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No evidence of tradeoffs in the evolution of sperm numbers and sperm size in mammals

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Short title: Sperm numbers and size in mammals

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Abstract

Postcopulatory sexual selection, in the form sperm competition, has influenced the evolution of several male reproductive traits. However, theory predicts that sperm competition would lead to tradeoffs between numbers and size of spermatozoa because increased costs per cell would result in a reduction of sperm number if both traits share the same energetic budget. Theoretical models have proposed that, in large animals, increased sperm size would have minimal fitness advantage compared with increased sperm numbers. Thus, sperm numbers would evolve more rapidly than sperm size under sperm competition pressure. We tested in mammals whether sperm competition maximizes sperm numbers and size, and whether there is a tradeoff between these traits. Our results showed that sperm competition maximizes sperm numbers in eutherian and metatherian mammals. There was no evidence of a tradeoff between sperm numbers and sperm size in any of the two mammalian clades since we did not observe any significant relationship between sperm numbers and sperm size once the effect of sperm competition was taken into account. Maximization of both numbers and size in mammals may occur because each trait is crucial at different stages in sperm's life; e.g., size-determined sperm velocity is a key determinant of fertilization success. In addition, numbers and size may also be influenced by diverse energetic budgets required at different stages of sperm formation.

Keywords: sexual selection, sperm competition, metabolic rate, energetic constraints, sperm size, sperm numbers

Introduction

One of the key predictions of sperm competition theory (Parker, 1970) is that males belonging to species with high levels of sperm competition should produce more sperm in order to outcompete their rivals in their quest to fertilize ova. If it is assumed that sperm competition resembles a raffle, in which sperm are equivalent to fertilizing opportunity increments, more sperm would increase the chances of fertilization (Parker, 1970; 1993). In line with this "raffle hypothesis", high levels of sperm competition associate with increases in testes mass relative to body size (Harcourt *et al.*, 1981; Gage, 1994; Birkhead & Møller, 1998; Simmons, 2001; Birkhead *et al.*, 2009; Soulsbury, 2010) and with high relative sperm numbers (Birkhead & Møller, 1998; Parker & Pizzari, 2010; Gomez Montoto *et al.*, 2011a) in many taxa.

Since the production of a vast amount of sperm would be highly expensive in terms of energy, and larger sperm may increase the energetic cost per cell, a "direct" energetic tradeoff would exist between sperm numbers and sperm size (Parker, 1982). This line of reasoning assumed that total energetic budget for sperm production equaled the number of sperm produced multiplied by the energetic cost of producing each sperm. Consequently, in order to produce more sperm there should be a reduction in the cost of producing each individual cell (i.e., via a reduction in sperm size).

Nevertheless, sperm size could provide an adaptive advantage. If sperm competition resembles a race in which the first sperm to reach the ovum engages in fertilization, an increase in sperm size (e.g., due to a longer flagellum) could provide increased sperm velocity, thus affecting the outcome of sperm competition (Gomendio & Roldan, 1991). This scenario adds a temporal component to the raffle hypothesis. Evidence supporting positive relationships between sperm competition levels and sperm size (Gage, 1994; Briskie *et al.*, 1997; Tourmente *et al.*, 2009; Tourmente *et al.*, 2011b; a), sperm size and sperm velocity

(Gomendio & Roldan, 2008; Fitzpatrick *et al.*, 2009; Lüpold *et al.*, 2009; Gomez Montoto *et al.*, 2011b; Tourmente *et al.*, 2011b), and sperm competition and sperm velocity (Fitzpatrick *et al.*, 2009; Kleven *et al.*, 2009; Gomez Montoto *et al.*, 2011b; Tourmente *et al.*, 2011b) has been found in many taxa, including mammals. Therefore, since faster sperm increase fertilization success in competitive scenarios (Birkhead *et al.*, 1999; Gage *et al.*, 2004; Gasparini *et al.*, 2010), males in species with high levels of sperm competition should maximize both sperm numbers and sperm size to increase their fitness.

Subsequent theoretical models developed the concept of an "indirect tradeoff" by placing the emphasis on total reproductive budget rather than just on sperm production (Parker, 1993). This tradeoff definition was expanded to incorporate a new partition of the total reproductive energetic budget between mate acquisition and total ejaculate investment (the latter encompassing sperm numbers and sperm size) (Parker, 1993; Parker *et al.*, 2010). Nevertheless, this model is still based on the idea of a direct tradeoff between sperm numbers and sperm size within the ejaculate investment component, for a given value of sperm competition risk. In addition, two sperm competition mechanisms have been defined on the basis of the volume scale differences between the ejaculate and the female reproductive tract. On the one hand, there is a raffle mechanism in which female reproductive tract volume and ejaculate volume scales differ considerably and there is no space constraint for fertilization (Parker, 1990). This mechanism describes conditions for externally fertilizing species and many vertebrates with internal fertilization, especially those with large bodies. On the other hand, a displacement mechanism, in which the volumes of the ejaculate and the female sperm storage organs are similar and, thus, successive ejaculates will volumetrically displace the preceding ones (Parker & Simmons, 1991). This mechanism describes the conditions prevalent in insects, in which females have sperm stores that can hold a small volume of ejaculate.

An early theoretical model on sperm size proposed that the effect of sperm size on sperm competition is mainly mediated by sperm density (Parker, 1993). This model predicted that, if the effect of sperm density in the female tract on sperm competitiveness is negligible, there would be a single optimal size for sperm, independently of the sperm competition level. In a later paper (Parker *et al.*, 2010), the probable effects of sperm density on sperm competitiveness were investigated in detail. Since sperm numbers ejaculated (and hence the density of competing sperm) often change with sperm competition level, this study (Parker *et al.*, 2010) examined how the balance between sperm size and number is expected to change with sperm competition. Applying the indirect tradeoff hypothesis to sperm competition mechanisms, theoretical studies proposed that (a) a tradeoff between sperm numbers and sperm size would occur; (b) fitness advantages related to sperm size would be mediated by "sperm density" (i.e., sperm numbers in relation to the space available for sperm competition); and (c) in the raffle models, an increase in sperm numbers would generally yield greater fitness gain than an increase in sperm size since sperm density is considered to be negligible in this mechanism due to the scaling difference between ejaculate volume and female reproductive tract volume (Parker *et al.*, 2010). Thus, in "raffle" models, the effect of sperm size on sperm competitiveness is assumed as a weak force "reduced mainly to effects on sperm motility and survival" (Immler *et al.*, 2011). A comparative study presented empirical support for these predictions by comparing the relationships between sperm numbers, sperm size and sperm competition risk in two taxa with different sperm competition mechanisms (birds: raffle; flies: displacement) (Immler *et al.*, 2011).

In mammals, sperm competition is associated with increases in both sperm numbers (Møller, 1989; Gomendio *et al.*, 1998; Gomez Montoto *et al.*, 2011a; Lüpold, 2013), and sperm size (Tourmente *et al.*, 2011b; a). However, the existence of a positive association between both sperm size and number with the risk of sperm competition does not allow any predictions about the existence of a tradeoff between these two traits, since the investment in one of them might be higher in relation to the investment in the other (Parker *et al.*, 2010). Mammals and birds share a number of similarities regarding the reproductive traits which were taken in account by the models cited above. Firstly, volume of the female reproductive tract is considerably higher than the volume of the ejaculate (Brillard & Bakst, 1990; Suarez & Pacey, 2006). Secondly, sperm swimming velocity is a main factor in fertility (Birkhead *et al.*, 1999; Malo *et al.*, 2005), is positively related to sperm size (Lüpold *et al.*, 2009; Gomez Montoto *et al.*, 2011b; Tourmente *et al.*, 2011b) and is positively selected by sperm competition (Kleven *et al.*, 2009; Gomez Montoto *et al.*, 2011b; Tourmente *et al.*, 2011b; a; Tourmente *et al.*, 2013). Thirdly, immotile sperm are not transported towards the egg despite passive sperm transport to the site of fertilization (Froman & Kirby, 2005; Suarez & Pacey, 2006). Therefore, according to current theoretical models, the existence of a tradeoff between sperm numbers and size in mammals would be anticipated.

