hogue, 2003	2.77815125
66	Hogue, 2003	2.84509804
67	Gittleman, 1985	4.799340549
68	Mills, 1982	4.779596491
69	Munoz-Garcia & Williams, 2005	4.602059991
70	Munoz-Garcia & Williams, 2005	4
71	Munoz-Garcia & Williams, 2005	3.278753601
72	Fitzgerald & Krausman, 2002	4.662757832
73	Munoz-Garcia & Williams, 2005	5.04359803
74	Munoz-Garcia & Williams, 2005	5.308564414
75	Munoz-Garcia & Williams, 2005	5.456922469
76	Munoz-Garcia & Williams, 2005	5
77	Munoz-Garcia & Williams, 2005	3.635986112
78	Larivière, 2001	4.071882007
79	Larivière, 2001	4.380211242
80	Larivière, 2003	3.477121255
81	Hwang & Larviere, 2005	3.954242509

DSC_Ord	Mass Cite	abslat	Lat cite.
55	Ernest, 2003	21.0695	Davies et al., 2007
56	Lindenfors et al., 2007	19.3189	Davies et al., 2007
57	Goldman, 1987	6.8295	Davies et al., 2007
58	Komers & Brotherton, 1997	7.0407	Davies et al., 2007
59	Carbone, Teacher & Rowcliffe, 2007	8.3455	Davies et al., 2007
60	Iossa et al., 2008	24.7767	Davies et al., 2007
61	Iossa et al., 2008	19.2639	Davies et al., 2007
62	Gittleman,1986	19.6367	Davies et al., 2007
63	Munoz-Garcia & Williams, 2005	18.8103	Davies et al., 2007
64	Iossa et al., 2008	19.5169	Davies et al., 2007
65	Hogue, 2003	19.6050	Davies et al., 2007
66	Ernest, 2003	20.7927	Davies et al., 2007
67	Iossa et al., 2008	5.1473	Davies et al., 2007
68	Mills, 1982	24.3033	Davies et al., 2007
69	Iossa et al., 2008	16.9368	Davies et al., 2007
70	Iossa et al., 2008	7.6294	Davies et al., 2007
71	Gittleman,1986; Munoz-Garcia & Williams, 2005	3.9279	Davies et al., 2007
72	Fitzgerald & Krausman, 2002	12.3317	Davies et al., 2007
73	Iossa et al., 2008	51.6632	Davies et al., 2007
74	Iossa et al., 2008	55.5510	Davies et al., 2007
75	Iossa et al., 2008	69	*DeMaster & Stirling, 1981
76	Munoz-Garcia & Williams, 2005	24.1600	Davies et al., 2007
77	Iossa et al., 2008	28.3890	Davies et al., 2007
78	Gittleman,1986; Larviere, 2001	8.8966	Davies et al., 2007
79	Lariviere, 2001	1.5520	Davies et al., 2007
80	Larivière, 2003	16.3285	Davies et al., 2007
81	Iossa et al., 2008	15.4319	Davies et al., 2007

DSC_Ord	DI3	DI Cite
55	0	Lindenfors, Dalen & Angerbjoern, 2003
56	0	Lindenfors, Dalen & Angerbjoern, 2003
57	0	Lindenfors, Dalen & Angerbjoern, 2003
58	0	Lindenfors, Dalen & Angerbjoern, 2003
59	0	Lindenfors, Dalen & Angerbjoern, 2003
60	0	Lindenfors, Dalen & Angerbjoern, 2003
61	0	Köhncke & Leonhardt, 1986
62	0	Lindenfors, Dalen & Angerbjoern, 2003
63	0	Lindenfors, Dalen & Angerbjoern, 2003
64	0	Lindenfors, Dalen & Angerbjoern, 2003
65	0	Lindenfors, Dalen & Angerbjoern, 2003
66	0	Lindenfors, Dalen & Angerbjoern, 2003
67	0	Lindenfors, Dalen & Angerbjoern, 2003
68	0	Mills, 1982; Lindenfors, Dalen & Angerbjoern, 2003
69	0	Lindenfors, Dalen & Angerbjoern, 2003
70	0	Lindenfors, Dalen & Angerbjoern, 2003
71	0	Lindenfors, Dalen & Angerbjoern, 2003
72	1	Dathe, 1963; McCusker, 1975
73	1	Hamlett, 1935; Wimsatt, 1963
74	1	Dittrich & Kronenberger, 1963; Craighead, Hornocker & Craighead, 1969
75	1	Dittrich, 1961; Volf, 1963
76	1	Laurie & Seidensticker, 1977; Puschman, Schuppel & Kronberger, 1977
77	1	Roberts & Gittleman, 1984
78	0	Thom, Johnson & Macdonald, 2004
79	0	Thom, Johnson & Macdonald, 2004
80	0	Lindenfors, Dalen & Angerbjoern, 2003
81	0	Lindenfors, Dalen & Angerbjoern, 2003

