

Age-related gestation length adjustment in a large iteroparous mammal at northern latitude

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Summary

1. There is considerable interest in patterns of age-dependent reproductive effort and reproductive timing of large iteroparous mammals living in strongly seasonal environments. Due to lack of data on both timing of mating and birth, there is generally little insight into whether variation in gestation length play a role for life-history patterns observed for large mammals at northern latitudes.
2. Based on data on both timing of mating and birth of 88 female reindeer (and paternity confirmed with DNA fingerprinting), we explore the view that adjustment of gestation length plays a role in the reproductive tactic.
3. Observed gestation lengths of reindeer varied between 211 and 229 days (mean of 221 days). Consistent with a dynamic view of gestation length, variation could be predicted from life-history traits. Gestation length was longer for male than female offspring, which is expected in polygynous species where males benefit more from extra allocation of maternal resources. Gestation length increased with maternal age both due to direct effects and indirect effects linked to earlier mating of older, heavier females. Early mating females increased gestation length. A relatively small effect of female age on birth mass operated through variation in gestation length.
4. Our analysis supports the view that adjustment of gestation length is a part of the reproductive tactic of large mammals in northern environments.

Key-words: life-history tactics, maternal allocation, *Rangifer tarandus*, reproductive synchrony, seasonality

Introduction

Studies of age-related reproductive effort in mammals typically report variation in traits such as likelihood of reproduction, calf body mass, calf survival or similar traits (Gaillard *et al.* 2000). Variation in these traits is the key to understand dynamics of the strongly age-structured populations of large mammals (Coulson *et al.* 2001). Reproductive effort in female mammals peaks in late gestation and during lactation (Clutton-Brock, Albon & Guinness 1989), but there is limited knowledge of how female age may affect relative allocation during different periods. Furthermore, it is usually assumed that timing of birth is regulated at time of oestrus or ovulation (Clutton-Brock, Guinness & Albon 1982; Langvatn *et al.* 2004), and selection for earlier calving has been reported with

recent climate change (Coulson *et al.* 2003). The role of phenotypic plasticity in gestation length among different aged females has largely been ignored in the literature. Until the landmark paper by Berger (1992), gestation length was considered to be fairly fixed (Clutton-Brock *et al.* 1982) and a constraint to seasonal breeding (Kiltie 1988). However, gestation length can be variable within species (Kiltie 1982, 1988).

The study of how gestation length adjustment may form part of an adaptive reproductive tactic facilitating reproductive synchrony or offspring viability has been hampered by the difficulty in obtaining the relevant data on timing of both mating and birth (Berger 1992). In the animal husbandry literature with abundant data, focus has been on estimating heritability of gestation length (Bourdon & Brinks 1982; Crews 2006). A few reports link gestation length to female age (e.g., Crews 2006), but without paying attention to the link between female age and the life-history timing aspects,

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possibly due to lack of seasonal breeding in cattle. Age may be predicted to have an important role for potential for gestation length adjustments, since females are ovulating earlier in autumn with increasing age until they reach senescence and delay again (Langvatn *et al.* 2004).

Here we analyse variation in gestation length of reindeer (*Rangifer tarandus* L.), and test the hypothesis that it is a reproductive tactic in the sense that it can be predicted from life-history traits such as age or body condition (Berger 1992). Offspring birth mass is tightly linked to lifetime reproductive success, in particular in males (Kruuk *et al.* 1999). We predict a longer gestation length for male than female offspring (Clutton-Brock *et al.* 1982) as, in highly polygynous species, male offspring benefit more from allocation of extra maternal resources than females (Kruuk *et al.* 1999). We predict increased gestation length with increasing female age, as ungulates allocate less in somatic growth and more into offspring, and since mating is earlier (Langvatn *et al.* 2004). We predict that increased gestation length is a way to increase offspring body mass and/or to achieve reproductive synchrony. We aim to quantify the path by which female age affect birth mass being either direct effects of superior condition or more indirectly due to earlier mating and longer gestation length.

Material and methods

STUDY AREA

The study was conducted at the Kutuharju Field Reindeer Research Station, in Kaamanen, Finland (69°N, 27°E). Most of the area is forested with pubescent and mountain birches (*Betula pubescens* Ehrh.; *Betula pubescens czerepanovii* Orlova) and Scots pine (*Pinus sylvestris* L.) interspersed with lakes and bogs. The terrain is hilly. The area is fenced (totalling ~40 km²) in several compartments, including two large rutting (September–November) enclosures, Lauluvaara (13.8 km²) and Siniöivi (15 km²), and one smaller calving paddock (0.5 km²). The herds were supplementary fed during winter only.