In this study, we analyzed the relationships between sperm numbers and sperm size in mammals by comparing two sister mammalian clades, Eutheria and Metatheria. We chose to compare these two groups because they present clear differences in sperm numbers, sperm size, and reproductive physiology, which could strongly influence responses of sperm phenotype to sperm competition. In general terms, Metatheria (i.e., marsupials) have lower relative testes mass (Tourmente *et al.*, 2011a), lower sperm numbers in relation to body size (Taggart *et al.*, 1998; Taggart *et al.*, 2003), longer sperm (Tourmente *et al.*, 2011a), and a higher efficiency of sperm transport, with an extremely high proportion of ejaculated sperm reaching the fertilization site in relation to Eutheria (i.e., the so-called placental mammals) (Taggart *et al.*, 1998; Taggart *et al.*, 2003). Furthermore, in Metatheria sperm storage in the female reproductive tract takes place during relatively long time periods (up to two weeks) in comparison to Eutheria (with the exception of Chiroptera) (Bedford *et al.*, 1984).

We predicted that a tradeoff between sperm numbers and sperm size exists among mammals but that, based on their reproductive physiology, there may be differences between Eutheria and Metatheria with regards to the relative increase in sperm size and numbers in response to sperm competition.

Materials and Methods

Sperm numbers, sperm size, and relative testes mass

Data on body mass (g), testes mass (g), total sperm length (µm), and number of sperm in caudae epididymides (x 10^6 sperm) were obtained from the literature for 64 species (21 families) of Eutheria and 14 species (7 families) of Metatheria (Table S1 for data and references). Only species for which all these measures were available were included. Data collection was restricted to studies on adult, healthy, reproductively mature individuals. In cases of experimental studies or clinical trials, only the values of control groups were used as data sources. Whenever possible, data were collected from studies in which the higher number of variables analyzed were present. In those cases in which different values for the same variable and species were available from different studies, averages were used to obtain a representative measure. For total sperm length, only species where this parameter was measured from digital images were incorporated into the dataset.

The measure of sperm number used in this study was the number of sperm in epididymal reserves (cauda epididymides), since it is a more robust parameter of sperm production than the number of sperm in the ejaculate, although both traits are strongly correlated (delBarco-Trillo *et al.*, 2013). We are aware that the number of sperm in epididymal reserves may not always be extremely accurate due to insufficient flushing of the epididymis, seasonal or age-related variation (Johnson & Thompson, 1983), and perceived risk of sperm competition during spermatogenesis (Ramm & Stockley, 2009). However, the number of sperm in the ejaculate is a less direct measure of sperm production and may be affected by many factors, namely number of copulations (Pierce *et al.*, 1990; Pitnick & Markow, 1994; Ambriz *et al.*, 2002), number of potential partners (Sheldon, 2000), sperm depletion in successive ejaculates (Preston *et al.*, 2001), differential sperm allocation through perceived sperm competition risk (Dewsbury, 1982; Wedell *et al.*, 2002; delBarco-Trillo, 2011; Kelly & Jennions, 2011), or the effect of electrostimulation (a common procedure in large mammals) in comparison to normal copulation (Mattner & Voglmayr, 1962; Aulerich *et al.*, 1972; Schneiders *et al.*, 2004).

Data analysis

To test the influence of sperm competition we used relative testes size as predictor of number of sperm in caudae epididymides (dependent variable). Since testes size relative to body mass is a reliable indicator of investment in sperm production, this trait is considered to be a very good proxy of sperm competition levels (Gage, 1994; Birkhead & Møller, 1998). Experimental selection assays in insects have demonstrated that lines subjected to high sperm competition environments evolve larger testes (Hosken & Ward, 2001), while lines subjected to enforced monogamy evolve reduced testes size and sperm production (Pitnick *et al.*, 2001). Moreover, a recent comparative study (Soulsbury, 2010) found that levels of multiple paternity correlate well with relative testes size in mammals. We performed multiple regression analyses introducing the following predictors in the model: body mass, testes mass, a quadratic term for testes mass, and the interaction between body mass and testes mass (Tomkins & Simmons, 2002; Immler *et al.*, 2011).

When testing the effect of sperm size on sperm numbers, we used two sets of predictor variables in order to control for the effect of body size (body mass and total sperm length as predictors), and sperm competition (body mass, testes mass and total sperm length as predictors) on sperm numbers. All variables were $\log_{10}$-transformed to meet parametric assumptions.

All regressions were performed using phylogenetic generalized least-squares analyses (PGLS) (Freckleton *et al.*, 2002) since species trait values may be similar as a result of phylogenetic association rather than selective evolution (Felsenstein, 1985; Harvey & Pagel, 1991). PGLSs incorporate phylogenetic interdependency among the data points by including the phylogenetic structure within a standard linear model as a covariance matrix that assumes a predetermined evolutionary model. PGLS estimates (via maximum likelihood) a phylogenetic scaling parameter lambda (λ) of the tree's branch lengths that fits evolution proceeding via Brownian motion. In our study, the length of all branches was set to 1. If λ values are close to 0, the variables are likely to have evolved independently of phylogeny, whereas λ values close to 1 indicate strong phylogenetic association of the variables. Additionally, we calculated the effect size "r" from t-values obtained from the PGLS model and the non-central confidence limits (CLs) for the z-transformed value of r (Nakagawa & Cuthill, 2007). The CLs value [values?] indicate that the effect size is statistically significant if 0 is not contained within the interval (Smithson, 2002).

All statistical analyses were performed using the CAPER v0.5 (Orme *et al.*, 2012) package for R (v3.0.1; R Foundation for Statistical Computing 2013). P values were considered statistically significant at $\alpha < 0.05$. Residual testes mass was calculated for each group as the residual of a log-log linear regression of testes mass on body mass (Eutheria: $p<0.0001$, $R^2=0.87$; Metatheria: $p<0.0001$, $R^2=0.89$) and used exclusively to illustrate the results. The phylogenetic reconstruction used in the PGLS analyses is included in the Supplementary Information (Fig. S1). This reconstruction was based on a mammalian supertree (Bininda-Emonds *et al.*, 2007) for the determination of the phylogenetic position of the higher groups (orders and families). Group-specific phylogenies were used to resolve the within group relationships for Artiodactyla (Prothero & Foss, 2007; Agnarsson & May-Collado, 2008), Carnivora (Flynn *et al.*, 2005), Soricomorpha (Dubey *et al.*, 2007), Lagomorpha (Robinson & Matthee, 2005), Rodentia (Pages *et al.*, 2010; Fabre *et al.*, 2012), and Metatheria (Meredith *et al.*, 2008; 2009).

Results

Data on sperm numbers, sperm size, body mass and testes mass are presented in Table S1. Eutherian mammals had a mean of 8.6×10^9 spermatozoa in their sperm reserves (i.e., sperm in caudae epididymides), ranging from 2.6×10^5 spermatozoa in the Damaraland mole rat (*Fukomys damarensis*) to 1.4×10^{11} spermatozoa in the sheep (*Ovis aries*) (a range of 6 orders of magnitude; Table S1). On the other hand, metatherian mammals showed a mean value of 4.6×10^8 sperm in cauda epididymides, which ranged from 1.2×10^6 spermatozoa in the fat-tailed dunnart (*Sminthopsis crassicaudata*) to 4.8×10^9 spermatozoa in the tammar wallaby (*Macropus eugenii*) (a range of 2 orders of magnitude; Table S1).

In both Eutheria and Metatheria, sperm number exhibited a strong negative allometric association with body mass (Eutheria: $\log_{10}$ sperm numbers = 6.52 + 0.67 * $\log_{10}$ body mass, $F_{1,62}= 90.95$, $p< 0.0001$, $R^2= 0.59$; Metatheria: $\log_{10}$ sperm numbers = 5.11 + 0.81 * $\log_{10}$ body mass, $F_{1,12}= 38.72$, $p< 0.0001$, $R^2= 0.76$). However, the log-log equation for Metatheria presents a higher slope and a lower intercept, which means that small metatherians would have lower sperm numbers than their eutherian counterparts of the same body mass. To show this, we calculated the "linearized mean body mass" (the antilogarithm of the mean $\log_{10}$-transformed body mass) for each group (Eutheria= 2851.02g, Metatheria= 2299.85g) and used the previously stated equations to predict the sperm numbers. Comparison of predicted sperm numbers between the two clades revealed that Metatheria had roughly one order of magnitude less sperm (6.66×10^7) than their Eutheria counterpart (7.02×10^8).