DSC_Ord	name	Common	Fam13	Diet6all	Diet7
82	l3_Lutra_lutra	Eurasian otter	9	0	1
83	lm_Hydrictis_maculicollis	spotted-necked otter	9	0	1
84	l2_Lontra_canadensis	American river otter	9	0	1
85	ll_Lontra_longicaudis	Neotropical river otter	9	0	1
86	el_Enhydra_lutris	Sea otter	9	0	1
87	gv_Galictis_vittata	Greater grison	9	4	1
88	pa_Poecilogale_albinucha	African striped weasel	9	4	1
89	zp_Pteronura_brasiliensis	Flat-tailed, giant otter	9	0	1
90	mv_Mustela_frenata	Long-tailed weasel	9	4	1
91	zg_Neovison_vison	American mink	9	4	1
92	mj_Mustela_altaica	Mountain weasel	9	4	1
93	za_Mustela_nivalis	Least weasel	9	4	1
94	zd_Mustela_putorius	Ferret	9	4	1
95	mx_Mustela_lutreola	European mink	9	4	1
96	my_Mustela_nigripes	Black-footed ferret	9	4	1
97	ze_Mustela_sibirica	weasel	9	4	1
98	mq_Mustela_erminea	Short-tailed weasel, Ermine	9	4	1
99	is_Ictonyx_striatus	Zorilla or striped pole cat	9	4	1
100	vp_Vormela_peregusna	Marbled polecat	9	4	1
101	eb_Eira_barbara	Tayra	9	1	1
102	mp_Martes_pennanti	Fisher	9	4	1
103	g6_Gulo_gulo	Woverine	9	4	1
104	ma_Martes_americana	American marten	9	4	1
105	m1_Martes_foina	Beech marten	9	1	1
106	mn_Martes_martes	Pine marten	9	4	1
107	mz_Martes_zibellina	Sable	9	4	1
108	M2_Martes_melampus	Japanese marten	9	1	1
109	mf_Martes_flavigula	yellow-throated marten	9	1	0

DSC_Ord	Diet_Cite	lmass
82	Munoz-Garcia & Williams, 2005	3.829303773
83	Hogue, 2003	3.602059991
84	Gittleman, 1985	3.794139356
85	Hogue, 2003	3.875061263
86	Munoz-Garcia & Williams, 2005	4.338456494
87	Yensen & Tarifa, 2003	3.371067862
88	Gittleman, 1985	2.410777233
89	Hogue, 2003	4.380211242
90	Munoz-Garcia & Williams, 2005	2.177824972
91	Munoz-Garcia & Williams, 2005	2.954121855
92	Gittleman, 1985	2.23299611
93	Munoz-Garcia & Williams, 2005	1.696793085
94	Kinlaw, 1995	2.769871904
95	Gittleman, 1985	2.643452676
96	Hillman & Clark, 1980	2.907948522
97	Gittleman, 1985	2.607455023
98	Munoz-Garcia & Williams, 2005	2.042693618
99	Hogue, 2003	2.883661435
100	Gittleman, 1985	2.734399743
101	Munoz-Garcia & Williams, 2005	3.653212514
102	Powell, 1981	3.414973348
103	Munoz-Garcia & Williams, 2005	4.213074737
104	Munoz-Garcia & Williams, 2005	2.782952519
105	Hogue, 2003	3.230448921
106	Munoz-Garcia & Williams, 2005	3.113943352
107	Hogue, 2003	3.028030081
108	Hogue, 2003	3.096910013
109	Gittleman, 1985	3.397940009