REINDEER DATA

During the period 1996–2005, we obtained both mating date and subsequent calf birth date (42 female offspring and 46 male offspring) for 88 offspring produced by 67 females aged between 1 and 12 at time of mating (Table S1). No twinning was recorded, and we seldom obtained data from the same female in consecutive years; only 3 females gave 3 data points and 15 females gave 2 data points. Data derive from a long-term experiment manipulating mainly the male segment of the population (Røed *et al.* 2007; see Table S2 for an overview of treatments). Data for females without access to males during first rut were not included (Holand *et al.* 2006). Females were kept within a smaller enclosure (0.5 km²) during the calving season, so that data on calf sex, birth date (daily observation) and mother–calf assignments were obtained and the calves were individually tagged. Adults are weighed by leading them into a cage with a balance, while newborn calves were weighted using a handheld spring scale.

Data on mating date were obtained by visual observations with binoculars in the field. A potential problem is that the mating observed is not the mating leading to fertilization. The potential

open window for fertilization is about 48 h (Ropstad 2000). We normally surveyed all the mating groups daily and observed each main group for an average of about 4 h during daylight. Therefore, multiple mating by the same male or mating by multiple males cannot be ruled out. In two cases, we observed mating by the same pair on two consecutive days. After mating by the dominant male, it was very unusual to observe another male mating despite other males' presence and attempts to do so. However, we did record a few cases of females mating with more than one male. We here only included observed mating dates if (i) all matings were recorded within a single day, and (ii) paternity analysis confirmed this male was the actual sire. The very low frequency of such observations clearly indicates bias is unlikely to be a problem.

Analyses of paternity were based on scoring polymorphic DNA microsatellites from blood samples obtained from all individuals. Fifteen reindeer-specific microsatellite loci (NVHRT-01, -03, -16, -21, -24, -30, -31, -34, -48, -73 and -76, Røed & Midthjell 1998, and RT-1, -5, -6, and -27, Wilson *et al.* 1997) were first employed on all the individuals within each treatment. For a few cases, where paternity could not be accurately resolved by use of these loci, up to eight additional microsatellite loci were analysed (see methods in Røed *et al.* 2002). For assigning paternity, we used the likelihood ratio approach in the computer program CERVUS (Marshall *et al.* 1998) with a criterion for assignment of paternities set at 95% confidence level.

STATISTICAL ANALYSES

We analysed direct and indirect effects (operating through date of mating) of female traits (age, body mass, primiparity/multiparity) on variation in gestation length (Julian mating date minus Julian calving date) and (log) birth mass (kilograms) using path analysis (Shipley 2002). Linear models were used throughout, as partial residuals and additive models (Wood 2006) showed no marked evidence of nonlinearity (see Figs S1 and S2). Parameter estimation was carried out with Bayesian methods (Gelman & Hill 2007) as some distributions were not normal (primiparous vs. multiparous). Such an approach requires assumptions of prior distributions for model parameters (regression coefficients and residual variances) and use of Bayes' theorem to derive the posterior distributions of parameters. The following prior distributions were used: normal for regression intercepts and slopes with a mean of 0 and a variance of 10⁶, and gamma for the inverse of residual variances with shape and scale parameters 0.001 and 0.001 (Gelman & Hill 2007). Markov chain Monte Carlo simulations were used to derive posterior distributions. Model fitting and model diagnostics (in particular the convergence of numerical simulations) were carried out using the library BRugs and coda in the R environment (Thomas *et al.* 2006; R Development Core Team 2007), using version R 2.7.2. An initial burn-in of 20 000 iterations was used, and posterior distributions of parameters were based on 100 000 more iterations. We used three chains to check for the stability of posterior distribution estimates (as recommended in Clark & Gelfand 2006) and assessed convergence and autocorrelation using the diagnostics tools available in BRugs and coda (e.g. convergence test based on Gelman & Rubin 1992). Female age (SD = 2.63), body mass (SD = 7.35), birth date (SD = 5.20) and mating date (SD = 5.45) was standardized before analysis (mean = 0, SD = 1). We used 95% credible intervals as a measure of uncertainty.

