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Twinning rate and foetal sex ratio of moose Alces alces in Estonia during low-density and increasing population phases in relation to maternal age

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My study was carried out to evaluate hypotheses about the effects of maternal age and population growth rate on twinning rate and offspring sex ratio in moose *Alces alces*. Reproductive tracts from 128 female moose of known age and with embryos present were collected across Estonia during the hunting seasons (1 October-15 November) of 1993-1999. Maternal age was treated as an indicator of condition, with younger mothers assumed to be in poorer condition than older mothers. The sex of embryos was determined using cytological methods. As indexed by sex ratio, there was a clear increase in maternal investment as maternal age increased. The sex ratio in younger mothers was nearly one male to three females, whereas in older mothers it was nearly equal. If the age of the mother is a good index of condition, this can be explained by the Trivers-Willard theory of condition-dependant sex ratios. Yet, because the Trivers-Willard theory predicts the sex ratio only of an individual, older females might compensate for the excess of female offspring among subadults according to Fischer's equal allocation theory. Population phases were distinguished using data on fecundity. In the increase phase there was strong agreement with the Trivers-Willard theory, but there was no clear agreement in the low-density phase. This was probably a result of the greater participation of young females in reproduction during the increase phase. Foetal sex ratio did not differ between different population growth phases.

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Fisher (1930) offered the first scientific explanation of the variation in sex ratio. Fisher's equal allocation theory states that if the cost of the offspring of both sexes is equal, then the sex ratio should also be equal. This is based on the presumption that for both sexes, average reproductive success is reciprocal to their frequency in the population. If the cost of the offspring of both sexes is not equal, then Fisher predicts that parents should divide reproductive investment equally between both sexes, having more offspring of the 'cheaper' sex.

Trivers & Willard (1973) suggested that when "females deviate from the mean adult female condition they should show an increasing tendency to bias the production of their young toward one sex or the other". For polygynous mammals in which male fighting ability determines their access to females, male reproductive success relies heavily on condition. This is in turn related to their mother's ability to invest in them during the pre- and postnatal periods. In this area, females in better condition are more successful. Therefore, females

in good condition should produce an increased proportion of sons (Trivers & Willard 1973).

When the size of a litter is not fixed, the relationship between maternal condition and sex ratio is more complicated. Williams (1979) created a model of sex ratio in relation to litter size for species with small litter size and modest cost differences between sons and daughters. According to his model, females should change their litter size and sex ratio in relation to their condition in the following sequence: ♀ < ♂ < ♀♀ < ♂♀ < ♂♂ < ♀♀♀ < ♀♀♂ and so on as the mother's condition improves. If the cost difference is greater, then the preceding sequence differs, as shown by Cassinello & Gomendio (1996).

Fisher (1930) proposed that if reproductive value declines with age, then parents should invest more in their offspring near the end of their reproductive age. Ultimately this leads to the situation that when a parent (usually the mother) reaches the stage of life in which prospective reproduction is unlikely, she should invest all her existing resources in her current offspring in order to maximise their future success. This theory (known as terminal investment) has been suggested to be true for female red deer *Cervus elaphus* (Clutton-Brock, Guinness & Albon 1982, see Clutton-Brock (1984) for in-depth analysis).

Twinning rate, i.e. the average amount of twins, is probably one of the most sensitive indicators of the reproductive abilities of a moose *Alces alces* population. It is closely related to age (as shown by Solberg, Sæther, Strand & Loison 1999), which is in accordance with theoretical models predicting that allocation of energy to reproduction should increase as the cost of reproduction is generally higher among young than among old animals (Clutton-Brock 1991).

One of the factors affecting moose reproduction is local resource competition (Clark 1978), which is closely related to density. For example, Ferguson (2002) found that moose in Newfoundland experienced relatively high densities and low primary production rate. In contrast, Ontario moose living at low densities (regulated by wolves *Canis lupus*) exhibited higher production rates. Also, in a study by Ericsson, Wallin, Ball & Broberg (2001), female moose living at very high densities (0.7-0.9 moose/km²) showed senescence in reproduction (as indexed by litter size) starting from 12 years of age. In a study of female moose in Estonia no signs of reproductive senescence were found in moose up to 19 years of age (A. Kirk, pers. comm.).

There is fairly little information about the influence of population density and dynamics on sex ratio and the twinning rate. Kruuk, Clutton-Brock, Albon, Pemberton & Guinness (1999) showed that the relationship between maternal dominance and offspring sex ratio in red deer disappeared at high population density. The proportion of males born each year declined with increasing population density; changes in sex ratio corresponded to reductions in fecundity. In addition, research on Siberian lemmings *Lemmus lemmus* (Erlinge, Hasselquist, Swensson, Frodin & Nilsson 2000) revealed that the reproductive behaviour of female lemmings differed significantly in relation to population cycle phase. In increase-phase populations, all captured females were reproducing and females started to reproduce early in life. During the peak phase, only 6% of young and 63% of winter-born females were reproducing; females did not start reproducing until they were 5-6 months old.

In their study of twinning and sex ratio in moose in Finland, Nygren & Kojola (1997) explained the lack of significant differences in their study by low moose density (lack of intense competition between the members of the more sedentary sex) and a moderate degree of polygyny.

