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REVIEW

Size is not everything: Nuanced effects of female multiple mating and annual litter number on testes size in terrestrial mammals

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Abstract

Sperm production represents a costly reproductive investment by males. High reproductive competition within the female reproductive tract may select for higher sperm counts or quality resulting in selection for larger testes size. In species where females mate multiply or have more offspring per litter (litter size), or more litters per year (litter rate), male reproductive competition may select for larger relative testes size (i.e., scaled by body mass). Given that different mating systems vary in the alternative forms of reproductive investment available to males, sperm production levels may vary with social system. Here, we examined the relationship between testes size and mating systems, litter size, and litter rate while considering male lifespan and investment in paternal care in 224 terrestrial mammalian species in 15 orders. Relative testes size was larger in species where females mated with multiple males. Furthermore, in species with multiple mating females, species with higher litter rates had larger testes compared to species with fewer litters per year. In contrast, in monogamous species, species that had multiple litters per year had smaller relative testes sizes compared to species with fewer litters per year. Neither longevity nor paternal care influenced testes size. Our results elucidate the effect of female reproductive strategies on relative testes size is nuanced and varies between mating systems. Our findings suggest that the interplay between male reproductive investment and female reproductive investment may be different within similar social mating systems.

Introduction

Sperm competition has received a lot of attention (reviewed in Simmons & Wedell, 2020) since the foundational work of Parker (1970). Sperm production represents a form of reproductive investment by males (Parker, 2020). Larger testes are associated with enhanced fertilization as a result of an increased number of sperm per insemination, and larger accessory glands are associated with ejaculate features that may enhance the performance and quality of sperm (Parker, 2020). In cases of high reproductive competition within the female reproductive tract, such as in species where females mate with multiple males, selection for higher sperm count or quality may exist (Parker, 2020). Thus, relative testes mass (i.e., scaled by body mass) has been used as an indicator of the intensity of sperm competition (Balshine et al., 2001; Gage & Freckleton, 2003; Parker et al., 1997; Simmons & Fitzpatrick, 2012) and is validated as a proxy for sperm competition (Lüpold et al., 2020; Rowley et al., 2019). Indeed, larger relative testes size is often associated with genetic and social mating systems in which females have multiple mates, such as in promiscuous, polyandrous, and polygynandrous mating systems (Baker et al., 2020; Ramm et al., 2005; Soulsbury, 2010). Furthermore, testes size varies with mating season length (Iossa et al., 2008; Soulsbury, 2010), reproductive lifespan (Iossa et al., 2008), ovulation mode (Iossa et al., 2008; Soulsbury, 2010), and other forms of paternal investment such as sexual ornaments and weaponry (Fitzpatrick et al., 2012; Lüpold et al., 2019) and parental care (Pitcher et al., 2005), demonstrating that the evolution of testes size is complex and driven by environmental and social factors.

Large testes sizes resulting in high sperm production levels may be advantageous when fertilization success is determined according to a ‘raffle principle’ (Parker et al., 1997). However, ejaculates are costly to produce (Dewsbury, 1982). As the number of mates a female has affects the degree of reproductive competition between males, the advantages of higher levels of sperm production may vary with mating systems (Soulsbury, 2010). As social systems may influence the opportunity for males to employ alternative forms of reproductive investment, sperm production levels may vary with social system. For example, in animals that live in pairs or groups, males may have the opportunity to contribute towards parental care, which may increase the survival of their offspring (Rymer & Pillay, 2018), and in species where females live in groups, males might have the opportunity to employ pre-copulatory strategies such as competing for territories and investing in weaponry, which may increase access to females (Lüpold et al., 2014). In some mating and social systems, sperm competition may be an important male investment because males are not able to employ other reproductive strategies (Lüpold et al., 2014).

For females, the number and mass of each offspring in one reproductive event (e.g., litter, clutch) represent resources allocated towards reproduction by females at a given time (Sibly et al., 2012). Single breeding opportunities, small litter size, and/or offspring mass may represent a limitation to the amount of resources that a female could allocate, which may be offset by the care of males (West & Capellini, 2016). For example, in mammalian species with paternal care, lactation time is shorter for females, the relative number of litters per year is higher, and the relative litter size is larger when males provision females, providing more opportunities to sire offspring (West & Capellini, 2016).

To males, litter size represents the number of offspring that a male could sire at a given copulation event (oestrus, female receptive period), and the total annual number of offspring represents offspring that could be sired over the course of the breeding season. An individual's investment in reproduction (e.g., testes size) may be limited by the need to invest resources into growth and maintenance (van Noordwijk & de Jong, 1986) or other 'expensive' tissues or activities (Isler & van Schaik, 2006) necessary for their life history. One strategy to maintain 'expensive' tissues or activities, is to limit the investment (e.g., energy) in those tissues/activities to particular periods. For example, as migratory birds have high energetic requirements associated with reproduction, plumage growth (i.e., feather moults), and migration, these key events occur at different periods throughout the year (Dawson, 2008). Therefore, it is important to consider investment within differing time scales when studying the evolution of reproductive investment (e.g., testes size).