Our analyses regarding the effect of sperm competition on sperm numbers revealed that in both Eutheria and Metatheria, there is a positive association between relative testes size and sperm numbers in caudae epididymides (Table 1). Neither the quadratic term for testes mass, nor the interaction between body mass and testes mass were significantly related to sperm numbers or sperm size in any of the two clades (Table S2). We next examined possible relationships between sperm size and sperm numbers. Since sperm size is negatively associated with body size in both Eutheria (Gomendio *et al.*, 2011) and Metatheria (Tourmente *et al.*, 2011a), as a consequence of a decrease in mass-specific metabolic rate in large-bodied animals, we included body size as a controlling variable in our analyses. We found that, after controlling for body size, sperm numbers in reserves and sperm size were positively associated in Eutheria (Table 1, Fig. 1A) but showed no significant relationship in Metatheria (Table 1, Fig. 1B).

According to recent theoretical models, the influence of sperm competition risk on both sperm numbers and sperm size should be taken into account to identify a possible tradeoff between these traits (Parker *et al.*, 2010). Thus, in order to accurately predict the possible effect of one sperm trait on the other, the effect of relative testes size was included in the model. When relative testes size was controlled for, no significant association between sperm numbers and sperm length was observed in either Eutheria or Metatheria (Table 1, Fig. 1C, D).

Further assessments of the relationships between sperm numbers and sperm size were performed by calculating two additional parameters (Parker, 1990; Parker & Simmons, 1991; Parker *et al.*, 2010; Immler *et al.*, 2011): (a) total sperm investment (i.e., sperm size * sperm numbers), which represents an approximation of the total energy a male spends in sperm production, and (b) relative investment in sperm size (i.e., sperm size / sperm numbers), which represents an approximation of the proportion between investment in sperm numbers and investment in sperm size. We found that total sperm investment was positively related to relative testes size in both Eutheria and Metatheria but that there were differences between clades when comparing increase rates (Eutheria, slope= 4.2121, $F_{1,61}$= 91.54, p<0.0001; Metatheria, slope= 2.9936, $F_{1,11}$= 11.68, p=0.0057; Table 2, Fig. 2A, B). On the other hand, relative investment in sperm size showed a significant negative relationship with relative testes size in Eutheria (slope= -0.0489, $F_{1,61}$= 71.80, p<0.0001; Table 2, Fig. 2C) but not in Metatheria (slope= -0.0398, $F_{1,11}$= 1.58, p=0.2352, Table 2, Fig. 2D). Overall, these results suggest that, in Eutheria, sperm numbers increase faster than sperm size in response to sperm competition.

Discussion

The results of this study showed that sperm competition maximizes sperm numbers in eutherian and metatherian mammals. Previous work on both clades also revealed a positive relationship between sperm competition and sperm size (Tourmente *et al.*, 2011b; a). Both an increase in sperm numbers and sperm size would promote a positive association between total sperm investment (sperm size * sperm numbers) and sperm competition levels (Parker, 1990; Parker & Simmons, 1991; Parker *et al.*, 2010; Immler *et al.*, 2011), which is supported for both Eutheria and Metatheria in our analyses.

Theoretical models (Parker, 1990; Parker *et al.*, 2010) predict that, in large vertebrates, the vast scale difference between the size of the sperm cell and that of the female reproductive tract favors a "raffle" sperm competition mechanism, and that competitive advantage may be gained mainly through increases in sperm numbers, which would tradeoff against sperm size. Our results regarding the relationship between sperm competition and relative investment in sperm size (sperm size/sperm numbers) in Eutheria suggest that, in this group, sperm numbers increase more rapidly than sperm size in response to sperm competition. At first glance, these trends appear to be consistent with those described for passerine birds (Immler *et al.*, 2011) and seem to adjust to theoretical models (Parker *et al.*, 2010). However, we did not find any significant relationship between sperm numbers and sperm size in any of the two mammalian clades once the effect of sperm competition was controlled for. That is, an increase in sperm size is not associated to a decrease in sperm numbers at a given level of sperm competition. In addition, studies in birds showed an “inverted U-shaped” relationship between sperm competition and sperm size, which led to the conclusion that little or no increase in sperm size at high sperm competition risks occurred as a result of a tradeoff between sperm numbers and sperm size (Parker *et al.*, 2010; Immler *et al.*, 2011). In contrast, sperm size was not significantly associated with the quadratic term of testes mass, nor with the interaction term between body mass and testes mass in any of the two clades analyzed. Thus, there is no evidence of a deceleration in the slope of increase of sperm size in relation to sperm competition in mammals. Moreover, extremes cases such as *Cricetulus griseus* (total sperm length: 258 μm) and *Tarsipes rostratus* (total sperm length: 337 μm) show the longest sperm, respectively, in Eutheria and Metatheria, along with extremely high relative testes size (Gomendio *et al.*, 2011; Tourmente *et al.*, 2011b; a).

Raffle models predict that sperm numbers would have a higher rate of increment than sperm size in response to sperm competition because of the differences in the relative competitive advantages provided by each trait in a tradeoff scenario. Since our results found no evidence of such tradeoff, we propose that a similar relationship between rates of increase of these two traits could exist in mammals because their morpho-physiological reproductive characteristics do not conform to certain assumptions of the general models (Parker *et al.*, 2010). These reproductive characteristics of mammals are discussed below.

Sperm numbers and size may be regulated by different energetic budgets

Sperm numbers and size are determined at different stages of spermatogenesis and are related to separate, independent processes of sperm formation. Sperm numbers may depend on testes architecture, kinetics of spermatogenesis, cell proliferation (i.e., the number of cell divisions of spermatogonia) and apoptosis (i.e., cell loss) resulting in a net number of cells after meiosis. These traits show considerable variations in mammals (Hess & Franca, 2008) and have been shown to covary with sperm competition levels (delBarco-Trillo *et al.*, 2013). Sperm size, on the other hand, depends exclusively on the post-meiotic differentiation process, when spermatids give rise to sperm cells. This process also presents a high degree of variability among mammals (Hess & Franca, 2008), although the possible associations between sperm competition and the duration of sperm differentiation remain to be established.

Differential constraints on sperm size and numbers

At least in Eutheria, longer sperm take longer to produce, and sperm competition selects for shorter sperm production times (Ramm & Stockley, 2010; delBarco-Trillo *et al.*, 2013). This suggests that to produce increasingly longer sperm in a competitive timeframe, an enhanced mass-specific metabolic rate is required. In both Eutheria and Metatheria, sperm size correlates negatively with body size, which is a consequence of differences in mass-specific metabolic rates (low in large species and high in small species) (Gomendio *et al.*, 2011; Tourmente *et al.*, 2011a). Thus, in large mammalian species the increase on sperm size in relation to high levels of sperm competition appears to be constrained by the influence of mass-specific metabolic rate (Gomendio *et al.*, 2011; Tourmente *et al.*, 2011a). On the other hand, an increase in sperm numbers can be attained simply by increasing the quantity of sperm-producing units (i.e., increasing testes size). Absolute body mass and testes mass are positively correlated with sperm numbers in sperm reserves in both Eutheria and Metatheria, meaning that larger bodied species have larger testes and hence more sperm. Moreover, a recent study found that sperm numbers in ejaculate, while increasing with relative testes size, appear not to be associated with metabolism (Lüpold, 2013). In addition, it should be pointed out that sperm size may be limited by factors other than energetic budget because size could relate to hydrodynamic, biomechanical (Ishijima, 2012), and biochemical (e.g., energy production, and ATP diffusion) (Takei *et al.*, 2014) constraints, beyond which motility would not be efficient. Therefore, if sperm numbers and sperm size have a diverse set of energetic constraints, a tradeoff between these two sperm traits is not required to explain a more pronounced increase in sperm numbers associated to sperm competition.