We also assessed if models with random terms for 'female ID' and 'treatment' gave better fit or changed uncertainty of parameter estimates. Note that inclusion of 'treatment' accounts for both between-year variation and differences between locations, in addition

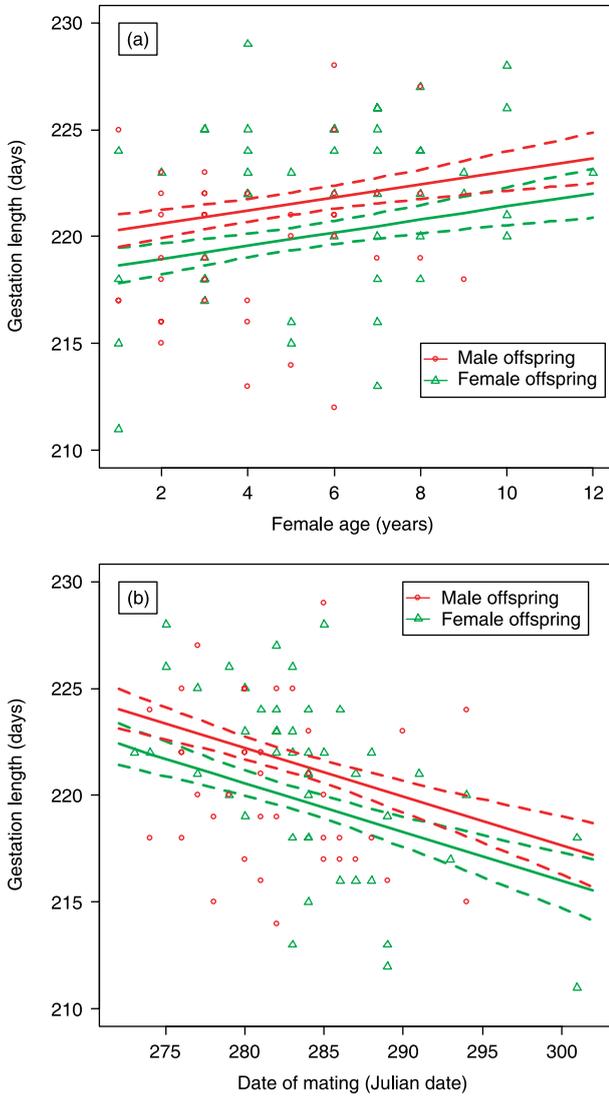


Fig. 1. Gestation length (days) as a function of (a) female age and (b) date of mating for reindeer in Kaamanen, Finland. Note that the predicted lines are adjusted for (a) date of mating (given for mean) and (b) female age (given for 5 years of age), while data points are raw data. Dotted lines indicate standard errors.

to the treatment effect (i.e., the male segment of the population). We estimated the variation related to these factors in a mixed model (using library 'ape').

We further tested whether overall synchrony differed for 'mating date' and 'birth date' by bootstrapping the ratio of variances of mating date and birth date (10 000 resamples were used).

Results

Observed gestation lengths varied between 211 and 229 days with a mean of 221 days. Gestation length increased with female age and was shorter when mothers gave birth to a female rather than a male offspring (Figs 1 & 2a). There was a nonsignificant tendency for an interaction; gestation length for male offspring was fairly stable with increasing female age, but with female offspring the gestation length increased more