The amount of resources that a female allocates towards reproduction may directly affect male fitness because this investment represents a male's opportunity to sire offspring. However, few studies have examined the relationship between male reproductive investment strategies and female reproductive strategies (Soulsbury, 2010). Soulsbury (2010) found a positive association between litter size and testes size. However, testes size also increased in mating systems with multiple paternity suggesting that the relationship between testes mass and litter size may differ between mating systems.

Mammals are excellent species for examining the association between male reproductive investment and female reproductive investment because of the variety of mating systems and social characteristics they exhibit. In mammals, testes size (Iossa et al., 2008; Soulsbury, 2010) and litter size vary (1–8 offspring) with smaller species (e.g., rodents) typically having at least two offspring per gestation (Carranza, 1996) and larger species having fewer offspring (Soulsbury, 2010). Mammals also vary in the reproductive strategies and parental care exhibited by males and females (Heldstab et al., 2019; Lukas & Clutton-Brock, 2013; Lukas & Huchard, 2019; Sinervo et al., 2020; West & Capellini, 2016).

We compiled the largest and most detailed dataset of relative testes size (RTS) in mammals until now, and using this dataset, we examined the association between RTS (male reproductive investment) and two litter characteristics representing female reproductive investment (litter size and litters per year). Additionally, we also examined the association between RTS and longevity, mating system, and paternal care. We hypothesized that the relationship between RTS and female reproductive output would differ between mammal species exhibiting different mating systems. Specifically, in species where females mate with multiple males per breeding attempt, we predicted larger RTS with smaller litter sizes and fewer total annual offspring, because smaller litters represent more intense competition due to fewer potential offspring available to be sired. In species where females mate with a single male per breeding attempt, we predicted larger RTS with larger litter sizes and greater numbers of total annual offspring, despite less competition with other males, because males would have an increased need to fertilize eggs. Also, to maximize fitness, males should invest in sperm production regardless of the number of breeding opportunities, so we predicted that RTS would not be associated with the number of litters/year. Given that longevity increases the time males have to obtain paternity and thus may represent opportunities to trade-off survival with reproduction (Stearns, 1992), we predicted that longer-lived species may have on average smaller RTS compared to shorter-lived animals. Furthermore, as paternal care represents a form of post-copulatory reproductive investment, in species with paternal care, males may be selected for traits indicative of the quality of caregiving. Thus, we predicted that RTS would be inversely related to the level of parental care; species that give more parental care will have smaller testes than species that give less parental care.

Materials and methods

Data collection

We obtained testes and body mass data from different sources (Breed & Taylor, 2000; Gage & Freckleton, 2003; Soulsbury, 2010). We also included the testes size data of squirrels from our own compiled dataset (see the data file on the GitHub repository for references, van der Marel et al., 2023). If multiple sources provided different testes size data for a species, we included both sources as a data point. We excluded mammalian orders that are completely aquatic, that is, Cetacea and Sirenia.

We obtained life history trait data, litter size, litter rate (number of litters per year), and longevity, for the terrestrial mammal species with testes size data from Capellini et al. (2015). The life history data were not complete; therefore, we included data from Myhrvold et al. (2015) or performed a literature search for the species in question (see the datafile on the GitHub repository for references, van der Marel et al., 2023).

For the mating system, we included data from Soulsbury (2010), Sinervo et al. (2020), and Baker et al. (2020). In the past, genetic mating systems have largely been defined by the number of mates that a male has obtained and have largely neglected the number of mates a female has obtained. However, an increasing number of molecular studies have found that some mammal mating systems that had been described as polygynous (e.g., dusky-footed woodrats, *Neotoma fuscipes*) have females that mate multiply or promiscuously (McEachern et al., 2009; Waterman, 2007). Indeed, promiscuous mating in mammals is quite common (Waterman, 2007; Wolff & Macdonald, 2004). As such, we have defined mating systems based on the number of mates that both the male and female have obtained. However, it is important to note that some studies define mating systems from the viewpoint of spatial relationships between individuals during the breeding season (e.g., territory range overlap, Lin et al., 2009); this definition is referred to as the social mating system. Thus, a species, or population of species, may be categorized as socially monogamous, even if the genetic evidence indicates that individuals mate with multiple partners (Clutton-Brock & Isvaran, 2006).

Therefore, we cross-referenced the mating system with our own literature search (see GitHub repository, van der Marel et al., 2023) to ensure that we defined the mating system based on genetic mating patterns. We classified the mating system as *monogamous*, one male, one female; *polygynous*, one male, multiple females; and *promiscuous*, multiple males, one or multiple females following Soulsbury (2010). If we had multiple mating systems for the same species from different sources, we considered them equally valid but only kept the recording of the mating system of the highest level of complexity following Baker et al. (2020). The order of complexity went from monogamy to polygyny, to promiscuity. To account for multiple mating of the females, we combined monogamous and polygynous mating systems as systems without multiple mating by the female and compared that to systems where females do mate multiply, that is, promiscuous mating systems.