Variable scaling of the female reproductive tract

The models described assume that the female reproductive tract is a uniform space, which increases proportionally to the size of the female. In mammals, the female reproductive tract generally comprises three major organs: the vagina, the uterus, and the oviducts, which contain both the sperm reservoir (at the lower isthmus) and the site of fertilization (ampulla) (Suarez, 2008). The size of the vagina and uterus exhibit considerable variation across mammalian species and are related to adult body mass, individual offspring size, and total litter mass (Austin & Short, 1985). However, the volume of the oviduct represents a small fraction of the total size of the tract. The oviduct volume represents about 0.2% of the total volume of the reproductive tract (estimated from (Peters & Ball, 1987; Kunhbar *et al.*, 2003)) and relates to body mass with an extremely low exponent (0.2809, (Gomendio & Roldan, 1993; Anderson *et al.*, 2006)). As an example, a cow has an oviduct 10-fold longer than a mouse, while having an approximately 13,000-fold body mass (Gomendio & Roldan, 1993). In this scenario, the effect of interspecific body mass variation on the scaling proportion between ejaculate size and the volume of the fertilization space would be very different according to the region of the female reproductive tract in which the sperm is located (extremely high in the uterus and vagina; much lower in the oviduct, the actual fertilization space in mammals).

Sperm numbers at the site of fertilization and polyspermy

Parker et al.'s raffle model assumes that the ejaculated sperm mass is affected only by passive sperm loss in its passage through the female reproductive tract, and that the amount of sperm lost is proportional to its size. In birds, this sperm loss is considerable since only 1-2% of the ejaculated sperm reaches the oviductal sperm storage tubules (Brillard & Bakst, 1990; Birkhead, 1992). In mammals, the sperm mass is rapidly transported (within minutes) from sperm deposition sites (uterus or vagina depending on the species) to the oviduct by muscular contractions. During this process, the sperm mass also sustains passive sperm loss, with a reduction of sperm numbers of approximately one order of magnitude (Suarez & Pacey, 2006). However, at least in the Eutheria, this reduction in sperm numbers is relatively low when compared to the reduction (5 to 6 orders of magnitude) that takes place when spermatozoa swim through the junction between the uterus and the oviduct (utero-tubal junction) (Hunter, 1993; Suarez & Pacey, 2006; Coy *et al.*, 2012). As a result, only a few thousands of sperm are trapped and stored in the oviductal reservoir (Suarez, 2008; Coy *et al.*, 2012). It is noteworthy that, while the number of ejaculated sperm in mammals presents considerable variation (a range of 5 orders of magnitude), and is related to body size [40], similar sperm numbers are found in the oviductal reservoir in many species with different body sizes (Harper, 1982). Following storage in the oviductal sperm reservoir, and after completing the process of capacitation, sperm are released in discrete small-numbered populations, which swim along the oviduct towards the site of fertilization (the oviductal ampulla) (Suarez, 2008; Hunter, 2012). A final step in the reduction of sperm numbers relates to the loss of cells that are not able to reach the ampulla, a process in which both thermotaxis

(Eisenbach & Giojalas, 2006) and chemotaxis (Guidobaldi *et al.*, 2012) are involved. Thus, as a result of these complex physiological steps, the sperm:egg ratios at the time of fertilization are close to unity (Hunter, 1996; Burkitt *et al.*, 2012; Coy *et al.*, 2012; Hunter, 2012). The most likely explanation for such dynamics of sperm storage and release in mammals is the need to prevent polyspermy (Coy & Aviles, 2010; Coy *et al.*, 2012; Hunter, 2012), which generally results in the death of the embryo (Snook *et al.*, 2011). On the contrary, polyspermy appears to be the rule in birds, and multiple sperm penetration in the egg's germinal disk (up to 60 in some cases (Perry, 1987)) does not lead to any developmental problem for the embryo (Snook *et al.*, 2011). Moreover, multiple sperm penetration in birds appears to be an adaptation to assure fertility, since there is a positive correlation between the number of sperm entering the ova and the ovum size (Birkhead *et al.*, 1994).

Taken together, differences in reproductive physiology suggest that, contrary to what happens in birds, in mammals sperm velocity (which is related to sperm size) would play a more essential role than sperm number in the last stage of the sperm journey to the site of fertilization. Thus, this final step in sperm transport would resemble a short race, with only a few participants, rather than a raffle.

Differences between Eutheria and Metatheria

In Eutheria sperm numbers tend to increase in a more pronounced way than sperm size under increasing levels of sperm competition. This pattern was not observed in Metatheria. A possible explanation for this difference may lay in fundamental differences that exist between Eutheria and Metatheria with regards to their relative investments in sperm numbers and sperm size. Metatheria have, on average, longer and fewer spermatozoa than their Eutheria counterparts (mean total sperm length, Metatheria = 163µm; Eutheria = 91µm) (Tourmente *et al.*, 2011b; a). Several species of Metatheria appear to have evolved extremely efficient mechanisms to transport sperm to the fertilization site (Taggart *et al.*, 2003), measures of sperm protection (such as the ability to store sperm in specialized crypts of the oviductal epithelium) (Taggart *et al.*, 1998), and the formation of sperm pairs (which appear to enhance velocity and/or protect the acrosomes) (Bedford *et al.*, 1984). Consequently, the proportion of ejaculated sperm reaching the oviduct is up to four orders of magnitude higher than in some Eutheria (Taggart *et al.*, 2003). Additionally, Metatheria have approximately 40% lower testes mass in relation to their body mass than Eutheria (Tourmente *et al.*, 2011a). These particular physiological characteristics (more efficient transport, high sperm survival rate, and sperm storage in the female reproductive tract) may have resulted in a lower selective pressure on the production of high sperm numbers in Metatheria when compared to Eutheria, which may explain the comparatively lower relative testes mass and sperm numbers in the former.

Conclusions

While we found some relationships that matched the prediction of the raffle model of sperm competition (Parker *et al.*, 2010) regarding the relationships between sperm numbers and size in mammals, we did not find evidence of a tradeoff between numbers and size when controlling for sperm competition levels. These relationships between sperm traits may be explained, in the absence of a tradeoff, as the product of two main features of mammalian reproductive physiology: (1) The number and size of spermatozoa are regulated by different molecular and cellular mechanisms underlying sperm proliferation and differentiation, which are subjected to different energetic budgets and constraints. (2) The extraordinary variation in the scaling between the volumes of the ejaculate and the female reproductive tract, and the extreme reduction in sperm numbers at the fertilization site make increasing sperm numbers and sperm size (through its influence on sperm velocity) adaptive advantages in different stages of fertilization. In this way, increased sperm numbers would be more important at the levels of the vagina and uterus, where sperm are likely to be diluted in a large environment, while increased sperm size would be key in the oviduct, where there are only a few sperm competing to reach the oocyte. These findings demand for revised or more detailed hypotheses in order to elucidate the relationship between sperm traits in a sperm competition context. Increased knowledge of the general and reproductive physiology of different taxa should be taken into account to perform general predictions regarding the evolutionary effects of sperm competition on sperm phenotype. In particular, physiological variables such as mass-specific metabolic rate, timing, duration and efficiency of spermatogenesis in general (and their different stages), accurate measures of the relative scaling of female reproductive tract and sperm:egg ratio at the site of fertilization, and effects of sperm design and physiology on sperm performance will be of foremost significance when predicting the coevolution of sperm numbers and sperm size.

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Table 1. Relation between sperm numbers, relative testes size and total sperm size in mammals. Phylogenetically controlled multiple regression analyses (PGLS). Superscripts following the λ value indicate significance levels (n.s. $p>0.05$; $*p<0.05$) in likelihood ratio tests against models with $\lambda = 0$ (first position) and $\lambda = 1$ (second position). Effect size r calculated from the t values and the non-central 95% confidence limits (CLs) for the z-transformed value of r are presented. Confidence intervals excluding 0 indicate statistically significant relationships. P-values and CL that indicate statistical significance are shown in bold. All variables were $\log_{10}$-transformed. n: number of species.

