

Article

Different maternal investment strategies for male and female calves in a polygynous mammal

Francisco CEACERO^{a,*}, Martina KOMÁRKOVÁ^b, Andrés J. GARCÍA^{c,d,e} and Laureano GALLEGO^e

^aDepartment of Animal Science and Food Processing, Faculty of Tropical AgriSciences, Czech University of Life Sciences, Kamýcká 129, 165 00 Praha 6 - Suchbátka, Czech Republic, ^bDepartment of Ethology, Institute of Animal Science, Přátelství 815, 104 01 Praha - Uhřetěves, Czech Republic, ^cInstituto de Investigación en Recursos Cinegéticos (IREC), Consejo Superior de Investigaciones Científicas—Universidad de Castilla-La Mancha—Junta de Comunidades de Castilla-La Mancha (CSIC-UCLM-JCCM), Ronda de Toledo s/n, 13071 Ciudad Real, Spain, ^dSección de Recursos Cinegéticos y Ganaderos, Instituto de Desarrollo Regional (IDR), 02071 Albacete, Spain, and ^eDepartamento de Ciencia y Tecnología Agroforestal y Genética, ETSIAM, Universidad de Castilla-La Mancha (UCLM), 02071 Albacete, Spain

*Address correspondence to Francisco CEACERO. E-mail: ceacero@ftz.czu.cz

Handling editor: Zhi-Yun Jia

Received on 2 December 2017; accepted on 25 June 2018

Abstract

Maternal effects occur when the phenotype of the mother influences that of the young to the detriment of her survival, growth or fitness. The investment of the mother can be affected by maternal body condition and/or experience. Trivers–Willard Hypothesis (TWH) and Local Resource Competition Hypothesis (LRCH) are the main hypotheses used to explain bias in birth sex-ratios in mammals, as well as for sex-biased maternal investment. Both hypotheses suggest that a different amount of investment must be expected according to the sex of the young. However, recent studies suggest that these differences are not in quantity but in the strategies: mechanisms and objectives may differ for each sex. We studied how maternal characteristics (age, body mass, body condition, and dominance status) influence relevant aspects of the birth and early growth of the calf (birth date, birth body mass, body mass at weaning, and body condition at weaning) separately for each sex; and how that investment is mediated by milk production and composition (lactose, fat, and protein). One hundred eighty-eight newborns from 75 captive red deer hinds aged from 2 to 19 years were analyzed. The main differential investment observed was related to birth date: when producing a female, hinds give birth earlier in the season only if they have a good body condition; however, when gestating a male it is the older hinds those which deliver earlier. Subsequently, milk production and composition are correlated with birth body mass in female calves, but to weaning body mass in males. Thus, only hind body mass affects the weaning body mass of female calves, compared with age and hind body mass in males. These results suggest that while TWH fits the maternal investment strategy found for male calves, it is LRCH which correlates with the maternal investment patterns observed for females.

Key words: birth date, body condition, local resources competition hypothesis, milk production, sex-ratio, Trivers–Willard hypothesis

In ungulates, as in other species, maternal effects occur when the phenotype of the mother influences that of the young (Wilson and Festa-Bianchet 2009). It is common that the maternal identity, probably mediated by genetics (Kruuk et al. 2000, 2015) exerts a strong influence, and yet there are several well-known maternal effects such as calf birth body mass, milk production, and even milk composition being related to maternal mass in most mammals (Kleiber 1961). However, there is still room for some plasticity, which can be modulated by the mother according to her maternal body condition, experience, or age. For example, better body condition in female ungulates gives rise to earlier births within the season independently of the sex of the calf (Coulson et al. 2003; Stopher et al. 2008; Plard et al. 2014a, 2014b). Mothers with higher body condition, experience or age may also regulate their investment during lactation through higher or better quality milk production, weaning a heavier offspring in better condition. Even if maternal investment in polygynous ungulates has been extensively studied, conclusions are still unclear: 2 opposing although not mutually excluding hypothesis allow predictions to be made in almost every direction; conclusions differ among studies focused on maternal traits around conception date vs. delivery date; and differ among studies focusing on calf traits at delivery vs. weaning.

Is that maternal investment equal for sons and daughters? The main hypotheses about adaptive sex allocation in polygynous mammals (the Trivers–Willard Hypothesis [thereafter TWH; Trivers and Willard 1973] and the Local Resource Competition Hypothesis [thereafter LRCH; Hamilton 1967; Clark 1978; Silk 1983]) both agree that mothers should invest differently in offspring of each sex depending on the conditions, but predict opposite results. In polygynous ungulates, and in the case that maternal investment would increase offspring quality persisting into the adulthood (as in red deer), TWH predicts that mothers of high quality should invest more in male than in female offspring, because only high quality males are expected to have a high reproductive success, whereas females have a more constant reproductive success. This has been evidenced in a number of species (e.g., *Rangifer tarandus* [reindeer; Trivers and Willard 1973], *Cervus elaphus* [red deer; Clutton-Brock et al. 1984], *Ammotragus lervia* [Saharan arrii; Cassinello and Gomendio 1996] and *Alces americanus* [Alaskan moose; Veeroja et al. 2010]). However, many other studies failed to find such support (reviewed by Hewison and Gaillard 1999), and a meta-analysis showed only a weak support for the correlation between maternal condition and sex ratio (Sheldon and West 2004). The explanation may be ecological and/or sociodemographic: LRCH suggests at population (Clark 1978) and individual levels (Silk 1983) that when the dispersal from the maternal group is restricted to males, then female offspring will reduce the maternal reproductive success if resources are limited. Thus, only high-quality mothers can successfully rear daughters (Clutton-Brock et al. 1982a).