We included paternal care data from Heldstab et al. (2019), Lukas and Huchard (2019), and Sinervo et al. (2020). Overall, all sources defined paternal care as help provided to the care of offspring (weaned or unweaned), which could constitute huddling, grooming or carrying offspring, and food provisioning. We excluded protection of the offspring as a form of paternal care as this behaviour could be confused with territorial behaviour or antipredator behaviour (West & Capellini, 2016) and is not considered to be energetically costly (Heldstab et al., 2019). Sinervo et al. (2020) included the protection of young as a form of paternal care and Lukas and Huchard (2019) were not clear in their description of whether they only included paternal care or also non-parental group members. As such, if we have paternal care data for the same species of different sources that did not correspond with each other, we followed Heldstab et al. (2019) followed by Lukas and Huchard (2019).

For the comparative analyses, we inferred the phylogenetic relatedness between species from the updated mammalian supertree (Upham et al., 2019). To represent phylogenetic uncertainty, we downloaded a sample of 100 phylogenetic trees of the selected species with testes size from http://vertlife.org/phylosubsets.

We obtained body mass and testes mass for 571 unique terrestrial mammal species. We found a slightly less than linear relationship between body mass and testes mass (Fig. S1), as found in previous studies (Hayward & Gillooly, 2011; Kenagy & Trombulak, 1986). Given that in 133 mammal species, males of smaller species had relatively larger testes than larger species (Kenagy & Trombulak, 1986), and smaller mammals have proportionally higher gonadal mass in relation to somatic mass in mammals (Hayward & Gillooly, 2011). We calculated relative testes size (RTS) following the equation $y = 0.035x^{0.72}$, where the mass of the testes is y and the body mass is x, as in Kenagy and Trombulak (1986). We note that this body mass-corrected testes size is the ratio of observed testes size to the testes size predicted by this equation (Kenagy & Trombulak, 1986). However, we believe this slightly less than linear relationship with body size may represent an ecological constraint to a minimum testis size, as seen in minimum sizes of expensive tissues such as brains (Quesada et al., 2011), and given the complex architecture of testes (Ramm & Schärer, 2014). Thus, accounting for the non-linear allometric relationship between testes and body mass may be important to consider.

Statistical analyses

We fit phylogenetically controlled multinomial generalized linear mixed models using R packages 'brms' v2.18.0 (Bürkner, 2017, 2018) in the Stan environment using the 'rstan' package v2.21.7 (Stan Development Team, 2022) in R version 4.1.2 (R Core Team, 2021). Figures were made using 'ggplot' (Wickham, 2016).

To examine the effect of environmental and social factors on testes size in mammals, we ran several models as follows:

(1) An intercept-only model (M1).
(2) Mixed effect model (M2) with species as a random effect to account for data non-independence (for some species, we had multiple observations of testes mass and body mass) and to account for the phylogenetic signal between species. Our global model included litter size, litters per year, longevity, paternal care, and mating system as predictor variables.
(3) Mixed effect model with added variability (M3) of the dependent variable within species (accounting for different measures of RTS per species) on one selected phylogenetic tree. However, this model did not converge; therefore, we did not include this analysis.
(4) An interaction model (M4), which included a three-way interaction between litter size, litters per year and mating system.

(5) In species that only have one offspring in a litter (monotocous species), we cannot detect the extent of female multimale mating (i.e., multiple paternity) and, thus, the level of post-copulatory selection (i.e., sperm competition). Therefore, models containing monotocous species may restrict our ability to detect a relationship between testes size and litter size. Thus, we also ran additional models on a subset of our data (M5): species with a litter size ≤1. As we only had one species with a litter size of one and a monogamous mating system, we used the dichotomous variable of whether females mate multiply as our dependent factor instead of using mating system. We excluded paternal care from these models as our dataset only included one species with paternal care. Similar to the above, we standardized litters per year and longevity and included species as a random effect. Our global model included litters/year, longevity, and female multiple mating (y/n) as predictor variables. Our interaction model included longevity and an interaction between litters/year and females multiple mating. We used the same model settings as before and ran the models over the 100 sampled phylogenetic trees.

(6) To account for the derived calculation of RTS, we also ran the interaction model (see M4) with RTS calculated as testes mass divided by body mass (M6) following Charlton and Reby (2016).

For each model, we checked for collinearity but none of our predictor variables had a variance inflation factor above 3, so we included all variables. We standardized the life history data by z-transformation. We looped the models over the 100 sampled phylogenetic trees. For our models, we used four chains, six cores, 5000 iterations, and 3000 as the warmup (burnin), and we set the ‘adapt_delta’ to 0.99 and the ‘max_treedepth’ to 15. We visually confirmed model convergence and assessed mixing by ensuring that the effective sample sizes for all parameters were above 400 and that the scale reduction factor was close to 1. We selected the model with the lowest loo information criterion (Vehtari et al., 2017). We also calculated the phylogenetic signal lambda for lognormal distribution for all models.

Results

We obtained RTS data for 571 mammalian species. However, values for all our predictor variables were available for 224 unique terrestrial mammalian species across 15 orders. The model with the lowest loo information criterion (LooIC) across models M1 to M4 was our three-way interaction model (M4, Table 1; see Supporting Information 2 for a full summary of the other models). Species with greater numbers of litters per year (Fig. 1, Table 2) and a promiscuous mating system (Fig. 2, Table 2) had larger RTS. We also found a significant interaction between litters per year and mating system (Fig. 3, Table 2). In species exhibiting polygynous or promiscuous mating systems, species that had more litters per year had larger RTS than species with fewer litters per year. We found the opposite trend for monogamous species: Species with a monogamous mating system, that had multiple litters per year, had smaller RTS than species with fewer litters per year. Our results showed weak phylogenetic signal across terrestrial mammals in both models (mean Pagel’s λ and 95% credible interval (CI) for interaction model = 0.19, 0.0–0.46; for global model = 0.23, 0.00–0.50).