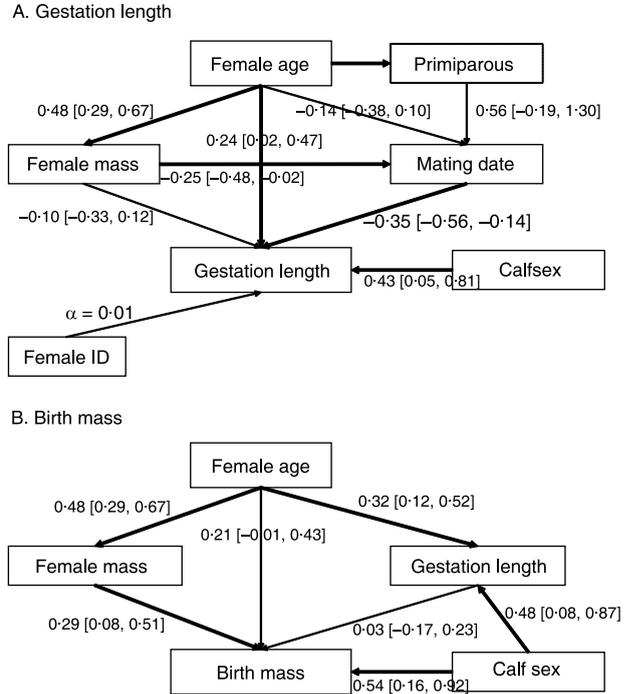


Fig. 2. Path analysis of the direct and indirect relationship of factors affecting (a) gestation length and (b) birth mass in female reindeer, Finland. 95% credible interval in brackets. Thick lines indicate that 95% credible interval does not overlap zero. Note that the relationship between whether female was primiparous or multiparous and age was estimated with a logistic link, however, the link is so strong (separation between young primiparous and old multiparous females) that sensible estimates are not achieved. Female ID was added as a random term, but had little impact on estimates and in particular standard errors.

markedly with female age. Date of mating strongly affected gestations lengths, earlier mating females extended gestation (Fig. 2b). Body mass was in turn the only strong predictor of mating date (Fig. 2a); heavy females mated earlier than light females. There was thus also a strong indirect effect of female age on gestation length operating through the effect body mass had on mating date. Due to the very strong correlation between female age and parity (all seven of the 1-year-olds and two out of the nine 2-year-olds were primiparous), the logistic regression of parity as a function of age did not provide sensible estimates (nearly complete separation: Heinze & Schemper 2002), but that did not affect other parameter estimates which showed good convergence properties (Gelman–Rubin upper limit close to 1) and were based on large effective sample size. Only 3.4% of the variation in gestation length was attributable to between-female variation, while 17.1% of the variation could be attributed to 'treatment'. Inclusion of these as random terms had very little impact on parameter estimates (see Fig. S3).

Synchrony for date of mating and date of birth were similar [variance mating date/variance birth date = 1.10, bootstrapped 95% CI (0.80; 1.50)], but note that data was pooled over all years. Observed variance ratios varied from year to year between 0.57 and 3.30 due to small sample size each year.

Birth mass correlated positively with gestation length; however, this was due to the correlation between gestation length and other factors (Fig. 2b). Birth mass increased with female body mass, was higher for male offspring, and tended to increase with female age as well. There was no direct effect of gestation length on birth mass after accounting for these factors (Fig. 2b).

Discussion

Our study highlights the role of gestation length adjustment as a reproductive tactic of large mammals in seasonal environments. Gestation length in reindeer was markedly related to maternal age, was shorter when females gave birth to female rather than male offspring, but only indirectly (through mating time) related to female condition. Our results thus differ in detail from the other extensive analysis of intraspecific gestation length (Berger 1992). Primiparous bison (*Bison bison* L.) females exceeded multiparous females in gestation length by 8 days, heavier females shortened gestation if mating late, but no age effect was found (Berger 1992). Since age, condition and primiparity are correlated, details in the species life history may determine which trait is picked up as the more important if these relationships are not modelled explicitly as in our case. It nevertheless points to a similar conclusion; that the intraspecific variation in gestation length can be related to the life history, as suggested also inter-specifically (Ryan, Knechtel & Getz 2007).

In iteroparous, large (female) mammals, fitness is closely linked to longevity. Longevity increases the number of reproductive seasons, and also experience plays a role in increasing reproductive success (Weladji *et al.* 2006). Living at northern latitudes, a key constraint is the trade-off between further allocations in current offspring during autumn, stopping lactating and gain condition to have a head start for the next breeding season, or to allocate more in own somatic growth for primiparous females (Hogg, Hass & Jenni 1992; Langvatn *et al.* 2004). Likely due to the trade-off between further somatic growth in younger females, female red deer (*Cervus elaphus* L.) are ovulating earlier with increasing age before senescence (Langvatn *et al.* 2004). Similarly in reindeer, the timing of mating was earlier with increasing age, being mainly linked to female body mass. We show for the first time an age effect on gestation length linked to timing of mating, but that there also were effects of female age on gestation length beyond this relationship (Fig. 2a). We interpret this as females breeding earlier in autumn are delaying birth and allocating more in offspring during pregnancy. As our data are transversal (rather than following individuals over time), we cannot rule out that those surviving to older age were initially higher quality individuals beyond the effect of body mass (Weladji *et al.* 2006; Weladji *et al.* 2008). Indeed, there seems to be a tendency for reduced variability at old age (Fig. 1), which may be due to older females being of better quality. In captive red deer, early mated females also extended gestation so that birth was delayed (García *et al.* 2006). Maternal investment pattern often differs between males and