Clade	Dependent variable	Independent variable	Slope	R^2	p	t	λ	r	CL(-)	CL(+)
Eutheria	Sperm numbers	Body mass	-0.7318	0.72	**<0.0001**	-5.0570	$0.999^{*,ns}$	-0.5435	**-0.8601**	**-0.3582**
(n=64)		Testes mass	1.8320		**<0.0001**	11.1430		0.8189	**0.9025**	**1.4044**
Metatheria	Sperm numbers	Body mass	-0.0959	0.88	0.7866	-0.2775	$0.001^{ns,ns}$	-0.0834	-0.6745	0.5074
(n=14)		Testes mass	1.4944		**0.0214**	2.6792		0.6284	**0.1478**	**1.3297**
Eutheria	Sperm numbers	Body mass	0.5710	0.25	**0.0003**	3.8675	$0.988^{*,ns}$	0.4438	**0.2259**	**0.7279**
(n=64)		Total sperm length	2.5975		**0.0132**	2.5543		0.3108	**0.0705**	**0.5724**
Metatheria	Sperm numbers	Body mass	0.5837	0.81	0.0640	2.0584	$0.001^{ns,ns}$	0.5273	-0.0045	1.1774
(n=14)		Total sperm length	-1.4523		0.4305	-0.8183		-0.2395	-0.8353	0.3467
Eutheria	Sperm numbers	Body mass	-0.6853	0.73	**<0.0001**	-4.5779	$0.999^{*,ns}$	-0.5088	**-0.8120**	**-0.3101**
(n=64)		Testes mass	1.7769		**<0.0001**	10.4166		0.8025	**0.8545**	**1.3564**
		Total sperm length	0.7466		0.2481	1.1663		0.1489	-0.1009	0.4010
Metatheria	Sperm numbers	Body mass	-0.4323	0.90	0.3138	-1.0606	$0.001^{ns,ns}$	-0.3180	-0.9204	0.2616
(n=14)		Testes mass	1.5858		**0.0146**	2.9477		0.6819	**0.2416**	**1.4235**
		Total sperm length	-1.9384		0.1879	-1.4134		-0.4081	-1.0242	0.1577

Table 2. Relation between sperm investment and relative testes size in mammals. Phylogenetically controlled multiple regression analyses (PGLS). Superscripts following the λ value indicate significance levels (n.s. $p>0.05$; $*p<0.05$) in likelihood ratio tests against models with $\lambda = 0$ (first position) and $\lambda = 1$ (second position). Effect size *r* calculated from the *t* values and the non-central 95% confidence limits (CLs) for the *z*-transformed value of *r* are presented. Confidence intervals excluding 0 indicate statistically significant relationships. *P*-values and CL that indicate statistical significance are shown in bold. All variables were $\log_{10}$-transformed. n: number of species.

Clade	Dependent variable	Independent variable	Slope	R^2	p	t	λ	r	CL(-)	CL(+)
Eutheria	Total sperm investment	Body mass	-2.0393	0.63	**<0.0001**	-5.2897	$0.980^{*,\mathrm{ns}}$	-0.5608	**-0.8913**	**-0.3766**
		Testes mass	4.2121		**<0.0001**	9.5670		0.7746	**0.7745**	**1.2892**
	Relative investment in sperm size	Body mass	0.0170	0.62	**0.0014**	3.3493	$0.999^{*,\mathrm{ns}}$	0.3941	**0.1593**	**0.6740**
		Testes mass	-0.0489		**<0.0001**	-8.4737		-0.7353	**-1.1975**	**-0.6828**
Metatheria	Total sperm investment	Body mass	-1.2102	0.57	0.0536	-2.1612	$0.999^{\mathrm{ns},\mathrm{ns}}$	-0.5459	-1.3056	0.0804
		Testes mass	2.9936		**0.0057**	3.4177		0.7176	**0.2098**	**1.5957**
	Relative investment in sperm size	Body mass	-0.0290	0.90	0.1669	-1.4801	$0.001^{\mathrm{ns},*}$	-0.4075	-1.1256	0.2603
		Testes mass	-0.0398		0.2352	-1.2557		-0.3541	-1.0631	0.3229

FIGURE LEGENDS

Figure 1. Relationships between sperm numbers and sperm size in mammals. Figure points are partial residuals estimated from multiple regression analysis using sperm numbers as dependent variable. A, B: body mass and total sperm length used as predictors. C, D: body mass, testes mass, and total sperm length used as predictors. All variables were $\log_{10}$-transformed. A, C: Eutheria. B, D: Metatheria.

Figure 2. Relationships between sperm investment and relative testes size in mammals. Figure points are partial residuals estimated from multiple regression analysis using total sperm investment (sperm size * sperm numbers) (A, B), or relative investment in sperm size (sperm size / numbers) (C, D) as dependent variable, and body mass and testes mass as predictors. All variables were $\log_{10}$-transformed. A, C: Eutheria. B, D: Metatheria.

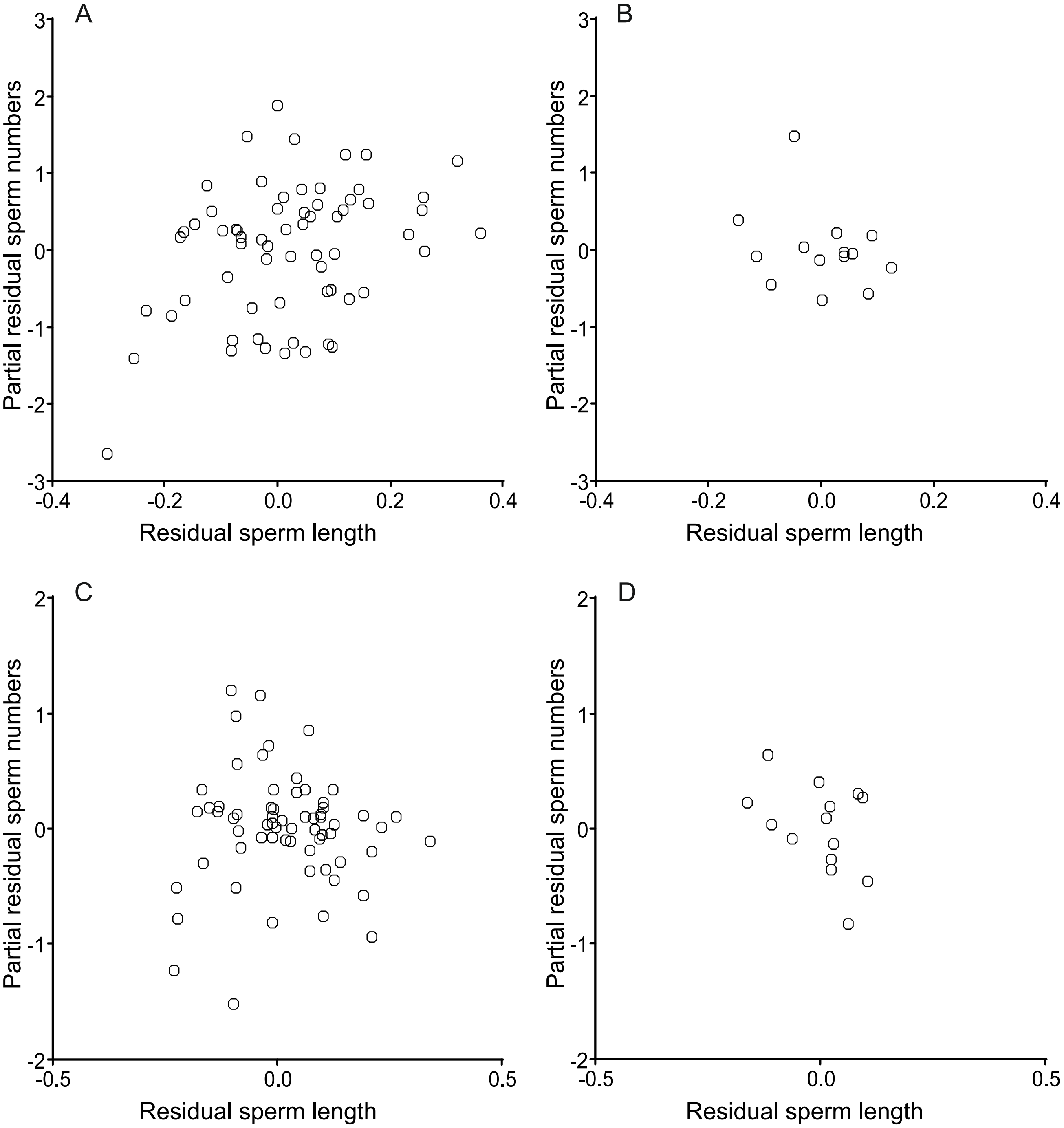
A
B
C
D
Partial residual sperm numbers
Residual sperm length
3
2
1
0
-1
-2
-3
-0.4
-0.2
0.0
0.2
0.4
-0.5
0.0
0.5