DSC_Ord	Mass Cite	abslat	Lat cite.
82	Iossa et al., 2008	36.6628	Davies et al., 2007
83	Gittleman,1986	8.1353	Davies et al., 2007
84	Iossa et al., 2008	47.6003	Davies et al., 2007
85	Lindenfors et al., 2007	6.0044	Davies et al., 2007
86	Iossa et al., 2008	39.5	*Estes, 1980
87	Yensen & Tarifa, 2003	3.5014	Davies et al., 2007
88	Gittleman,1986	16.1351	Davies et al., 2007
89	Iossa et al., 2008	8.5269	Davies et al., 2007
90	Iossa et al., 2008	19.5261	Davies et al., 2007
91	Iossa et al., 2008	48.1689	Davies et al., 2007
92	Gittleman,1986	45.0976	Davies et al., 2007
93	Iossa et al., 2008	51.8416	Davies et al., 2007
94	Iossa et al., 2008	47.7336	Davies et al., 2007
95	Gittleman,1986	54.3300	Davies et al., 2007
96	Iossa et al., 2008	6.8793	Davies et al., 2007
97	Gittleman,1986	38.4662	Davies et al., 2007
98	Iossa et al., 2008	50.8943	Davies et al., 2007
99	Iossa et al., 2008	6.1004	Davies et al., 2007
100	Gittleman,1986	40.5977	Davies et al., 2007
101	Iossa et al., 2008	4.4947	Davies et al., 2007
102	Iossa et al., 2008	52.5442	Davies et al., 2007
103	Iossa et al., 2008	60.6779	Davies et al., 2007
104	Iossa et al., 2008	55.5693	Davies et al., 2007
105	Lindenfors et al., 2007	45.1125	Davies et al., 2007
106	Iossa et al., 2008	53.3589	Davies et al., 2007
107	Lindenfors et al., 2007	55.6909	Davies et al., 2007
108	Lindenfors et al., 2007	37.4879	Davies et al., 2007
109	Ernest, 2003	20.7013	Davies et al., 2007

DSC_Ord	DI3	DI Cite
82	0	Lindenfors, Dalen & Angerbjoern, 2003
83	0	Thom, Johnson & Macdonald, 2004
84	1	Ferguson et al., 1996
85	1	Cubas et al., 1993; Jacome & Paera, 1995
86	1	Novikov, 1956; Sinha, Conoway & Kenyon, 1966
87	0	Yensen & Tarifa, 2003
88	0	Thom, Johnson & Macdonald, 2004
89	0	Lindenfors, Dalen & Angerbjoern, 2003
90	1	Lindenfors, Dalen & Angerbjoern, 2003
91	1	Lindenfors, Dalen & Angerbjoern, 2003
92	0	Thom, Johnson & Macdonald, 2004
93	0	Ferguson, Virgl & Larivière, 1996
94	1	Hogue but see Thom et al 2004's data.
95	1	Thom, Johnson & Macdonald, 2004
96	0	Lindenfors, Dalen & Angerbjoern, 2003
97	0	Thom, Johnson & Macdonald, 2004
98	1	Watzka,1940; Deanesly, 1943; Enders, 1952
99	0	Lindenfors, Dalen & Angerbjoern, 2003
100	1	Thom, Johnson & Macdonald, 2004
101	0	Lindenfors, Dalen & Angerbjoern, 2003
102	1	Enders & Pearson, 1943; Powell, 1981
103	1	Wright & Rausch, 1955; Rausch & Pearson, 1972
104	1	Ashbrook & Hansson, 1930; Pearson & Enders, 1944
105	1	Thom, Johnson & Macdonald, 2004
106	1	Prell, 1927; Stubbe, 1968
107	1	Thom, Johnson & Macdonald, 2004
108	1	Thom, Johnson & Macdonald, 2004
109	1	Thom, Johnson & Macdonald, 2004