We obtained values for our predictor variables for 88 unique terrestrial mammalian species which typically have only a single offspring per reproductive attempt (average litter size of ≤1) (Table 3). Our results were not fundamentally different from models using our full dataset (see Supporting Information 2 for a full summary). RTS was larger in species where females multiple mated, that is, promiscuous species (Table 4; Fig. 4). We also found a weak phylogenetic signal for both the global and interaction model across the 88 terrestrial mammals (mean Pagel’s λ and 95% CI = 0.29, 0.00–0.65, and 0.26 [0.00–0.60], respectively).

Finally, the results of the interaction model with RTS calculated as testes mass/body mass (M6) instead of a derived calculation did not differ from our original model (M4) results. Litter rate, a promiscuous mating system, and the interaction between litter rate and polygynous and promiscuous species influenced testes size (Table S5).

Discussion

We examined the effects of litter size, litter rate, longevity, mating system, and paternal care on relative testes size (an indicator of sperm competition intensity) in 224 unique terrestrial mammalian species across 15 orders. As hypothesized, we found that the effects of female reproductive strategies on testes size were nuanced and varied with the mating system, but the effects were different than predicted. In contrast to our predictions, we found that litter size did not affect RTS, while litter rate did affect RTS. Furthermore, we found no evidence for an inverse relationship between paternal care and testes size. Also, we found no relationship between longevity and testes size, indicating no trade-off in this form of reproductive investment nor in traits related to longevity such as life history (Stearns, 1992). The results were consistent between models

Table 1 Loo Information Criterion of the models describing relative testes size in 224 terrestrial mammals

Model	Predictor variables	LooIC mean (lwr95CI – upr95CI)	Number of species
Intercept-only	1	341.5 (337.9–345.2)	224
Global model	Litter size + litters/year + longevity + mating system + paternal care	330.8 (326.9–335.4)	224
Interaction model	Litter size × litters/year × mating system + longevity + paternal care	320.4 (316.6–324.8)	224