Other published studies have highlighted the possibility that both models may appear simultaneously within the same species. Landete-Castillejos et al. (2001a) found that mouflon *Ovis gmelini* mothers were more likely to have female offspring with increasing age (LRCH), but males when increasing mass within a given age class (TWH). Isaac et al. (2005) also found that patterns of variation in sex allocation in common brushtail possums *Trichosurus vulpecula* can be explained by the interaction of TWH and LRCH. In any case, most of the studies mentioned focused on the causation of sex determination (the influence of maternal traits on primary or secondary sex-ratio), but few investigated the proximate mechanisms driving specific investment to female or male offspring

(Veller et al. 2016). Biases in maternal investment (more than variations in the sex-ratio) are expected for species with long periods of infant dependency (Hrdy 1987; Hewison and Gaillard 1999). As Kruuk et al. (2015) recently highlighted, what is good for son is not necessarily good for daughters, and thus, “what defines a high-quality mother depends on the sex of the offspring.” Thus, further empirical work is necessary to understand the associations between maternal and offspring phenotype and sex-biased investment (Räsänen and Kruuk 2007).

There are several mechanisms by which females can differentially influence their offspring according to sex. In ungulates inhabiting seasonal environments, the first 2 mechanisms are timing of birth and birth mass; both highly influencing growth, survival, and fitness (Clutton-Brock et al. 1982b; Adam and Moir 1987; Festa-Bianchet 1988; Fisher et al. 1989; Green and Rothstein 1993; Clements et al. 2011). Modulation of gestation length may be a mechanism, with a dependency on factors like age and body condition (Mysterud et al. 2009) and highly mediated by nutrition in the last third of gestation (Verme 1965; Asher et al. 2005). The next key period is lactation, with milk production and/or composition being the investment mechanism. There is a greater protein and/or lipid content in the milk for male offspring compared with that for female (Landete-Castillejos et al. 2005 for red deer; Hinde 2007, 2009 for rhesus macaques; Robert and Braun 2012 for tammar wallabies; Quesnel et al. 2017 for kangaroos). Mediated by these mechanisms, the final investment can be measured at weaning, that is, once the young become independent of the mother.

In this study, using a captive population of red deer, we investigated how 4 traits of the mother (age, social rank, body mass, and body condition) influence the early growth of her male and female offspring by different maternal investment strategies. The mechanisms we focused on included giving birth earlier, delivering a heavier calf, and producing more and/or higher quality milk; where weaning a heavier calf, or weaning a calf in better body condition were the goals sought by the hind. A large long-term dataset was analyzed as a whole, and also separately for male and female calves, in order to understand maternal investment in these 3 scenarios (full dataset and, especially, male and female calves separately), and how the observed strategies and goals fit to the predictions of TWH and LRCH models for sex-biased maternal investment in ungulates. Our prediction is that the amount of maternal investment may (or not) be different for male and female calves, but the investment strategies must differ as the goals are different: for female calves, survival may be enough; but for males only a high-quality calf is relevant in terms of fitness.

Material and Methods

This study was carried out at the Deer Experimental Farm of the University of Castilla-La Mancha in Albacete, Spain. Data were collected in the years 2005, 2006, 2008, 2009, and 2012, involving 188 births from 75 hinds aged from 2- to 19-years old. All the hinds involved in the study were reared in captivity and belong to a pure breed of the subspecies *Cervus elaphus hispanicus*. The species is a seasonal breeder with one calf per season (twins are very rare, and did not occur during the study). The rearing system resembles that occurring in the wild, with social groups of hinds, frequently relatives, of different ages, and their calves (Carranza 1988). Animals were kept in 10 000 m² enclosures with pasture, and were supplemented with diets based on suggestions by Brelurut et al. (1990) using barley straw and hay from barley, alfalfa, oat, and sweet

beetroot (16% protein). Animals always had *ad libitum* access to food and water. The irrigated pasture included tall fescue, *Festuca arundinacea* (52.4%); cocksfoot, *Dactylis glomerata* (28.6%); lucerne, *Medicago sativa* (14.3%); and white clover, *Trifolium repens* (4.8%).

All enclosures are connected to a handling premise by a 5-m wide corridor, where animals are routinely weighed once a week on a ± 50 g electronic balance. Animals are habituated to this weekly low-intensity handling routine (Ceacero et al. 2014). By this method, 3 body mass measures were collected: hind body mass, recorded in the first handling session after parturition (i.e., no longer than 7 days); and calf and hind body mass at weaning, when calves are individually forcibly weaned 18 weeks after birth. In the same handling sessions, the body condition of the animals (after birth for hinds and just before weaning for calves and hinds) was recorded. The body condition score was measured by rump palpation, ranging from 1 (very poor condition—cachexia) to 5 (very good condition—fat), with the scale divided in quarters of unit, according to that which is widely used by deer managers (Audigé et al. 1998; Carrión et al. 2008). Wings of the pelvis, sacrum, rump, and spine are used as landmarks to detect the accumulation of muscles and fat. Values close to 5 or below 2 are not recommended due to negative effects on reproduction. In order to increase the accuracy of this measurement, instead of using only the value recorded after delivery and before weaning, we used the mean of the values recorded in the 2 first handlings after delivery, and the 2 handlings before the weaning.

Deliveries occur during May and June. Delivery date was recorded for every hind, and calves were weighed within their first 24 h of life and marked with ear tags (Allflex GTLF1; 76×57 mm). During the same handling session, newborns were weighed on a tensiometric digital scale (± 10 g). Multiple factors influencing mating and occurring throughout the gestation period can affect the mean birth date across years. Thus, in order to ensure that the birth date data can be comparable between years, we calculated the relative birth date as the number of days since the first delivery occurred in the season within the herd.