Figure 1

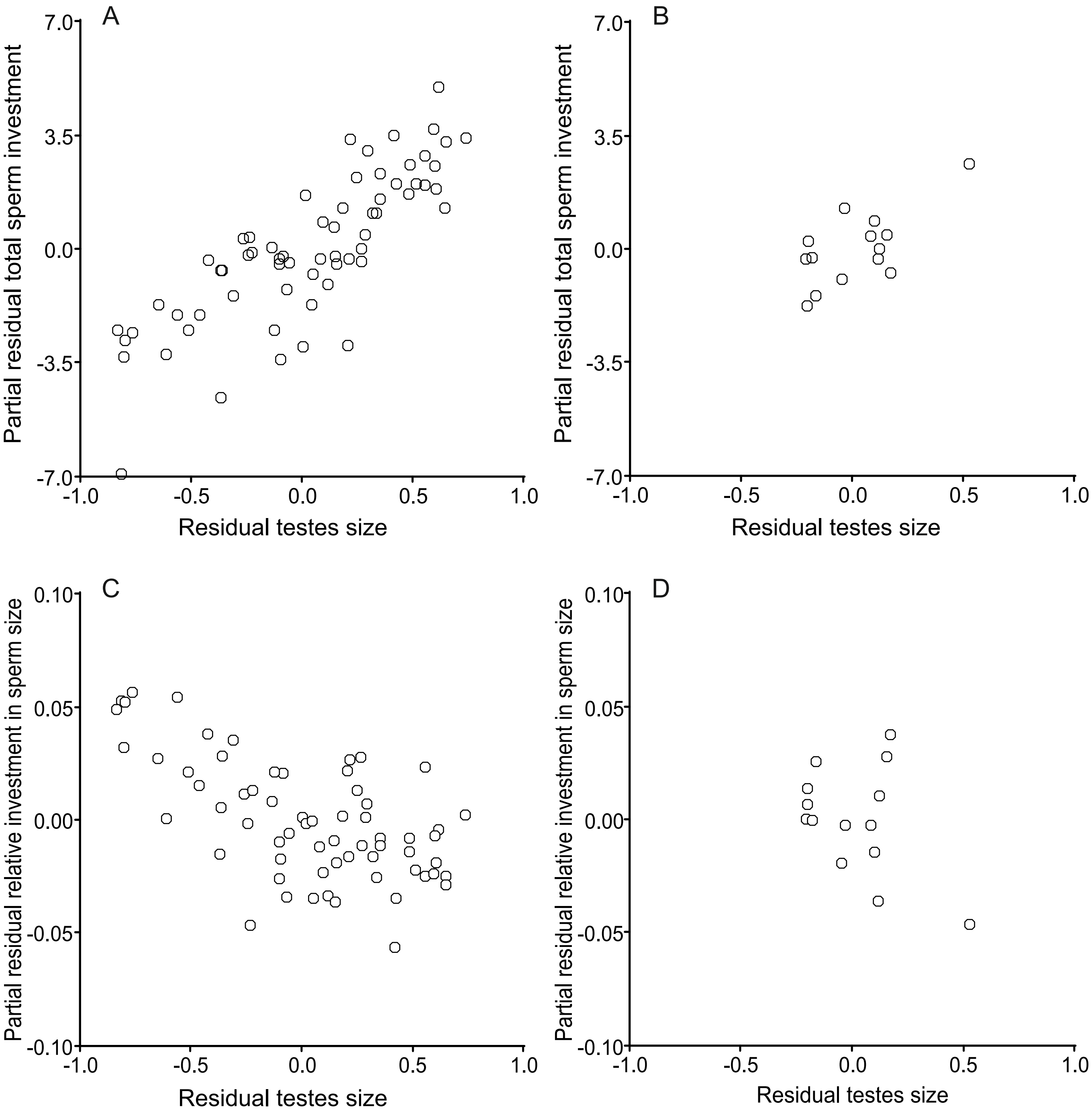
A
B
C
D
Partial residual total sperm investment
Partial residual relative investment in sperm size
Residual testes size
7.0
3.5
0.0
-3.5
-7.0
0.10
0.05
0.00
-0.05
-0.10
-1.0
-0.5
0.0
0.5
1.0

Figure 2

No evidence of tradeoffs in the evolution of sperm numbers and sperm size in mammals

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Supporting Information

Table S1. Sperm size, sperm numbers, body mass, and testes mass in 78 species of mammals.

Table S2. Alternative models for the relation between sperm numbers, sperm size and relative testes size in mammals.

Figure S1. Phylogenetic reconstruction for the 78 mammal species utilized in the PGLS analysis.

Table S1. Sperm size, sperm numbers, body mass, and testes mass in 78 species of mammals. Abbreviations: TSL: total sperm length (μm). SN: sperm numbers in caudae epididimydes (x 10^6). BM: body mass (g). TM: testes mass (g).

Species	Clade	Order	Family	TSL	SN	BM	TM	Ref. TSL	Ref. SN	Ref. BM & TM
Bos taurus	Eutheria	Artiodactyla	Bovidae	53.53	51775.00	680385.00	681.00	(1, 2)	(22, 23)	(69)
Bubalus bubalis	Eutheria	Artiodactyla	Bovidae	69.35	6545.45	680000.00	652.00	(3)	(3, 24)	(70)
Capra hircus	Eutheria	Artiodactyla	Bovidae	59.39	23680.00	25420.00	156.80	(1)	(25)	(71)
Connochaetes taurinus	Eutheria	Artiodactyla	Bovidae	46.77	24882.00	227000.00	306.00	(1)	(26)	(13)
Ovis aries	Eutheria	Artiodactyla	Bovidae	64.70	145500.00	57172.73	222.99	(1, 2)	(27, 28)	(71)
Taurotragus oryx	Eutheria	Artiodactyla	Bovidae	65.31	5700.00	408500.00	203.20	(4, 5)	(29)	(29)
Camelus dromedarius	Eutheria	Artiodactyla	Camelidae	47.21	2197.40	800000.00	121.70	(1, 2)	(30)	(71)
Odocoileus virginianus	Eutheria	Artiodactyla	Cervidae	52.00	8130.00	44800.00	85.50	(1, 5)	(31)	(69)
Hippopotamus amphibius	Eutheria	Artiodactyla	Hippopotamidae	33.49	11030.00	1600000.00	650.00	(1, 5)	(32)	(13)
Sus scrofa	Eutheria	Artiodactyla	Suidae	54.60	124733.33	39700.00	128.20	(1, 2)	(27, 28, 33)	(72)
Canis familiaris	Eutheria	Carnivora	Canidae	61.40	2119.00	21620.00	27.66	(4, 6)	(34, 35)	(35)
Mustela vison	Eutheria	Carnivora	Mustelidae	43.00	100.00	2387.60	5.40	(1)	(36)	(73, 74)
Ursus arctos	Eutheria	Carnivora	Ursidae	70.35	640.00	180900.00	68.60	(4, 2, 7)	(37)	(75)
Lepus californicus	Eutheria	Lagomorpha	Leporidae	50.32	1042.00	2250.00	8.48	(8)	(8)	(8)
Oryctolagus cuniculus	Eutheria	Lagomorpha	Leporidae	58.02	1286.00	2888.00	6.06	(1)	(8, 27, 34, 38)	(69)
Romerolagus diazi	Eutheria	Lagomorpha	Leporidae	54.61	310.00	486.00	1.88	(8)	(8)	(8)
Elephantulus myurus	Eutheria	Macroscelidea	Macroscelididae	76.60	2.59	60.00	0.08	(2)	(39, 40)	(40)
Equus asinus	Eutheria	Perissodactyla	Equidae	64.15	60935.00	290000.00	202.34	(5, 9)	(41)	(71)
Equus caballus	Eutheria	Perissodactyla	Equidae	60.60	59487.67	468000.00	416.00	(1, 2)	(27, 28, 41)	(69)
Macaca mulatta	Eutheria	Primates	Cercopitecidae	74.10	10358.00	10430.00	76.00	(1, 4)	(42)	(69)
Homo sapiens	Eutheria	Primates	Hominidae	56.90	304.64	63540.00	50.20	(4)	(34, 43)	(69)
Chionomys nivalis	Eutheria	Rodentia	Arvicolidae	105.23	129.40	43.64	0.85	(10)	(44)	(44)
Microtus arvalis	Eutheria	Rodentia	Arvicolidae	91.66	41.60	36.40	0.28	(10)	(44)	(44)
Microtus cabrerae	Eutheria	Rodentia	Arvicolidae	85.12	7.60	44.27	0.14	(10)	(44)	(44)
Microtus duodecimcostatus	Eutheria	Rodentia	Arvicolidae	62.69	4.50	29.76	0.08	(10)	(44)	(44)
Microtus lusitanicus	Eutheria	Rodentia	Arvicolidae	86.02	27.70	17.73	0.09	(10)	(44)	(44)
Microtus ochrogaster	Eutheria	Rodentia	Arvicolidae	94.40	149.89	45.40	0.52	(11)	(45)	(76)
Myodes glareolus	Eutheria	Rodentia	Arvicolidae	83.91	43.20	25.65	0.40	(10)	(44)	(44)
Fukomys damarensis	Eutheria	Rodentia	Bathyergidae	42.90	0.26	194.50	0.18	(12)	(46)	(46)
Mesocricetus auratus	Eutheria	Rodentia	Cricetidae	186.70	1130.00	108.00	3.17	(13)	(27, 28, 42)	(69)