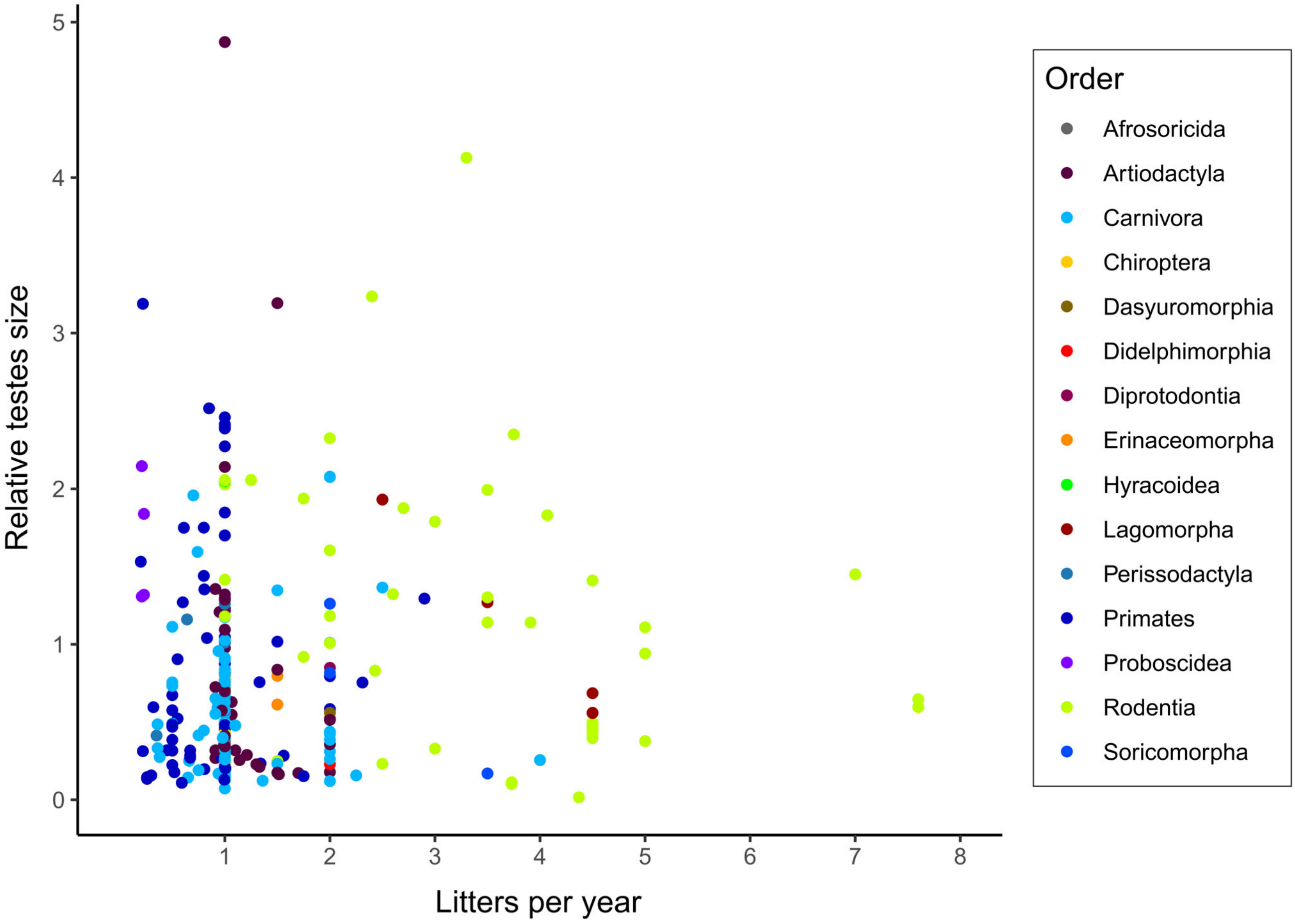

Figure 1 Relative testes size and litters per year for 224 terrestrial mammals. Colours represent species by order.

using all 224 species regardless of litter size and the subset of 88 species that typically have only a single offspring per reproductive attempt. Overall, our results highlight the importance of considering the effect of female reproductive strategies in light of the different mating systems influencing sperm competition intensity.

Our results are concordant with earlier studies that found larger RTS in birds (Baker et al., 2020) and mammals (Iossa et al., 2008; Kenagy & Trombulak, 1986; Soulsbury, 2010) in species where females mate with multiple males. In these mating systems, the sperm of multiple males compete to fertilize the female's eggs, which results in more intense sperm competition than in systems where only one male mates with the female. We note that sperm competition can still occur in monogamous systems as a result of within-ejaculate sperm competition (Sutter & Immler, 2020). However, there may be a mixed relationship between testes size and mating system when examining this relationship within particular lineages of animals. For example, no relationship between testes size and mating system in carnivore mammals exists (Iossa et al., 2008). However, Iossa et al. (2008) focused on social mating systems, but in carnivorous mammals, some species classed as socially monogamous have females that mate multiply (e.g., red foxes, Baker et al., 2004). Thus, the observed difference in the relationship between testes size and mating system observed in our study may be partially attributed to defining a mating system socially versus genetically.

Contrary to our prediction, we found a positive relationship between litter rate and RTS in promiscuous and polygynous species. This relationship may be possible in species with prolonged or continual breeding (e.g., tropical species, such as Cape ground squirrels, *Xerus inauris*; Waterman, 1996) because reproductive investment can be spread over several breeding opportunities, increasing the selective pressure to maintain sperm production continuously or over a prolonged period. For example, continually breeding Cape ground squirrels maintain large testes throughout the year and have among the largest testes in the Sciurids (Kenagy & Trombulak, 1986; Manjerovic et al., 2008). Indeed, mammal testes size was larger in species with longer mating seasons (Soulsbury, 2010). However, in carnivores, testes size was larger in species with shorter mating seasons, which is associated with higher female mating synchrony (Iossa et al., 2008), demonstrating that the effect of female reproductive features may differ between different mammalian orders.

Table 2 Full summary of the factors affecting relative testes size in terrestrial mammals (interaction model)

Parameter	Mean	lwr95CI	upr95CI
Intercept	**−1.09**	**−2.09**	**−0.16**
Litter size	0.14	−0.36	0.67
Litters per year	**−0.93**	**−1.38**	**−0.46**
Mating system (polygynous)	0.42	−0.03	0.86
Mating system (promiscuous)	**0.70**	**0.26**	**1.13**
Longevity	−0.01	−0.19	0.18
Paternal care present	−0.15	−0.49	0.18
Litter size:litters/year	0.35	−0.41	1.11
Litter size:polygynous mating system	−0.06	−0.61	0.48
Litter size:promiscuous mating system	−0.13	−0.64	0.39
Litters/year:polygynous mating system	**1.09**	**0.60**	**1.59**
Litters/year:promiscuous mating system	**0.97**	**0.51**	**1.44**
Litter size:litters/year:polygynous mating system	−0.40	−1.22	0.40
Litter size:litters/year:promiscuous mating system	−0.36	−1.13	0.43
Phylo SD	**0.30**	**0.00**	**0.52**
Species SD	**0.06**	**0.03**	**0.09**

All estimates are summarized by the mean and 95% credible interval ("lwr95CI" – "upr95CI") of the posterior distribution of the model run across 100 sampled phylogenetic trees. The factors highlighted in bold represent the variables that have an influential effect ($P \leq 0.05$) on relative testes size.