During July and August, after all the hinds in the breeding groups had given birth, we observed their social interactions in order to determine their social rank. A total of 20–40 observation hours per group (depending on size) were carried out in 2-h periods, covering the entire daylight time, but with greater effort concentrated during the most active periods (early in the morning and evening). All interactions were registered avoiding any interference on the behavior of the individuals, according to the focal group sampling method (Altmann 1974; Ceacero et al. 2007). Following Thouless and Guinness (1986), agonistic interactions were considered as those occasions when one hind attacked another or made a ritualized gesture associated with attacks that led to the submissive animal to move away. Social rank for each individual was calculated as a linear hierarchy by winner-loser outcome of interactions on MatMan 1.1.4 matrix manipulation and analysis program (Noldus, Wageningen, The Netherlands) as explained by de Vries (1998). By this method, the hierarchy is reorganized by a 2-step iterative procedure (1000 sequential trials) to order individuals by first minimizing the number of inconsistencies (I) and then the strength of these inconsistencies (SI). Because matrix size varied between groups, social ranks were transformed according to the Formula $1 - (\text{rank}/N_i)$, where N_i is the number of animals in the group. Therefore, social ranks ranged [0, 1]. Finally, to follow normality requirements social rank was transformed into the arcsine of the square root.

Hinds were milked in Weeks 2, 4, 6, 10, 14, and 18 of lactation. Hinds were isolated from calves for 6 h without pre-isolation milking for ethical reasons. Individuals were milked under anesthesia (xylazine at 0.5 mg/kg BW; ketamine at 1 mg/kg; reversed with yohimbine 0.25 mg/kg BW) using a milking machine set up of 50/50 massage/milking ratio and 44 kPa of vacuum. Once anesthesia was induced, 10 i.u. of oxytocin were injected into the right jugular vein 1 min before the start of milking to induce milk let-down. The values obtained throughout these 6 milking sessions are representative of the lactation curve of the species, and thus, allow the calculation of total milk yield for the whole lactation period (production, protein, lactose, and fat) using the algorithm proposed by García et al. (1999). Daily milk production is calculated by multiplying the obtained values by 4, and fitting the resulting data to Wood's (1967) gamma function: $y_t = a t^b e^{-ct}$, where y_t is the average daily yield on the t -th day and a , b , and c are constants. This model is more suitable than others, because the Gamma function can produce curves of both Types I and II, which are the typical for the species (García et al. 1999). Total milk, protein, lactose, and fat produced during the 18 weeks of lactation were used in the statistical analyzes.

Sampling frequencies and handling procedures were designed to reduce the stress of the animals, according to the European and Spanish laws and current guidelines for ethical use of animals in research (ASAB 2012), and we followed Spanish and European guidelines and laws in the use of animals in research. The research protocols used were approved by the Committee of Ethics in Animal Experimentation from the University of Castilla-La Mancha, Spain, with the authorization number 1002.04.

Statistical analysis

The analyzes were designed to interpret the influence of 4 traits of the hinds (age, body mass, body condition, and social status) on productive parameters (4 milk production traits: total milk, protein, lactose, and fat production during the 18 weeks of the lactation period) and on calves' early development (4 growth traits: relative birth date, birth body mass, weaning body mass, and condition at weaning). In order to understand the differences in these relationships when calves are males or females, all the analyzes described were conducted for the whole dataset. Subsequently, the same analyzes were conducted separately for each sex, in order to understand how the maternal investment strategies operate within each sex.

Table 1 shows descriptive statistics for the variables used, with Student t -tests highlighting differences for male and female calves. Levene's test for homogeneity of variances was considered for this analysis.

Pearson's correlations showed the relationships among the variables (among the hind traits: Supplementary Table S1; among the calf traits: Supplementary Table S2; between the hind and calf traits: Supplementary Table S3; and between milk production and composition and the hinds' and calf traits: Supplementary Table S4). Although tests for Variance Inflation Factor (VIF) did not detect multicollinearity among the hind traits, they were all significantly correlated (Supplementary Table S1), and for this reason the rest of the analyzes were designed to address with this characteristic of the data.

For each calf trait and each milk production and composition variable, 4 Generalized Linear Mixed Models were built: one for each single maternal trait. For the whole dataset, the maternal trait always entered the model in interaction with sex. In the models for each sex, the traits of the hinds were entered as a single covariable. All models included hind ID and year as random factors. Among those models where the maternal trait was significant, the best

Table 1. Descriptive statistics (mean, standard deviation [SD], and sample size) for the variables used in the study, separated for male and female calves: hind traits at delivery, calf traits at birth and weaning, milk production and composition and change in hind traits throughout the lactation period

	Male calves (♂)		Female calves (♀)		
	<i>n</i>	Mean ± SD	<i>n</i>	Mean ± SD	<i>t</i> -test
Hind rank	102	0.514 ± 0.304	85	0.481 ± 0.29	<i>t</i> = -0.716 ^{ns}
Hind age (year)	102	9.25 ± 3.82	85	9.21 ± 4.36	<i>t</i> = -0.072 ^{ns}
Hind body mass (kg)	102	106.4 ± 11.8	85	105.5 ± 13.1	<i>t</i> = -0.501 ^{ns}
Hind body condition	102	3.90 ± 0.41	85	3.80 ± 0.48	<i>t</i> = -1.485 ^{ns}
Relative delivery date	102	17.5 ± 10.4	85	17.6 ± 13.1	<i>t</i> = -0.055 ^{ns}
Calf birth body mass (kg)	96	8.53 ± 0.89	76	7.87 ± 0.83	<i>t</i> = -5.115***
Milk production (L)	81	274.6 ± 62.7	60	272.2 ± 59.2	<i>t</i> = -0.223 ^{ns}
Milk protein (kg)	81	18.4 ± 3.7	60	18.2 ± 3.7	<i>t</i> = -0.338 ^{ns}
Milk lactose (kg)	81	12.2 ± 2.9	60	12.2 ± 2.7	<i>t</i> = -0.001 ^{ns}
Milk fat (kg)	81	25.3 ± 5.9	60	25.1 ± 5.1	<i>t</i> = -0.182 ^{ns}
Calf weaning body mass (kg)	94	50.36 ± 5.76	66	43.0 ± 4.8	<i>t</i> = -8.951***
Calf weaning body condition	76	3.08 ± 0.31	60	3.05 ± 0.34	<i>t</i> = -0.538 ^{ns}
Hind's body mass change (kg)	100	-3.42 ± 7.4	84	-4.15 ± 9.3	<i>t</i> = -0.559 ^{ns}
Hind's body condition change	101	-0.104 ± 0.479	84	-0.063 ± 0.455	<i>t</i> = -0.581 ^{ns}

Student's *t*-test shows differences between them. Significant relationships are highlighted in bold and significance at 0.001 level is indicated as ***.

model was selected using Akaike's Information Criterion (AIC). AIC weights (AIC_w) were calculated following Wagenmakers and Farrell (2004), in order to assess which of the selected significant models had the higher probability of actually being the best one. Analyses were performed in IBM® SPSS® Statistics (version 20.0 for Windows, IBM, USA).