females in highly polygynous ungulates, as small male offspring have a particularly low reproductive success (Kruuk *et al.* 1999). Indeed, female reindeer extended gestation with male offspring; gestation length was similarly two days longer for male than female offspring in red deer (Clutton-Brock *et al.* 1982) and 1.26 days longer in cattle (Crews 2006). No such effects was found in bison (Berger 1992).

The above results thus provide new insight into how ungulates in northern environments adjust gestation length (Fig. 2a). Without controlling for other factors, gestation length was positively correlated with birth mass. Offspring being smaller at birth are also smaller in autumn (Berger 1992; Holand *et al.* 2006), and subsequently more prone to dying during the following winter. However, many traits are correlated. When linking the different paths by which maternal traits can affect offspring birth mass into a quantitative path analysis framework, female body condition and age, operating via other pathways than gestation length, were quantitatively more important than gestation length for resulting body mass. More data from each year would be required to properly address whether achieving synchrony might be a key to the delayed birth of early breeding females as suggested by Berger (1992).

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References

- Berger, J. (1992) Facilitation of reproductive synchrony by gestation adjustment in gregarious mammals: a new hypothesis. *Ecology*, **73**, 323–329.
- Bourdon, R.M. & Brinks, J.S. (1982) Genetic, environmental and phenotypic relationships among gestation length, birth weight, growth traits and age at first calving in beef cattle. *Journal of Animal Science*, **55**, 543–553.
- Clark, J.S. & Gelfand, A.E. (2006) *Hierarchical Modelling for the Environmental Sciences*. Oxford University Press, Oxford, UK.
- Clutton-Brock, T.H., Guinness, F.E. & Albon, S.D. (1982) *Red Deer: Behaviour and Ecology of Two Sexes*. Edinburgh University Press, Edinburgh.
- Clutton-Brock, T.H., Albon, S.D. & Guinness, F.E. (1989) Fitness costs of gestation and lactation in wild mammals. *Nature*, **337**, 260–262.
- Coulson, T., Catchpole, E.A., Albon, S.D., Morgan, B.J.T., Pemberton, J.M., Clutton-Brock, T.H., Crawley, M.J. & Grenfell, B.T. (2001) Age, sex, density, winter weather, and population crashes in Soay sheep. *Science*, **292**, 1528–1531.
- Coulson, T., Kruuk, L.E.B., Tavecchia, G., Pemberton, J.M. & Clutton-Brock, T.H. (2003) Estimating selection on neonatal traits in red deer using elasticity path analysis. *Evolution*, **57**, 2879–2892.
- Crews, D.H. (2006) Age of dam and sex of calf adjustments and genetic parameters for gestation length in Charolais cattle. *Journal of Animal Science*, **84**, 25–31.
- Gaillard, J.-M., Festa-Bianchet, M., Yoccoz, N.G., Loison, A. & Toigo, C. (2000) Temporal variation in fitness components and population dynamics of large herbivores. *Annual Review of Ecology and Systematics*, **31**, 367–393.
- García, A.J., Landete-Castillejos, T., Carrión, D., Gaspar-López, E. & Gallego, L. (2006) Compensatory extension of gestation length with advance of conception in red deer (*Cervus elaphus*). *Journal of Experimental Zoology*, **305A**, 55–61.