DSC_Ord	name	Common	Fam13	Diet6all	Diet7
110	m3_Meles_meles	Eurasian badger	9	1	1
111	tt_Taxidea_taxus	American badger	9	1	1
112	mc_Mellivora_capensis	Honey badger	9	4	1
113	bg_Bassaricyon_gabbii	Olingo	10	1	0
114	b1_Bassariscus_astutus	Ringtail	10	1	0
115	nn_Nasua_narica	White-nosed Coati	10	1	0
116	n1_Nasua_nasua	South American Coati	10	1	0
117	NO_Nasuella_olivacea	Mountain coati	10	1	0
118	pe_Procyon_cancrivorus	Crab-eatting racoon	10	1	0
119	pk_Procyon_lotor	Northern raccoon	10	1	1
120	PX_Procyon_pygmaeus	racoon	10	1	0
121	p6_Potos_flavus	Kinkajou	10	1	0
122	m6_Mephitis_macroura	Hooded skunk	11	2	1
123	m7_Mephitis_mephitis	Striped skunk	11	1	1
124	sp_Spilogale_putorius	Eastern spotted skunk	11	4	1
125	s1_Spilogale_pygmaea	pygmy spotted skunk	11	2	1
126	At_Arctocephalus_townsendi	Guadalupe Fur Seal	12	0	1
127	nc_Neophoca_cinerea	Australian sea lion	12	0	1
128	or_Odobenus_rosmarus	Walrus	12	2	1
129	cq_Cystophora_cristata	Hooded seal	12	0	1
130	p4_Pusa_sibirica	Baikal seal	12	0	1
131	ml_Mirounga_leonina	Southern elephant seal	12	0	1
132	vf_Vulpes_ferrilata	Tibetan sand fox	13	4	1
133	zk_Lycalopex_culpaeus	Culpeo	13	4	1
134	c3_Cerdocyon_thous	Crab-eatting fox	13	4	1
135	c4_Chrysocyon_brachyurus	Maned wolf	13	1	0
136	cm_Canis_mesomelas	Black-backed jackal	13	4	1
137	cl_Canis_latrans	Coyote	13	4	1

DSC_Ord	Diet_Cite	Imass
110	Munoz-Garcia & Williams, 2005	4.113943352
111	Munoz-Garcia & Williams, 2005	3.781755375
112	Vanderhaar & Hwang, 2003	4
113	Kays, 2000	3.094296397
114	Hogue, 2003	2.989227274
115	Gittleman, 1985	3.574031268
116	Munoz-Garcia & Williams, 2005	3.67669361
117	Hogue, 2003	3.602059991
118	Munoz-Garcia & Williams, 2005	3.797267541
119	Munoz-Garcia & Williams, 2005	3.643452676
120	de Villa-Meza et al., 2011	3.342422681
121	Munoz-Garcia & Williams, 2005	3.477121255
122	Hwang & Larivière, 2001	2.984527313
123	Hogue, 2003	3.260369565
124	Munoz-Garcia & Williams, 2005	2.708845638
125	Medellín, Ceballos, & Zarza, 1998; Cantu-Salazar et al., 20	2.414973348
126	Belcher & Lee, 1998	5
127	Ling, 1992	4.901094877
128	Fay, 1985	5.812913357
129	Kovacs & Lavigne, 1986	5.326335861
130	Thomas et al., 1982	4.912044847
131	Ling & Brydan, 1992	5.762978491
132	Clark et al., 2008	3.84509804
133	Gittleman, 1985	4.113943352
134	Munoz-Garcia & Williams, 2005	3.812913357
135	Dietz, 1985	4.361727836
136	Walton & Joly, 2003	3.989004616
137	Munoz-Garcia & Williams, 2005	4.071882007

DSC_Ord	Mass Cite	abslat	Lat cite.
110	Iossa et al., 2008	43.4738	Davies et al., 2007
111	Iossa et al., 2008	38.4514	Davies et al., 2007
112	Iossa et al., 2008	4.1354	Davies et al., 2007
113	Kays, 2000	5.0814	Davies et al., 2007
114	Iossa et al., 2008	29.6519	Davies et al., 2007
115	Gittleman,1986	21.2671	Davies et al., 2007
116	Munoz-Garcia & Williams, 2005	11.5516	Davies et al., 2007
117	Hogue, 2003	3.9150	Davies et al., 2007
118	Munoz-Garcia & Williams, 2005	11.1700	Davies et al., 2007
119	Iossa et al., 2008	32.9902	Davies et al., 2007
120	de Villa-Meza et al., 2011	20.4286	Davies et al., 2007
121	Munoz-Garcia & Williams, 2005	0.3082	Davies et al., 2007
122	Hwang & Larivière, 2001	23.5802	Davies et al., 2007
123	Iossa et al., 2008	43.1537	Davies et al., 2007
124	Iossa et al., 2008	36.5975	Davies et al., 2007
125	Medellín, Ceballos, & Zarza, 1998; Cantu-Salazar et al.,	17.2027	Davies et al., 2007
126	Belcher & Lee, 2002	28.0431	*Belcher & Lee, 1998
127	Ling, 1992	31.6667	*Ling, 1992
128	Fay, 1985	70	*Fay, 1985
129	Kovacs & Lavigne, 1986	58.5	*Kovacs, & Lavigne, 1986
130	Lindenfors et al., 2007	53.445	*Thomas et al., 1982
131	Lindenfors et al., 2007	53	*Ling & Brydan, 1992
132	Clark et al., 2008	32.4049	Davies et al., 2007
133	Lindenfors et al., 2007	26.0947	Davies et al., 2007
134	Munoz-Garcia & Williams, 2005	18.5072	Davies et al., 2007
135	Gittleman,1986	17.3286	Davies et al., 2007
136	Gittleman,1986	3.3610	Davies et al., 2007
137	Iossa et al., 2008	39.7078	Davies et al., 2007