A positive association between RTS and litter rate would be expected if it is costly for males to invest in testes (Hayward & Gillooly, 2011; Schulte-Hostedde et al., 2005). If large testes size represents an 'expensive' investment, then investment in large testes may result in a trade-off with the maintenance of other tissues ('expensive-tissue hypothesis'; Aiello & Wheeler, 1995) or other energetically expensive costs of body maintenance, locomotion, or reproduction ('energy trade-off hypothesis'; Isler & van Schaik, 2006). Our findings could suggest that, on average, testes are 'expensive' enough for promiscuous and polygynous species to decrease size investment in species with fewer litters per year (resulting in fewer opportunities for males to mate). However, if testes tissue is inexpensive to maintain, while the activities associated with reproductive activity are expensive (Isler & van Schaik, 2006), then perhaps the combined effect of maintaining high sperm production and behavioural competition to inseminate females (e.g., competitive searching for females over large areas, Waterman, 1998) would drive the observed trend between RTS and litter rate in promiscuous and polyandrous species. However, testes may not actually be expensive to maintain in a variety of mammals, including rodents, ungulates, primates, and carnivores (Lemaître et al., 2009). Thus, future work could include mating season length to study the relationship between testes size and litter rate in more detail.

We did not find the same positive association between litter rate and RTS in monogamous species, but rather a trend for the opposite pattern. The lack of an effect in monogamous

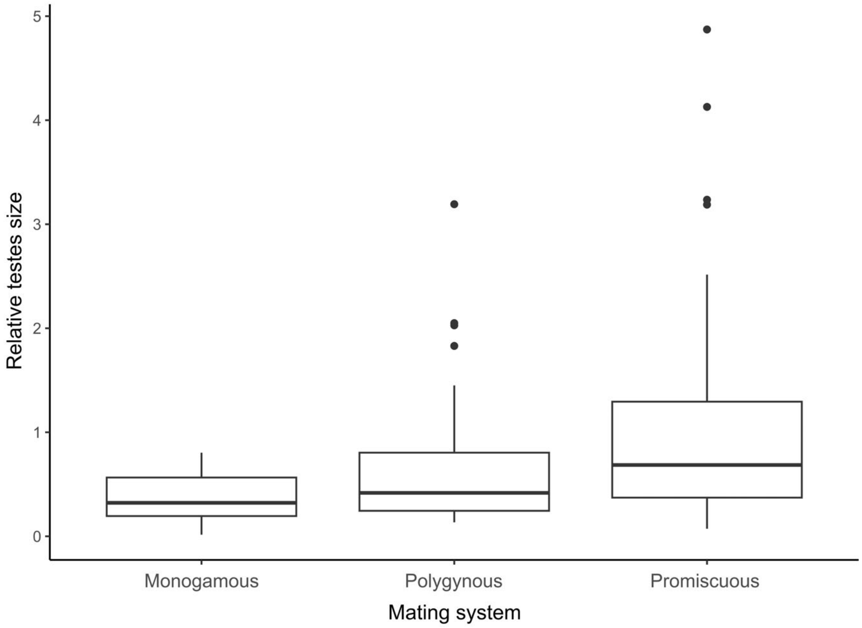

Figure 2 Relative testes size and mating system across 224 terrestrial mammalian species. The dark line is the median, the box edges are the upper and lower quartiles, the whiskers are 50% from the median, and the closed circles are the outliers, calculated as the values smaller or larger than 1.5 times the box length (i.e., upper–lower quantile).

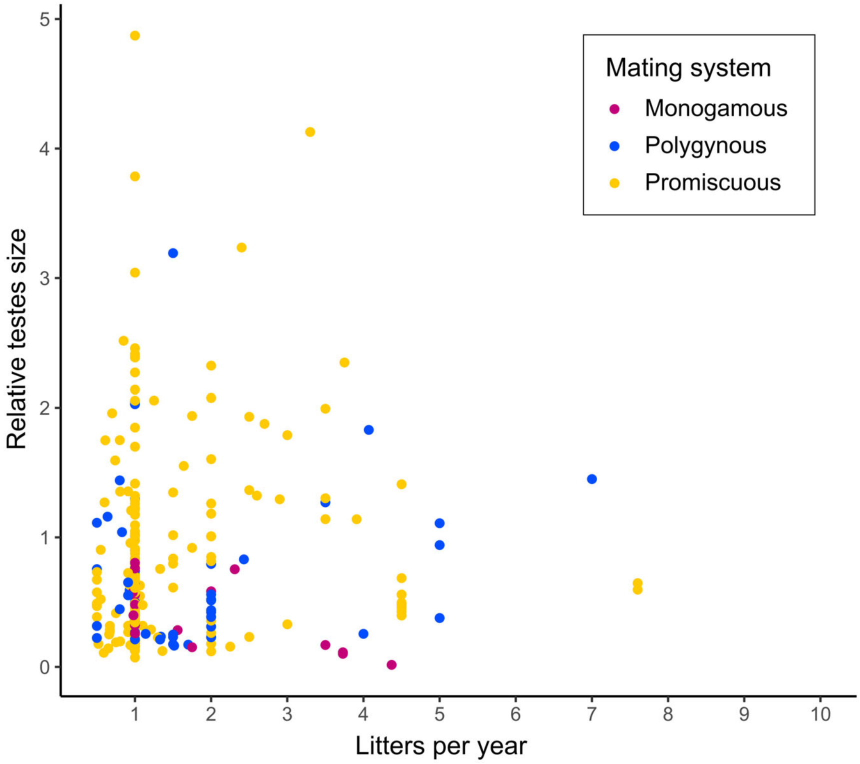

Figure 3 The interaction between litters per year and mating system on relative testes size in 224 terrestrial mammals.