- Gelman, A. & Hill, J. (2007) *Data Analysis Using Regression and Multilevel Hierarchical Models*. Cambridge University Press, Cambridge, UK.
- Gelman, A. & Rubin, D.B. (1992) Inference from iterative simulation using multiple sequences. *Statistical Science*, **7**, 457–511.
- Heinze, G. & Schemper, M. (2002) A solution to the problem of separation in logistic regression. *Statistics in Medicine*, **21**, 2409–2419.
- Hogg, J.T., Hass, C.C. & Jenni, D.A. (1992) Sex-biased maternal expenditure in Rocky Mountain bighorn sheep. *Behavioral Ecology and Sociobiology*, **31**, 243–251.
- Holand, Ø., Mysterud, A., Røed, K.H., Coulson, T., Gjostein, H., Weladji, R.B. & Nieminen, M. (2006) Adaptive adjustment of offspring sex ratio and maternal reproductive effort in an iteroparous mammal. *Proceedings of the Royal Society of London, Series B*, **273**, 293–299.
- Kiltie, R.A. (1982) Intraspecific variation in the mammalian gestation period. *Journal of Mammalogy*, **63**, 646–652.
- Kiltie, R.A. (1988) Gestation as a constraint on the evolution of seasonal breeding in mammals. *Evolution of Life Histories of Mammals. Theory and Pattern*. (ed. M.S. Boyce), pp. 257–289. Yale University Press, New Haven and London.
- Kruuk, L.E.B., Clutton-Brock, T.H., Rose, K.E. & Guinness, F.E. (1999) Early determinants of lifetime reproductive success differ between the sexes in red deer. *Proceedings of the Royal Society B: Biological Sciences*, **266**, 1655–1661.
- Langvatn, R., Mysterud, A., Stenseth, N.C. & Yoccoz, N.G. (2004) Timing and synchrony of ovulation in red deer constrained by short northern summers. *American Naturalist*, **163**, 763–772.
- Marshall, T.C., Slate, J., Kruuk, L.E.B. & Pemberton, J. (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7**, 639–655.
- R Development Core Team (2007) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Røed, K.H. & Midtjell, L. (1998) Microsatellites in reindeer, *Rangifer tarandus*, and their use in other cervids. *Molecular Ecology*, **7**, 1771–1788.
- Røed, K.H., Holand, Ø., Smith, M.E., Gjostein, H., Kumpula, J. & Nieminen, M. (2002) Reproductive success in reindeer males in a herd with varying sex ratio. *Molecular Ecology*, **11**, 1239–1243.
- Røed, K.H., Holand, Ø., Mysterud, A., Tverdal, A., Kumpula, J. & Nieminen, M. (2007) Male phenotypic quality influences offspring sex ratio in a polygynous ungulate. *Proceedings of the Royal Society B: Biological Sciences*, **274**, 727–733.
- Ropstad, E. (2000) Reproduction in female reindeer. *Animal Reproduction Science*, **60** (S1), 561–570.
- Ryan, S.J., Knechtel, C.U. & Getz, W.M. (2007) Ecological cues, gestation length, and birth timing in African buffalo (*Syncerus caffer*). *Behavioral Ecology*, **18**, 635–644.
- Shipley, B. (2002) *Cause and Correlation in Biology: A User's Guide to Path Analysis, Structural Equations and Causal Inference*. Cambridge University Press, Cambridge, UK.
- Thomas, A., O'Hara, R.B., Ligges, U. & Sturtz, S. (2006) Making BUGS Open. *R News*, **6**, 12–17.
- Weladji, R.B., Gaillard, J.-M., Yoccoz, N.G., Holand, Ø., Mysterud, A., Loison, A., Nieminen, M. & Stenseth, N.C. (2006) Good reindeer mothers live longer and become better in raising offspring. *Proceedings of the Royal Society B: Biological Sciences*, **273**, 1239–1244.
- Weladji, R.B., Loison, A., Gaillard, J.-M., Holand, Ø., Mysterud, A., Yoccoz, N.G., Nieminen, M. & Stenseth, N.C. (2008) Heterogeneity in individual quality overrides costs of reproduction in female reindeer. *Oecologia*, **156**, 237–247.
- Wilson, G.A., Strobeck, C., Wu, L. & Coffin, J.W. (1997) Characterization of microsatellite loci in caribou, *Rangifer tarandus*, and their use in other artiodactyls. *Molecular Ecology*, **6**, 697–699.
- Wood, S. (2006) *Generalized Additive Models: An Introduction with R*. Chapman & Hall, Boca Raton, Florida.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1 Sample size (*n*) per female age class for reindeer in Finland

Table S2 Reindeer breeding herd structure during the rut for the manipulated treatments of males present

Fig. S1 Gestation length (days) as a function of female age (yrs) and date of mating (Julian date) for reindeer in Kaamanen, Finland using AM plot within the R environment.

Fig. S2 AM plots within the R environment showing absence of marked non-linear effects.

Fig. S3 Path analysis of the direct and indirect relationship of factors affecting gestation length in female reindeer, Finland.

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