Results

Maternal traits in the studied animals were not different for male and female calves. Milk production and composition, relative birth date and body condition of the calves were also similar between both sexes. Only body mass at birth and weaning was higher for male calves, as expected in dimorphic species. During the lactation period hinds lost an amount of body mass and condition, which confirm that investment occurred; however, the loss was equal for male and female calves indicating that the total investment was similar for both sexes, at least during lactation (Table 1).

Pearson's correlations showed that the 4 studied hind traits at parturition, which may be involved in the different strategies of maternal investment, are highly correlated (Supplementary Table S1; Supplementary Figure S1). Correlations among calf traits were weaker (Supplementary Table S2), except those between weaning body mass with birth body mass and relative birth date. Correlations between hind and calf traits highlighted some initial differences according to sex (Supplementary Table S3): hind body mass correlated with calf birth and weaning body mass; however, relative birth date correlated with hind body condition for females, but with hind age for males. Differences between male and female calves were even greater for milk production and composition (Supplementary Table S4). For males, milk production and composition correlated mainly with calf traits (relative birth date, weaning body mass, and weaning body condition); whilst contrastingly, in females milk production and composition mainly correlated with hind traits at delivery (social rank and body mass) and calf birth body mass.

Mixed models clarified the effect of hind traits on milk production and calf growth, after adjustment for repeated observations for

the same females (Table 2). Relative birth date was better explained by the age of the hind for male calves ($P = 0.017$; Figure 1) whereas hind age did not significantly explain the relative birth date of female calves but instead the body condition of the hind for female calves ($P = 0.039$; Figure 2). Birth body mass is influenced by hind body mass both for male ($P < 0.001$) and female calves ($P = 0.009$). Body mass of male calves at weaning is related to the age ($P = 0.026$) and body mass ($P = 0.043$) of the hind, but only by hind body mass in female calves ($P = 0.001$). Finally, calf body condition at weaning was not significantly affected by any of the studied hind traits, neither in male nor female calves. Other relationships between hind and calf traits not shown here or in Table 2 were not significant in the mixed models. In general, total milk production ($P = 0.003$), protein ($P = 0.010$), and fat ($P = 0.002$) increased with the body mass of the hind. Fat content also decreased with age ($P = 0.049$), with almost the same pattern for male and female calves.

Discussion

Our results confirm that maternal investment in captive red deer does occur: hinds have lower body mass and condition after lactation, and it is already known that reproduction negatively influences the subsequent gestation (Carrión et al. 2010). In general, this investment follows well-known rules when we consider the whole dataset: older hinds give birth earlier (Plard et al. 2014b) and heavier hinds have heavier calves at birth, heavier calves at weaning, and produce more milk with higher quality composition (Kleiber 1961). However, the body condition of calves is the same at weaning for males and females, and the loss of body weight and condition by the hind is the same for hinds rearing calves of one sex or another. This means that the amount of investment, at least up to the weaning period, is very similar for male and female calves.

Nevertheless, when we focused our analyzes separately for male and female calves the patterns observed highlighted important differences in investment strategies. For male calves, as the age of the hind increases the delivery of the calf comes earlier in the season; thereafter, milk production and composition seem focused on



Figure 1. Correlation between relative birth date and age of the hind. Empty squares and solid line refer to male calves; black triangles and dotted line refer to female calves. The correlation was significant for male calves, but not for females (see [Supplementary Table S4](#)).

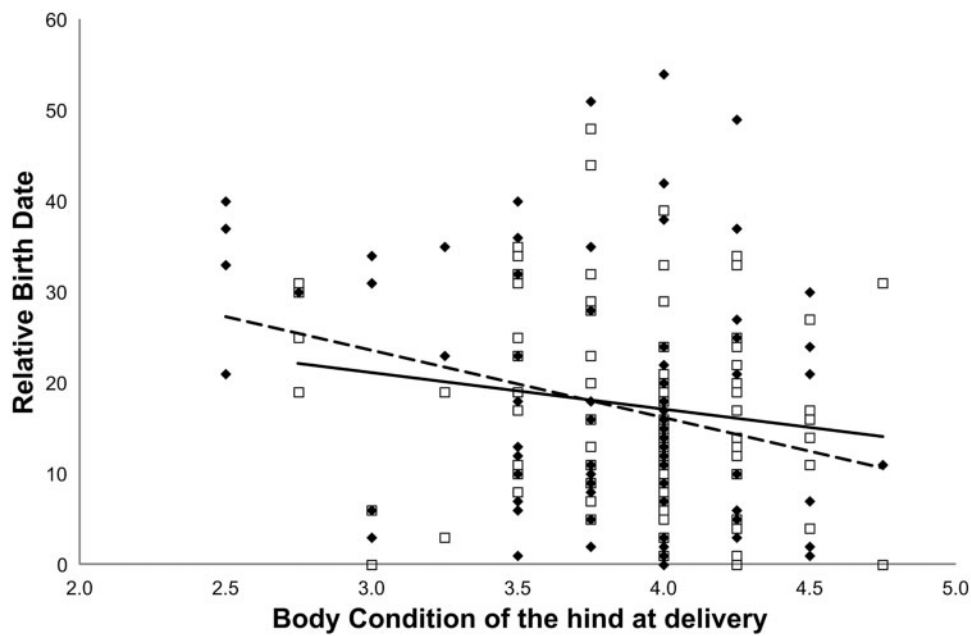


Figure 2. Correlation between relative birth date and body condition of the hind at delivery. Empty squares and solid line represent male calves; black triangles and dotted line refers to female calves. The correlation was significant for female calves, but not for males (see [Supplementary Table S4](#)).

maximizing body mass and body condition at weaning, leading to heavier calves at weaning in older hinds. When the calf is female, earlier births only happen when hinds have a good body condition, heavier calves are delivered by high-ranked hinds, and milk production and composition is better in high ranked hinds, linked to the body mass of the calf at birth. Thus, the results clearly show that even if the total amount of investment is similar for both sexes, the strategies followed are different, probably because of the different goals, as will be discussed further.