Peromyscus californicus	Eutheria	Rodentia	Cricetidae	72.00	248.00	34.20	0.20	(1)	(47)	(77)
Peromyscus leucopus	Eutheria	Rodentia	Cricetidae	74.80	91.17	24.21	0.38	(1)	(48)	(77, 78)
Ctenomys talarum	Eutheria	Rodentia	Ctenomyidae	49.28	3.22	118.00	0.35	(11)	(49)	(49)
Apodemus sylvaticus	Eutheria	Rodentia	Muridae	126.15	110.10	30.43	0.96	(10)	(44)	(44)
Conilurus penicillatus	Eutheria	Rodentia	Muridae	124.00	1908.00	184.00	4.39	(1)	(50)	(79)
Hydromys chrysogaster	Eutheria	Rodentia	Muridae	115.00	1156.00	745.00	11.77	(1)	(50)	(79)
Melomys littoralis	Eutheria	Rodentia	Muridae	110.00	333.00	61.00	1.68	(1)	(50)	(2)
Mus musculus musculus	Eutheria	Rodentia	Muridae	124.64	23.20	21.13	0.13	(10)	(44)	(44)
Mus pahari	Eutheria	Rodentia	Muridae	137.56	9.10	30.08	0.12	(14)	(44)	(44)
Mus spicilegus	Eutheria	Rodentia	Muridae	103.54	99.40	14.49	0.41	(10)	(44)	(44)
Mus spretus	Eutheria	Rodentia	Muridae	111.49	48.00	17.01	0.29	(10)	(44)	(44)
Notomys alexis	Eutheria	Rodentia	Muridae	102.50	2.23	34.00	0.05	(1)	(51-53)	(69)
Notomys cervinus	Eutheria	Rodentia	Muridae	115.00	21.40	33.00	0.20	(1)	(53)	(69)
Notomys fuscus	Eutheria	Rodentia	Muridae	106.00	2.00	44.00	0.07	(1)	(53)	(69)
Notomys mitchelli	Eutheria	Rodentia	Muridae	98.00	1.80	39.00	0.06	(1)	(53)	(69)
Pseudomys apodemoides	Eutheria	Rodentia	Muridae	120.00	10.00	31.00	0.14	(1)	(53)	(69)
Pseudomys australis	Eutheria	Rodentia	Muridae	122.50	900.65	59.00	2.24	(1)	(51-53)	(69)
Pseudomys hermannsburgensis	Eutheria	Rodentia	Muridae	117.00	20.00	18.00	0.16	(1)	(53)	(69)
Pseudomys nanus	Eutheria	Rodentia	Muridae	127.00	387.00	78.00	1.87	(1)	(53)	(69)
Pseudomys shortridgei	Eutheria	Rodentia	Muridae	96.00	51.00	78.00	0.35	1, 2	(53)	(69)
Rattus colletti	Eutheria	Rodentia	Muridae	158.00	323.00	154.00	1.94	(1)	(50)	(79)
Rattus fuscipes	Eutheria	Rodentia	Muridae	162.00	387.00	110.00	4.26	(1)	(50)	(16)
Rattus norvegicus	Eutheria	Rodentia	Muridae	189.40	303.68	379.63	3.06	(1)	(54-58)	(57, 69, 80-82)
Rattus tunneyi	Eutheria	Rodentia	Muridae	155.00	130.00	243.00	4.87	(15)	(50)	(50)
Rattus villosissimus	Eutheria	Rodentia	Muridae	147.00	195.00	207.00	2.15	(16)	(50)	(50)
Rhabdomys pumilio	Eutheria	Rodentia	Muridae	117.00	161.00	70.33	1.28	(1)	(59)	(59)
Uromys caudimaculatus	Eutheria	Rodentia	Muridae	106.00	1342.00	820.00	7.20	(1, 2)	(50)	(2)
Tupaia belangeri	Eutheria	Scadentia	Tupaiidae	73.05	6.32	141.00	1.49	(17)	(17)	(17)
Blarina brevicauda	Eutheria	Soricomorpha	Soricidae	70.00	60.00	24.00	0.30	(2)	(60)	(60)
Crocidura russula	Eutheria	Soricomorpha	Soricidae	102.70	3.89	13.40	0.04	(18)	(61)	(18)
Neomys fodiens	Eutheria	Soricomorpha	Soricidae	93.90	30.10	16.70	0.25	(18)	(61)	(18)
Sorex araneus	Eutheria	Soricomorpha	Soricidae	83.90	7.42	10.90	0.17	(18)	(61, 62)	(18)
Suncus murinus	Eutheria	Soricomorpha	Soricidae	113.30	3.30	66.10	0.15	(18, 19)	(61)	(69)
Talpa europaea	Eutheria	Soricomorpha	Talpidae	90.00	5292.00	92.31	1.80	(20)	(60)	(60)

Antechinus stuartii	Metatheria	Dasyuromorpha	Dasyuridae	271.10	2.07	40.00	0.64	(1)	(23, 63)	(65)
Dasyuroides byrnei	Metatheria	Dasyuromorpha	Dasyuridae	254.80	1.70	123.60	1.38	(1)	(23, 64)	(65)
Sminthopsis crassicaudata	Metatheria	Dasyuromorpha	Dasyuridae	264.90	1.22	14.60	0.17	(2)	(65)	(65)
Monodelphis domestica	Metatheria	Didelphiomorpha	Didelphidae	211.90	4.20	110.00	0.57	(2)	(65)	(65)
Macropus eugenii	Metatheria	Diprotodontia	Macropodidae	106.40	4851.00	7050.00	32.90	(1)	(66)	(83)
Macropus rufus	Metatheria	Diprotodontia	Macropodidae	123.90	388.00	39825.00	38.18	(1)	(64)	(63)
Wallabia bicolor	Metatheria	Diprotodontia	Macropodidae	109.50	482.00	31500.00	14.75	(1)	(23, 64)	(63)
Trichosurus vulpecula	Metatheria	Diprotodontia	Phalangeridae	94.17	218.33	3350.00	8.26	(1)	(23, 63, 64)	(63)
Aepyprymnus rufescens	Metatheria	Diprotodontia	Potoroidae	106.40	57.80	2400.00	4.68	(21)	(63)	(63)
Potorous tridactylus	Metatheria	Diprotodontia	Potoroidae	165.80	38.80	1280.00	4.38	(21)	(63)	(63)
Lasiorhinus latifrons	Metatheria	Diprotodontia	Vombatidae	79.50	177.33	28290.00	13.80	(1)	(23, 64, 67)	(63, 84)
Vombatus ursinus	Metatheria	Diprotodontia	Vombatidae	93.60	146.50	40100.00	18.42	(1)	(68)	(63)
Isoodon macrourus	Metatheria	Peramelemorphia	Peramelidae	171.10	101.70	2300.00	4.72	(1)	(23, 64, 63)	(63)
Isoodon obesulus	Metatheria	Peramelemorphia	Peramelidae	167.00	56.00	978.30	3.88	(1)	(64)	(63)

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Table S2. Alternative models for the relation between sperm numbers, sperm size and relative testes size in mammals. Phylogenetically controlled multiple regression analyses (PGLS). Superscripts following the λ value indicate significance levels (n.s. $p>0.05$; $*p<0.05$) in likelihood ratio tests against models with $\lambda = 0$ (first position) and $\lambda = 1$ (second position). Effect size r calculated from the t values and the non-central 95% confidence limits (CLs) for the z-transformed value of r are presented. Confidence intervals excluding 0 indicate statistically significant relationships. P-values and CL that indicate statistical significance are shown in bold. All variables were $\log_{10}$-transformed. n: number of species. Testes mass^2: quadratic term of testes mass. Body mass * Testes mass: interaction between body mass and testes mass.