Table 3 Loo Information Criterion of the models describing relative testes size in terrestrial mammals across 16 orders that have a litter size of 1

Model	Predictor variables	LooIC mean (lwr95CI – upr95CI)	Number of species
Global model	Litters/year + female multiple mating + longevity	74 (72.7–75.4)	88
Interaction model	Litters/year × female multiple mating + longevity	75.3 (74.1–76.9)	88

Table 4 Full summary of the factors affecting relative testes size in a subset of terrestrial mammals with a litter size ≤1 (global model)

Parameter	Mean	lwr95CI	upr95CI
Intercept	**−0.99**	**−1.53**	**−0.38**
Litters per year	0.07	−0.11	0.25
Longevity	0.14	−0.07	0.35
Female multiple mating present	**0.54**	**0.22**	**0.87**
Phylo SD	0.33	0	0.58
Species SD	0.03	0	0.07

All estimates are on the log-odds scale and summarized by the mean and 95% credible interval ("lwr95CI" – "upr95CI") of the posterior distribution of the model ran across 100 sampled phylogenetic trees. The factors highlighted in bold represent the variable that has an influential effect ($P \leq 0.05$) on relative testes size.

systems may have been due to low sample sizes ($N = 18$ species (8%) in our dataset). The low proportion of monogamous species in our dataset is not surprising, given that in many lineages of mammals, females mate multiply (e.g., rodents, Waterman, 2007; primates, Stumpf et al., 2011). We would, however, expect monogamous systems to have differing energetic expenditures regarding testes maintenance, so future studies should consider the effect of energy expenditure/allocation on testes in differing mating systems.

In contrast to our predictions and Soulsbury (2010), we did not find a significant relationship between litter size and RTS. However, litter size was included in our top model and showed a positive slope. Furthermore, the inclusion of differing life history variables in our study (e.g., litters per year) versus Soulsbury (e.g., multiple paternity rates) may have influenced the relationship between litter size and RTS. Additionally, our study examines a larger number of mammal species including Soulsbury's (2010) dataset, and as such may include more of the variability in testes size measured in mammals. However, it is important to note that Soulsbury (2010) specifically examined the relationship between multiple paternity and testes size, and thus, the sample size was limited by fewer molecular paternity studies in mammals at that time. Clearly, the relationship between testes size and life history variables is complex.

Although we had predicted that longer-lived species would have smaller RTS compared to shorter-lived animals, we found no relationship between longevity and RTS, as has been found in terrestrial carnivores (Iossa et al., 2008). However, a lack of

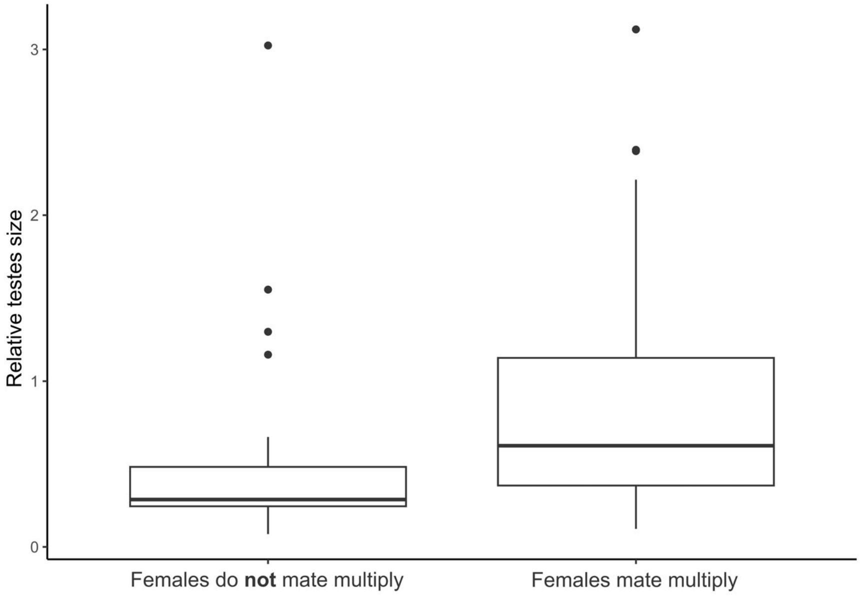

Figure 4 The effect of the presence of multiple mating of females (promiscuity) or absence (monogamy or polygyny) on relative testes size in 88 terrestrial mammals that have a litter size ≤1. The dark line is the median, the box edges are the upper and lower quartiles, the whiskers are 50% from the median, and the closed circles are the outliers, calculated as the values smaller or larger than 1.5 times the box length (i.e., upper–lower quantile).

relationship between longevity and RTS is still surprising given that long-lived species are predicted to invest more in survival while shorter-lived species invest more in current reproduction (Stearns, 1992). Indeed, in bats, longer life spans are associated with fewer offspring (Garbino et al., 2021). Furthermore, Hamel et al. (2010) found more evidence of reproductive costs in longer-lived ungulate species and greater survival costs in shorter-lived rodent species (Hamel et al., 2010). Our results thus suggest that RTS in mammals may not be influenced by an individual's lifespan but instead by reproductive opportunities within each breeding season, consistent with our finding of a positive relationship between the number of litters per year and RTS.