Maternal investment at birth

It seems clear that maternal investment should start as soon as possible, and so our results confirm the well-known fact that investment through birth body mass is linked mainly to the mother’s body mass ([Liggins 1979](#); [Asher et al. 2005](#)). However, one of the most interesting results is the sex-related difference observed in the strategy towards the second mechanism of early maternal investment: advance in birth date is linked to age when the calf is male, but to body condition when it is female. Not surprisingly, age and body condition

Table 2. Summary of the significant mixed models explaining the influence of hind traits (age, social rank, body mass, and body condition at parturition) on milk production or calf traits

	Hind trait	<i>df</i>	<i>F</i>	<i>p</i>	AIC ^a	AIC _w (%) ^a
Relative birth date						
All dataset pooled (♂+♀)	Body condition×sex	2,161.6	3.335	0.038	–	–
Only males (♂)	Age	192.2	5.955	0.017	–	–
Only females (♀)	Body condition	148.8	4.483	0.039	–	–
Birth body mass						
All dataset pooled (♂+♀)	Age×sex	2,135.3	13.646	<0.000	471.648	0.31
	Rank×sex	2,163.8	10.845	<0.000	466.800	3.52
	Body mass×sex	2,156.2	25.450	<0.000	460.293	91.09
	Body condition×sex	2,148.2	13.221	<0.000	466.067	5.08
Only males (♂)	Body mass	173.4	13.439	<0.001	–	–
Only females (♀)	Body mass	166.8	7.189	0.009	–	–
Total milk production						
All dataset pooled (♂+♀)	Body mass×sex	2,131.1	4.350	0.015	–	–
Only males (♂)	^b					
Only females (♀)	^b					
Total milk protein						
All dataset pooled (♂+♀)	Body mass×sex	2,130.1	3.422	0.036	–	–
Only males (♂)	^b					
Only females (♀)	Body mass	150.1	4.241	0.045	–	–
Total milk lactose						
All dataset pooled (♂+♀)	^b					
Only males (♂)	^b					
Only females (♀)	^b					
Total milk fat						
All dataset pooled (♂+♀)	Body mass×sex	2,129.7	4.799	0.010	–	–
Only males (♂)	Age	172.5	4.881	0.030	–	–
Only females (♀)	Age	118.2	5.928	0.025	–	–
Weaning body mass						
All dataset pooled (♂+♀)	Age×sex	2,135.9	26.907	< 0.000	1076.387	< 0.01
	Rank×sex	2,158.4	38.608	< 0.000	1051.996	4.56
	Body mass×sex	2,145.7	55.108	< 0.000	1048.253	29.60
	Body condition×sex	2,135.3	44.026	< 0.000	1046.654	65.85
Only males (♂)	Age	166.5	5.166	0.026	593.0	78.67
	Body mass	172.6	4.242	0.043	595.6	21.33
Only females (♀)	Body mass	147.5	12.718	0.001	–	–
Weaning body condition						
All dataset pooled (♂+♀)	^b					
Only males (♂)	^b					
Only females (♀)	^b					

Four models were built for each milk or calf characteristic but only those where the hind trait was significant are shown. When 2 models were significant, they were compared through weighed Akaike's Information Criterion which shows the probability of each model to be the best one. The same procedure was performed 3 times for the whole dataset and separately for male and female calves.

^aDashes indicate that only one significant effect of hind characteristic was found in milk production or calf characteristics, and thus, there is no reason for comparison of models.

^bNo significant model was found for any hind trait for this dataset.

have been repeatedly reported as the main variables affecting birth date (Guinness et al. 1978; Bon et al. 1993; Plard et al. 2014b); however, here we show for the first time how these maternal traits work differentially according to the sex of the calf. In most group-living mammals, including red deer, early birth means a substantial advantage for the survival, reproductive, and social successes of males (Guinness et al. 1978; Albon et al. 1987; Landete-Castillejos et al. 2001b; Plard et al. 2014b): early born animals grow faster (Clutton-Brock et al. 1982b; Adam and Moir 1987; Fisher et al. 1989), have better chances for obtaining extra milk through allosuckling (Landete-Castillejos et al. 2000), have more time to grow before the in winter hiatus due to changes in the photoperiod (Scott et al. 2013), etc.

It may be argued that birth date is affected by conception date, but this is highly improbable in a captive population with hinds in

good body condition kept in small groups with one stag during the rut season. Thus, compensating gestation length seems to exist (Berger 1992; Landete-Castillejos et al. 2001b; Holand et al. 2004; García et al. 2006; Mysterud et al. 2009), frequently linked to increased nutritional resources during the last third of gestation (Clements et al. 2011; Asher et al. 2005). Similarly, it could be suggested that there is a bias in the results due to paternal influence (Douhard et al. 2016), but this is also improbable because few stags participated in mating every year, and all were of a very high-quality. Nevertheless, before senescence female red deer ovulate earlier (Langvatn et al. 2004), and these hinds mate with the best males, with greater fertility (Malo et al. 2005a, 2005b) and higher content of Y-chromosome bearing sperm (Gomendio et al. 2006; Edwards and Cameron 2014). This would explain the earlier birth date

observed for males (according to TWH), whereas a greater percentage of females are conceived later and an advance in birth date would only happen if the hind achieves a good body condition in the last part of gestation (according to LRCH).