Clade	Dependent variable	Independent variable	Slope	R^2	p	t	λ	r	CL(-)	CL(+)
Eutheria	Sperm numbers	Body mass	-0.7417	0.73	**0.0005**	-3.6646	$0.999^{*,ns}$	-0.4277	**-0.7080**	**-0.2061**
(n=64)		Testes mass	2201.8000		0.2152	1.2528		0.1597	-0.0899	0.4120
		Testes mass^2	-1100.0000		0.2156	-1.2517		-0.1595	-0.4119	0.0901
		Body mass * Testes mass	-0.0003		0.9958	-0.0053		-0.0007	-0.2516	0.2503
	Sperm numbers	Body mass	-0.7066	0.72	**0.0009**	-3.5087	$0.999^{*,ns}$	-0.4126	**-0.6897**	**-0.1878**
		Testes mass	1.8501		**<0.0001**	9.5596		0.7770	**0.7867**	**1.2886**
		Body mass * Testes mass	-0.0118		0.8566	-0.1815		-0.0234	-0.2744	0.2275
	Sperm size	Body mass	-0.0432	0.09	0.2934	-1.0601	$0.999^{*,ns}$	-0.1356	-0.3874	0.1145
		Testes mass	-42.2989		0.9053	-0.1195		-0.0154	-0.2664	0.2355
		Testes mass^2	21.1929		0.9051	0.1198		0.0155	-0.2355	0.2664
		Body mass * Testes mass	-0.0088		0.5041	-0.6722		-0.0865	-0.3376	0.1643
	Sperm size	Body mass	-0.0439	0.09	0.2773	-1.0963	$0.980^{*,ns}$	-0.1401	-0.3920	0.1099
		Testes mass	0.0870		**0.0274**	2.2617		0.2803	**0.0370**	**0.5389**
		Body mass * Testes mass	-0.0086		0.5070	-0.6675		-0.0859	-0.3370	0.1649
Metatheria	Sperm numbers	Body mass	-0.1076	0.88	0.8080	-0.2502	$<0.001^{ns,*}$	-0.0789	-0.6700	0.5119
(n=14)		Testes mass	284.2800		0.9413	0.0757		0.0239	-0.5670	0.6149
		Testes mass^2	-141.3900		0.9416	-0.0753		-0.0238	-0.6148	0.5672
		Body mass * Testes mass	0.0073		0.9690	0.0400		0.0126	-0.5783	0.6036
	Sperm numbers	Body mass	-0.1033	0.88	0.8036	-0.2554	$<0.001^{ns,*}$	-0.0805	-0.6716	0.5103
		Testes mass	1.4800		0.0548	2.1746		0.5666	0.0516	1.2335
		Body mass * Testes mass	0.0072		0.9678	0.0414		0.0131	-0.5779	0.6041
	Sperm size	Body mass	-0.1797	0.84	0.0740	-2.0212	$<0.001^{ns,*}$	-0.5386	-1.1931	-0.0112
		Testes mass	-385.2988		0.6317	-0.4961		-0.1550	-0.7472	0.4347
		Testes mass^2	192.6532		0.6317	0.4961		0.1550	-0.4347	0.7472
		Body mass * Testes mass	0.0115		0.7691	0.3026		0.0953	-0.4954	0.6865
	Sperm size	Body mass	-0.1856	0.83	0.0533	-2.1899	$<0.001^{ns,*}$	-0.5693	-1.2375	-0.0556
		Testes mass	0.0239		0.8702	0.1677		0.0530	-0.5380	0.6440
		Body mass * Testes mass	0.0116		0.7561	0.3193		0.1005	-0.4902	0.6918

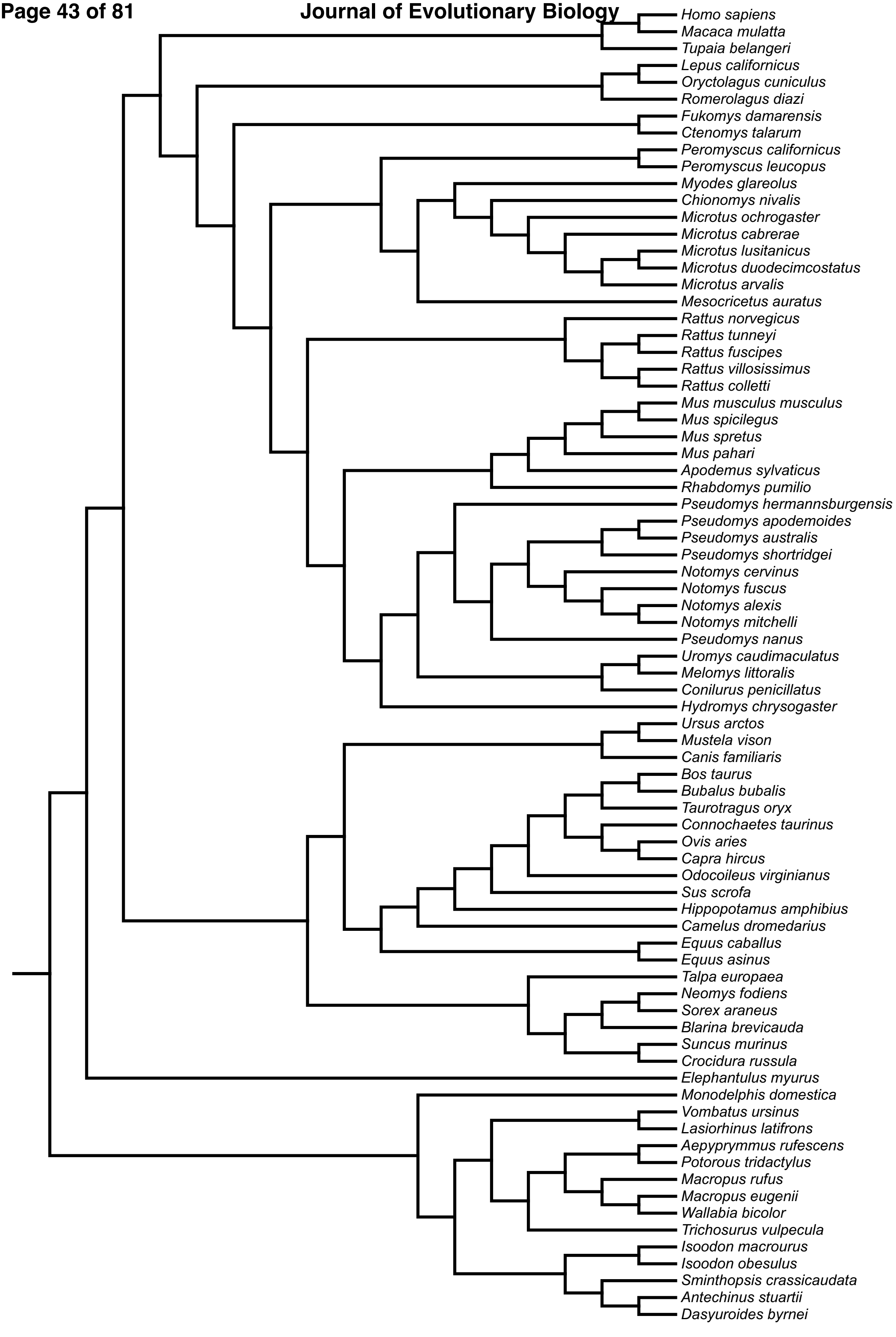

Figure S1. Phylogenetic reconstruction for the 78 mammal species utilized in the PGLS analysis.

Figure S1