Contrary to our prediction of smaller testes sizes in species that give more paternal care, we found no evidence of a relationship between paternal care and RTS. The absence of this relationship may be because paternal care is only present in 21.9% of species in our dataset, which is a bit higher than in mammalian species where approximately 10% provide parental care (West & Capellini, 2016; Woodroffe & Vincent, 1994). However, as our study broadly covered many mammal lineages, the association between parental care and RTS is likely nuanced and species specific. For instance, in mammals, paternal care is associated with greater female reproductive success (West & Capellini, 2016) and evolves when the benefits outweigh the costs of caring, for males. Indeed, in birds, testes size was smaller in species where males participated in feeding their offspring (Pitcher et al., 2005). Perhaps, the costs in species with paternal care are on average low enough in mammals for the males to be able to also allocate resources to sperm production, which may result in an absence of a relationship between RTS and paternal care.

Study limitations

We used RTS as a proxy for sperm competition. Other characteristics of male gonads, such as ejaculate composition (Perry et al., 2013; Ramm, 2020), sperm length and quality (Lüpold et al., 2020), and the size of other reproductive tissues (e.g., Sertoli cells, Russell et al., 1990; seminal vesicles, Lemaître et al., 2011) may be additional indicators of sperm competition intensity (Lüpold et al., 2009, 2020), and may affect a male's reproductive success (Anderson & Dixson, 2002). Additionally, as testes are also used for testosterone production, testes size may be correlated with testosterone production, especially in species where males compete through overt aggression for access to females (Preston et al., 2012). Also, different factors, such as the number of rival males, the option to use mating plugs, male condition and age, or social status, can have an effect on resource allocation in the gonad (Bayram et al., 2020; Ramm, 2020). Furthermore, variation in RTS may differ within species (Firman & Simmons, 2008) and may be driven by a variety of ecological factors (Firman et al., 2015). Our study covers many mammalian species; therefore, the lack of relationship may be due to variations in female reproductive strategies among different mammal lineages, and thus, studying this relationship with further lineage or other groupings of mammals (e.g., guilds) may have revealed different patterns.

Beyond the influence of female mating multiply on testes size, females may also play an active role in determining the success of a male's sperm (Firman, 2020) through mechanisms such as cryptic female choice (Thornhill, 1983) or through female reproductive fluids (Gasparini et al., 2020). For example, in cryptic female choice, females control which male sires offspring in a larger litter rather than siring being determined by competition among the sperm. Females may benefit from sperm competition by 'selecting' sperm that increases the chance of obtaining high genetic quality offspring (Firman, 2020). Potentially, a female's reproductive state may also influence ejaculate composition and function, which may in turn affect a male's chances of fertilizing an egg (Perry et al., 2013). Thus, females not only play a role in sperm competition but may influence the evolution of male reproductive anatomy and physiology.

We focused our study on post-copulatory sexual selection; yet, many species also use pre-copulatory strategies (Dines et al., 2015; Ferrandiz-Rovira et al., 2014), and a trade-off between pre- and post-copulatory sexual selection likely varies among different lineages because of differences in life history traits and ecological conditions (Dines et al., 2015; Simmons et al., 2017). For example, where female monopolization is not possible or not common, mate searching and post-copulatory selection may be more important (Lüpold et al., 2014).

Conclusion

We used the largest dataset for mammals to date for this meta-analysis, including relative testes size data for 631 mammalian species resulting in 224 unique terrestrial mammalian species across 15 orders to examine the relationships between RTS and litter size and rate. We found that testes size was larger in species where females mate with multiple males and when there are more opportunities within a breeding season/or year (higher litter rate) to mate, reflecting more intense sperm competition in these species. In contrast, in monogamous species, species that had multiple litters per year had smaller relative testes sizes compared to species with fewer litters per year. Overall, our extensive dataset shows that the effect of female reproductive strategies on relative testes size is nuanced and varies between mating systems.

Author contributions

All authors contributed to the conception of the study and data collection. AM analysed the data and prepared the figures and supplementary material; AM and MHW drafted the manuscript; and all authors edited and approved its final version.

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Conflict of interest

We declare that we have no conflict of interest.

Data availability statement

Data and code are available on the GitHub repository https://github.com/annemarievdmarel/meta-analysis_mammal_testessize (van der Marel et al., 2023).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. The relationship of log-transformed testes mass on log-transformed body mass for 571 terrestrial mammal species.

Table S1. Full summary of the intercept-only model (M1) describing relative testes size in terrestrial mammals.

Table S2. Full summary of the factors affecting relative testes size in terrestrial mammals (global model, M2).

Table S3. Full summary of the factors affecting relative testes size in terrestrial mammals excluding the potential outlier the tenrec (interaction model with LooIC of 317.6).

Table S4. Full summary of the factors affecting relative testes size in a subset of terrestrial mammals with a litter size ≤ 1 (interaction model, M5).

Table S5. Full summary of the factors affecting relative testes size calculated as testes divided by body mass (interaction model, M6, with LooIC of 596.1 [592.2–600.5]).