In this context, the benefits of early birth for female calves are not so clear. It has been argued that early born females are likely to achieve greater adult body mass (Côté and Festa-Bianchet 2001; Plard et al. 2014b), and our results seem to support a similar effect on weaning body mass. Nevertheless, since females are not the dispersing sex in red deer, it makes sense only for females in good condition; and this is supported by our results. Another benefit for early-born female offspring may be through higher rank acquisition (Guilhem et al. 2002). In any case, the fact that advancing birth date may be beneficial for both sexes (even if for different reasons), may explain why most studies in polygynous ungulates failed to find differences in gestation length between sexes (reviewed in Clements et al. 2011). Therefore, our results showing that the hind factors affecting birth date are different according to the sex of the calf are beneficial in the interpretation of past and future studies on this topic.

Maternal investment during lactation

Calf growth is closely linked to the availability of food resources for the calf. In the wild, this is commonly associated with vegetation phenology (Braza et al. 2000; Côté and Festa-Bianchet 2001), but not by milk production and composition which cannot be easily measured in wild animals. In our study, food resources for calves and hinds are continuously high. Even in such a supportive environment, sex-biased differences in milk production, and composition lead to investment strategies different for each sex. This has already been demonstrated in red deer where milk yield and energy density were biased in favor of male offspring (Birgersson et al. 1998; Landete-Castillejos et al. 2005; see Hinde 2007, 2009, e.g., in primates). However, we did not find such a pattern. Nonetheless, for female calves, milk production, and composition correlated with birth body mass, suggesting that milk is simply designed to supply the needs of the calf, that is, to support survival. On the contrary, for male calves, milk production, and composition correlated with weaning body mass and body condition, suggesting that milk is designed to maximize the quality and therefore fitness of the animal at the end of the maternal investment period. This confirms our prediction: the amount of investment does not need to be different for each sex but instead mediated by the factors needed to attain the different goals.

Social rank seems, in general, a bad predictor of maternal investment in calf performance at birth and weaning (Supplementary Figure S2). However, it positively affects milk production when the calf is a female. In reindeer, Holand et al. (2004) described the influence of social rank on maternal investment around parturition and weaning, but these effects were sex-independent. Moore et al. (2015) recently suggested that high stress derived from low dominance elevate glucose levels favoring the production of males in wallabies, and thus glucose can also be a good candidate to explain the effect of rank on milk production for female calves observed in this study (Bell and Bauman 1997). These results and others indicate that the influence of social rank on sex-biased maternal investment needs further investigation (Wilson and Festa-Bianchet 2009).

Implications for TWH and LRCH

One of the most interesting issues arising from this study is how the results fit the 2 main hypotheses on sex ratio/maternal investment:

TWH and LRCH. Results for male calves (Target: to produce heavy calves at weaning; Mechanisms: advancing birth date, delivering a heavy calf, and increasing milk quality and production; Greater investment driven by age) follow the predictions expected by TWH, a hypothesis developed mainly to explain the differential investment biased to the sex with greater reproductive potential (males). On the contrary, results for female calves (Target: adequate birth body mass; Mechanisms: adjust milk production and quality to such birth body mass; Greater investment driven by body condition and social rank) follow the predictions expected by LRCH, a hypothesis developed to explain the differential investment biased to the not dispersing sex in philopatric societies (in our case, females; indeed, this is also called “the advantaged-daughter hypothesis”). Sex-related adjustment in maternal investment may only happen if such differential allocation entails fitness consequences (Fisher 1930; Maynard-Smith 1980; Clutton-Brock 1991; Hewison and Gaillard 1999), as in our study. Thus, it is not surprising that when we look for the factors affecting maternal investment separately for each sex, the results fit with the predictions made by the hypotheses developed to explain sex-biased investment in that sex. These results highlight that both hypotheses (TWH and LRCH) are not mutually exclusive, and must be considered simultaneously in studies on species that fulfill the predictions of both.

Acknowledgements

This paper has been funded by projects AGL2012-38898 (Ministry of Economy and Competitiveness, Spain) and IGA-20185017 (Faculty of Tropical AgriSciences, Czech Republic) and MZE-RO0718 (Ministry of Agriculture, Czech Republic). The authors wish to thank Chris Johnson for professional language editing, and 3 anonymous reviewers for improving the quality of the manuscript.

Supplementary material

Supplementary material can be found at <http://www.oxfordjournals.org/>.

References

- Adam CL, Moir CE, 1987. A note on the effect of birth date on the performance of suckled red deer calves and their dams on low-ground pasture. *Anim Prod* 44:330–332.
- Albon SD, Clutton-Brock TH, Guinness FE, 1987. Early development and population dynamics in red deer. II. Density-independent effects and cohort variation. *J Anim Ecol* 56:69–81.
- Altmann J, 1974. Observational study of behaviour: sampling methods. *Behaviour* 49:227–267.
- ASAB, 2012. Guidelines for the treatment of animals in behavioural research and teaching. *Anim Behav* 83:301–309.
- Asher GW, Mulley RC, O’Neill KT, Scott IC, Jopson NB et al., 2005. Influence of level of nutrition during late pregnancy on reproductive productivity of red deer: i. Adult and primiparous hinds gestating red deer calves. *Anim Reprod Sci* 86:261–283.
- Audigé L, Wilson PR, Morris RS, 1998. A body condition score system and its use for farmed red deer hinds. *New Zeal J Agr Res* 41:545–553.
- Bell AW, Bauman DE, 1997. Adaptations of glucose metabolism during pregnancy and lactation. *J Mammary Gland Biol* 2:265–278.
- Berger J, 1992. Facilitation of reproductive synchrony by gestation adjustment in gregarious mammals: a new hypothesis. *Ecology* 73:323–329.
- Birgersson B, Tillbom M, Ekvall K, 1998. Male-biased investment in fallow deer: an experimental study. *Anim Behav* 56:301–307.
- Bon R, Dardaillon M, Estevez I, 1993. Mating and lambing periods as related to age of female mouflon. *J Mammal* 74:752–757.