DSC_Ord	DI3	DI Cite
110	1	Fries, 1880; Neal & Harrison, 1958; Canivenc, 1966; Canivenc & Bonnin,
111	1	Hamlett, 1932b; Wright, 1966
112	1	Rosevear, 1974
113	0	Lindenfors, Dalen & Angerbjoern, 2003
114	0	Ferguson, Virgl & Larivière, 1996
115	0	Lindenfors, Dalen & Angerbjoern, 2003
116	0	Lindenfors, Dalen & Angerbjoern, 2003
117	0	Lindenfors, Dalen & Angerbjoern, 2003
118	0	Lindenfors, Dalen & Angerbjoern, 2003
119	0	Lindenfors, Dalen & Angerbjoern, 2003
120	0	de Villa-Meza et al., 2011
121	0	Lindenfors, Dalen & Angerbjoern, 2003
122	1	Thom, Johnson & Macdonald, 2004
123	1	Lindenfors, Dalen & Angerbjoern, 2003
124	0	Lindenfors, Dalen & Angerbjoern, 2003
125	0	Thom, Johnson & Macdonald, 2004
126	1	Riedman, 1990
127	1	Riedman, 1990
128	1	Fay, 1981, 1982, 1985
129	1	Ørtisland, 1964
130	1	Kozhov, 1947; Riedman, 1990
131	1	Harrison, Matthews & Roberts, 1952; Laws, 1956
132	0	Lindenfors, Dalen & Angerbjoern, 2003
133	0	Lindenfors, Dalen & Angerbjoern, 2003
134	0	Lindenfors, Dalen & Angerbjoern, 2003
135	0	Lindenfors, Dalen & Angerbjoern, 2003
136	0	Lindenfors, Dalen & Angerbjoern, 2003
137	0	Ferguson, Virgl & Larivière, 1996

DSC_Ord	name	Common	Fam13	Diet6all	Diet7
138	c2_Canis_lupus	Gray Wolf	13	4	1
139	cs_Canis_simensis	Simian jackal	13	4	1
140	cj_Cuon_alpinus	Dhole	13	2	1
141	l6_Lycaon_pictus	African wild dog	13	4	1
142	sv_Speothos_venaticus	Bush dog	13	4	1
143	np_Nyctereutes_procyonoides	Racoon dog	13	1	1
144	vb_Vulpes_bengalensis	Bengal fox	13	4	1
145	v2_Vulpes_corsac	Corsac fox	13	4	1
146	vr_Vulpes_rueppellii	Sand fox	13	1	1
147	v4_Vulpes_vulpes	Red fox	13	4	1
148	Al_Vulpes_lagopus	Arctic fox	13	4	1
149	vd_Vulpes_macrotis	Kit fox, desert fox	13	4	1
150	vv_Vulpes_velox	Swift fox	13	4	1
151	va_Vulpes_cana	Blanford's fox	13	1	1
152	v5_Vulpes_zerda	Fennec fox	13	1	1
153	v1_Vulpes_chama	Cape fox	13	4	1
154	v3_Vulpes_pallida	Pale fox	13	4	1
155	o3_Otocyon_megalotis	Bat-eared fox	13	1	1
156	uc_Urocyon_cinereoargenteus	Grey fox	13	4	1
157	ul_Urocyon_littoralis	Island grey fox	13	4	1