- Braza F, San José C, Aragón S, 2000. Variation of male-biased maternal investment in fallow deer *Dama dama*. *J Zool* 250:237–241.
- Brelurut A, Pingard A, Thériez M, 1990. *Le Cerf Et Son Élevage*. Paris: INRA.
- Carranza J, 1988. Dominance relationships in female groups of red deer: seasonal changes. *Acta Theriol* 33:435–442.
- Carrión D, García AJ, Gaspar-López E, Landete-Castillejos T, Gallego L, 2008. Development of body condition in hinds of Iberian red deer during gestation and its effects on calf birth weight and milk production. *J Exp Zool* 309A:1–10.
- Carrión D, Gaspar-López E, Landete-Castillejos T, Gallego L, Garcia AJ, 2010. Effect of reproductive rest on the subsequent breeding in Iberian red deer hinds *Cervus elaphus hispanicus*. *Mamm. Biol* 75:211–218.
- Cassinello J, Gomendio M, 1996. Adaptive variation in litter size and sex ratio at birth in a sexually dimorphic ungulate. *P R Soc B Biol Sci* 263: 1461–1466.
- Ceacero F, Landete-Castillejos T, García AJ, Estevez JA, Gallego L, 2007. Kinship discrimination and effects on social rank and aggressiveness levels in Iberian red deer hinds. *Ethology* 113:1133–1140.
- Ceacero F, Landete-Castillejos T, Bartošová J, García AJ, Bartoš L et al., 2014. Habituating to handling: factors affecting pre-orbital gland opening in red deer calves. *J Anim Sci* 92:4130–4136.
- Clark AB, 1978. Sex ratio and local resource competition in a prosimian primate. *Science* 201:163–165.
- Clements MN, Clutton-Brock TH, Albon SD, Pemberton JM, Kruuk LE, 2011. Gestation length variation in a wild ungulate. *Funct Ecol* 25: 691–703.
- Clutton-Brock TH, Albon SD, Guinness FE, 1982a. Competition between female relatives in a matrilineal mammal. *Nature* 300:178–180.
- Clutton-Brock TH, Guinness FE, Albon SD, 1982b. *Red Deer: Behavior and Ecology of Two Sexes*. Edinburgh: Edinburgh Univ. Press.
- Clutton-Brock TH, Albon SD, Guinness FE, 1984. Maternal dominance, breeding success and birth sex ratios in red deer. *Nature* 308:358–360.
- Clutton-Brock TH, 1991. *The Evolution of Parental Care*. Princeton (NJ): Princeton University Press.
- Côté SD, Festa-Bianchet M, 2001. Birthdate, mass and survival in mountain goat kids: effects of maternal characteristics and forage quality. *Oecologia* 127:230–238.
- Coulson T, Kruuk LEB, Tavecchia G, Pemberton JM, Clutton-Brock TH, 2003. Estimating selection on neonatal traits in red deer using elasticity path analysis. *Evolution* 57:2879–2892.
- Douhard M, Festa-Bianchet M, Coltman DW, Pelletier F, 2016. Paternal reproductive success drives sex allocation in a wild mammal. *Evolution* 70: 358–368.
- Edwards AM, Cameron EZ, 2014. Forgotten fathers: paternal influences on mammalian sex allocation. *Trends Ecol Evol* 29:158–164.
- Festa-Bianchet M, 1988. Birthdate and survival in bighorn lambs *Ovis canadensis*. *J Zool* 214:653–661.
- Fisher RA, 1930. *The Genetical Theory of Natural Selection*. Oxford: Clarendon.
- Fisher MW, Fennessy PF, Davis GH, 1989. A note on the induction of ovulation in lactating red deer hinds prior to the breeding season. *Anim Prod* 49: 134–138.
- García A, Landete-Castillejos T, Molina A, Albiñana B, Fernández C et al., 1999. Lactation curves in captive Iberian red-deer *Cervus elaphus hispanicus*. *J Anim Sci* 77:3150–3155.
- García AJ, Landete-Castillejos T, Carrion D, Gaspar-Lopez E, Gallego L, 2006. Compensatory extension of gestation length with advance of conception in red deer *Cervus elaphus*. *J Exp Zool A Comp Exp Biol* 305:55–61.
- Gomendio M, Malo AF, Soler AJ, Fernández-Santos MR, Esteso MC et al., 2006. Male fertility and sex ratio at birth in red deer. *Science* 314: 1445–1447.
- Green WCH, Rothstein A, 1993. Persistent influences of birth date on dominance, growth and reproductive success in bison. *J Zool* 230:177–186.
- Guilhem C, Gerard JF, Bideau E, 2002. Rank acquisition through birth order in mouflon sheep *Ovis gmelini* ewes. *Ethology* 108:63–73.
- Guinness FE, Gibson RM, Clutton-Brock TH, 1978. Calving times of red deer *Cervus elaphus* on Rhum. *J Zool* 185:105–114.
- Hamilton WD, 1967. Extraordinary sex ratios. *Science* 156:477–488.
- Hewison AJM, Gaillard JM, 1999. Successful sons or advantaged daughters? The Trivers-Willard model and sex biased maternal investment in ungulates. *Trends Ecol Evol* 14:229–234.
- Hinde K, 2007. First time macaque mothers bias milk composition in favour of sons. *Current Biol* 17:958–959.
- Hinde K, 2009. Richer milk for sons but more milk for daughters: sex-biased investment during lactation varies with maternal life history in rhesus macaques. *Am J Human Biol* 21:512–519.
- Holand Ø, Weladji RB, Gjøstein H, Kumpula J, Smith ME et al., 2004. Reproductive effort in relation to maternal social rank in reindeer *Rangifer tarandus*. *Behav Ecol Sociobiol* 57:69–76.
- Hrdy SB, 1987. Sex-biased parental investment among primates and other mammals: a critical evaluation of the Trivers-Willard hypothesis. In: Gelles R, Lancaster JB, editors. *Child Abuse and Neglect: Biosocial Dimensions*. New York: Aldine.
- Isaac JL, Krockenberger AK, Johnson CN, 2005. Adaptive sex allocation in relation to life-history in the common brushtail possum *Trichosurus vulpecula*. *J Anim Ecol* 74:552–558.
- Kleiber M, 1961. *The Fire of Life: An Introduction to Animal Energetics*. New York: John Wiley & Sons.
- Kruuk LEB, Clutton-Brock TH, Slate J, Pemberton JM, Brotherstone S et al., 2000. Heritability of fitness in a wild mammal population. *P Nat Acad Sci USA* 97:698–730.
- Kruuk LE, Livingston J, Kahn A, Jennions MD, 2015. Sex-specific maternal effects in a viviparous. *Fish Biol Lett* 11:20150472.
- Landete-Castillejos T, Garcia A, Garde J, Gallego L, 2000. Milk intake and production curves and allosuckling in captive Iberian red deer *Cervus elaphus hispanicus*. *Anim Behav* 60:679–687.
- Landete-Castillejos T, Garcia A, Langton S, Inglis I, Gallego L et al., 2001a. Opposing offspring sex ratio variations with increasing age and weight in mouflon mothers *Ovis musimon*. *Acta Vet Hung* 49:257–268.
- Landete-Castillejos T, García AJ, Gallego L, 2001b. Calf growth in captive Iberian red deer *Cervus elaphus hispanicus*: effect of birth date and hind milk production and composition. *J Anim Sci* 79:1085–1092.
- Landete-Castillejos T, Garcia AJ, Lopez-Serrano FR, Gallego L, 2005. Maternal quality and differences in milk production and composition for male and female Iberian red deer calves *Cervus elaphus hispanicus*. *Behav Ecol Sociobiol* 57:267–274.
- Langvatn R, Mysterud A, Stenseth NC, Yoccoz NG, 2004. Timing and synchrony of ovulation in red deer constrained by short northern summers. *Am Nat* 163:763–772.
- Liggins GC, 1979. Initiation of parturition. *Br Med Bull* 35:145–150.
- Maynard-Smith J, 1980. A new theory of sexual investment. *Behav Ecol Sociobiol* 7:247–251.
- Malo AF, Garde JJ, Soler AJ, García AJ, Gomendio M et al., 2005a. Male fertility in natural populations of red deer is determined by sperm velocity and the proportion of normal spermatozoa. *Biol Reprod* 72:822–829.
- Malo AF, Roldan ER, Garde J, Soler AJ, Gomendio M, 2005b. Antlers honestly advertise sperm production and quality. *P R Soc B Biol Sci* 272:149–157.
- Moore EP, Hayward M, Robert KA, 2015. High density, maternal condition, and stress are associated with male-biased sex allocation in a marsupial. *J Mammal* 96:1203–1213.
- Mysterud A, Roed KH, Holand O, Yoccoz NG, Nieminen M, 2009. Age-related gestation length adjustment in a large iteroparous mammal at northern latitude. *J Anim Ecol* 78:1002–1006.
- Plard F, Gaillard JM, Coulson T, Hewison AM, Delorme D et al., 2014a. Mismatch between birth date and vegetation phenology slows the demography of roe deer. *PLoS Biol* 12:e1001828.
- Plard F, Gaillard JM, Coulson T, Hewison AJ, Delorme D et al., 2014b. Long-lived and heavier females give birth earlier in roe deer. *Ecography* 37: 241–249.
- Quesnel L, MacKay A, Forsyth DM, Nicholas KR, Festa-Bianchet M, 2017. Size, season and offspring sex affect milk composition and juvenile survival in wild kangaroos. *J Zool* 302:252–262.
- Räsänen K, Kruuk LEB, 2007. Maternal effects and evolution at ecological time-scales. *Funct Ecol* 21:408–421.

- Robert KA, Braun S, 2012. Milk composition during lactation suggests a mechanism for male biased allocation of maternal resources in the tammar wallaby *Macropus eugenii*. *PLoS One* 7:e51099.
- Scott IC, Asher GW, Barrell GK, Juan JV, 2013. Voluntary food intake of pregnant and non-pregnant red deer hinds. *Livestock Sci* 158:230–239.
- Silk JB, 1983. Local resource competition and facultative adjustment of sex ratios in relation to competitive abilities. *Am Nat* 121:56–66.
- Sheldon BC, West SA, 2004. Maternal dominance, maternal condition, and offspring sex ratio in ungulate mammals. *Am Nat* 163:40–54.
- Stopher KV, Pemberton JM, Clutton-Brock TH, Coulson T, 2008. Individual differences, density dependence and offspring birth traits in a population of red deer. *P R Soc B Biol Sci* 275:2137–2145.
- Thouless CR, Guinness FE, 1986. Conflict between red deer hinds: the winner always wins. *Anim Behav* 34:1166–1171.
- Trivers RL, Willard DE, 1973. Natural selection of parental ability to vary the sex ratio of offspring. *Science* 179:90–92.
- Veeroja R, Kirk A, Tilgar V, Säde S, Kreitsberg M et al., 2010. Conception date affects litter type and foetal sex ratio in female moose in Estonia. *J Anim Ecol* 79:169–175.
- Veller C, Haig D, Nowak MA, 2016. The Trivers-Willard hypothesis: sex ratio or investment? *P R Soc B Biol Sci* 283:20160126.
- Verme LJ, 1965. Reproduction studies on penned white-tailed deer. *J Wildlife Manag* 29:74–79.
- de Vries H, 1998. Finding a dominance order most consistent with a linear hierarchy: a new procedure and review. *Anim Behav* 55:239–245.
- Wagenmakers EJ, Farrell S, 2004. AIC model selection using Akaike weights. *Psychonom Bull Rev* 11:192–196.
- Wilson AJ, Festa-Bianchet M, 2009. Maternal effects in wild ungulates. In: Maestripieri D, Mateo JM, editors. *Maternal Effects in Mammals*. Chicago (IL): University of Chicago Press.
- Wood PDP, 1967. Algebraic model of the lactation curve in cattle. *Nature* 216:164–165.