DSC_Ord	Diet_Cite	lmass
138	Munoz-Garcia & Williams, 2005	4.542514216
139	Sillero-Zubiri & Gottelli, 1994	4.102947968
140	Cohen, 1978	4.105850674
141	Gittleman, 1985	4.433503097
142	Beisiegel & Zuercher, 2005	3.77815125
143	Gittleman, 1985; Ward & Wurster-Hill, 1990	3.626545659
144	Gompper & Vanak, 2006	3.255272505
145	Hogue, 2003	3.431363764
146	Munoz-Garcia & Williams, 2005	3.352182518
147	Munoz-Garcia & Williams, 2005	3.753008215
148	Munoz-Garcia & Williams, 2005	3.653212514
149	McGrew, 1979; Munoz-Garcia & Williams, 2005	3.371067862
150	Egoscue, 1979	3.441957361
151	Munoz-Garcia & Williams, 2005	3
152	Munoz-Garcia & Williams, 2005	3.079181246
153	Hogue, 2003	3.602059991
154	Hogue, 2003	3.40654018
155	Gittleman, 1985	3.618048097
156	Fritzell & Haroldson, 1982	3.625312451
157	Morre & Collins, 1995	3.276615733

DSC_Ord	Mass Cite	abslat	Lat cite.
138	Iossa et al., 2008	45.1627	Davies et al., 2007
139	Sillero-Zubiri & Gottelli, 1994	6.9203	Davies et al., 2007
140	Cohen, 1978	23.3800	Davies et al., 2007
141	Iossa et al., 2008	4.7406	Davies et al., 2007
142	Iossa et al., 2008	7.3321	Davies et al., 2007
143	Ward & Wurster-Hill, 1990; Iossa et al., 2008	38.2637	Davies et al., 2007
144	Gittleman,1986	21.2774	Davies et al., 2007
145	Lindenfors et al., 2007	41.9679	Davies et al., 2007
146	Munoz-Garcia & Williams, 2005	24.6153	Davies et al., 2007
147	Iossa et al., 2008	43.1073	Davies et al., 2007
148	Iossa et al., 2008	66.5356	Davies et al., 2007
149	McGrew, 1979	32.77	Jones et al. PanTHERIA data, 2009
150	Egoscue, 1979	33.2438	Davies et al., 2007
151	Munoz-Garcia & Williams, 2005	32.2136	Davies et al., 2007
152	Iossa et al., 2008	25.7361	Davies et al., 2007
153	Gittleman,1986	25.1815	Davies et al., 2007
154	Ernest, 2003	15.0064	Davies et al., 2007
155	Gittleman,1986	0.6535	Davies et al., 2007
156	Iossa et al., 2008	28.4605	Davies et al., 2007
157	Lindenfors et al., 2007	34.0208	Davies et al., 2007

DSC_Ord	DI3	DI Cite
138	0	Ferguson, Virgl & Larivière, 1996
139	0	Lindenfors, Dalen & Angerbjoern, 2003
140	0	Lindenfors, Dalen & Angerbjoern, 2003
141	0	Lindenfors, Dalen & Angerbjoern, 2003
142	0	Lindenfors, Dalen & Angerbjoern, 2003
143	0	Ward & Wurster-Hill, 1990
144	0	Lindenfors, Dalen & Angerbjoern, 2003
145	0	Lindenfors, Dalen & Angerbjoern, 2003
146	0	Lindenfors, Dalen & Angerbjoern, 2003
147	1	Hayssen, van Tienhoven & van Tienhoven 1993
148	0	Ferguson, Virgl & Larivière, 1996
149	0	Lindenfors, Dalen & Angerbjoern, 2003
150	0	Lindenfors, Dalen & Angerbjoern, 2003
151	0	Lindenfors, Dalen & Angerbjoern, 2003
152	0	Lindenfors, Dalen & Angerbjoern, 2003
153	0	Lindenfors, Dalen & Angerbjoern, 2003
154	0	Lindenfors, Dalen & Angerbjoern, 2003
155	0	Lindenfors, Dalen & Angerbjoern, 2003
156	0	Ferguson, Virgl & Larivière, 1996
157	0	Ferguson, Virgl & Larivière, 1996

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