The purpose of my study was to examine the effect of population phase and maternal age on the foetal sex ratio and twinning rate in moose.

Material and methods

My study is based on female moose harvested all over Estonia during the hunting seasons (1 October-15 November) of the seven successive years 1993-1999.

Hunters collected ovaries, uteri (with embryos, if present), and lower jaws with teeth for age determination.

A total of 612 samples were studied. My research is based on individuals whose age was known and in which embryos were present (N = 128). Of moose cows, 6.8% were not fertilised (all of which were yearlings). Moose cows without embryos > 4 weeks old and moose cows from which I did not have a lower jaw bone were excluded from data analysis (because no determination of embryo sex or moose cow age was possible), but all individuals were included in the analysis of population phase.

Jüri Tõnisson from the Estonian Center for Forest Protection determined the age of moose cows based on lower jaw tooth wear; in some cases annual rings in tooth cement of cross-section of incisors were used. As stated by Markgren (1969), the accuracy of these methods decreases with the increasing moose age; and this was taken into consideration during data analysis by grouping older individuals (> 10 year old) into a single age group.

Several studies have shown a strong positive relationship between an ungulate mother's age and her fecundity (Sand 1996, Sæther & Haagenrud 1983, Caughley 1977). In my study, maternal age was assumed to be an indicator of condition.

The age of embryos was determined using methods described by Markgren (1969), and so was the sexing of older embryos (> 7weeks old). However, most of the collected embryos were younger than six weeks so an alternative sexing method was employed for these. A sample was taken from the embryo and cells were fixed and stained on a slide with 0.005% methyl-blue, and then examined within a few minutes on a microscope and scored for the presence or absence of a Barr body. A Barr body is an (inactive) body of the two X-chromosomes in female somatic cells that is visualised as a densely staining body within the nucleus. It is also called sex chromatin. A similar method was employed by King (1984). The suitability of the two sexing methods was tested on 15 embryos using both sex determination methods, and the results were fully identical.

Data analysis was performed in SAS 6.12 and Statistica 5.5. Discriminant function analysis was used to distinguish population phases. Year was applied as the discriminant and fecundity (ovulation rate) as the discriminative variable. Data on fecundity were based on Kirk & Tõnisson (2000), who used the same data set as I used in my study. Mean ages and mean numbers of embryos were compared using Mann-Whitney U-test unless otherwise indicated. All probabilities (P) were two-tailed.

The number of moose in Estonia is based on official counts performed by the Ministry of the Environment.

Results

The mean age of the moose cows included in my analysis was 4.57 years (SE = 0.23), with age varying from 1.5 to 13.5 years. The mean ages of mothers of singletons (N = 75) and mothers of twins (N = 53) were 3.97 (SE = 0.28) and 5.40 (SE = 0.38), respectively (Table 1). The difference in maternal age was significant (t-test: P < 0.001). There was also a clear increase in maternal
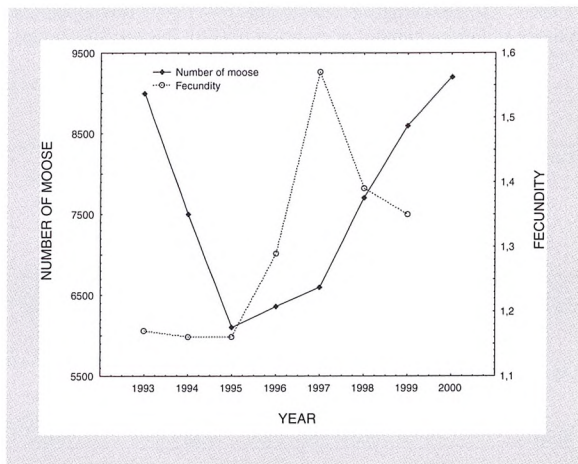

Figure 1. Number of moose in Estonia during 1993-2000, and their fecundity.

age in relation to the cost of a litter (as defined in Williams 1979; Spearman rank correlations: R = 0.3034; P = 0.0005).

Cows were divided into two age classes: young (1.5-3.5 years old) and prime-aged (> 4.5 years old). The age of 4.5 was selected as the beginning of the prime age class because younger cows (1.5-3.5 years old) had significantly fewer embryos. The mean number of embryos for young moose was 1.29 and for prime-aged moose it was 1.57, a significant difference (P = 0.001). There was no further change in the number of embryos or the amount of male embryos among cows > 4.5 years old; nor were there any signs of senescence in older cows.

Population data were divided into two phases using the number of moose in Estonia and fecundity: low-density (T = 1.27 for the years 1993-1996) and increase phase (T = 2.77 for the years 1997-1999; df = 1, F = 12.6; P = 0.01). The year 1993 was assigned to the low-density phase due to similarity with the years 1994-1996 in terms of fecundity. For comparison, the year 2000 is included in Figure 1.

When comparing the low-density and increase phases (Table 2), the increase in mean maternal age in relation to the cost of litters is present in the increase phase (Spearman: R = 0.428; P = 0.0001), whereas in the low-

Table 1. Mean age and numbers (/N) for the two age categories of Estonian moose cows during 1993-1999.

| Age in years | Singletons | | Twins | | |
	♀	♂	♀♀	♀♂	♂♂
2.5-3.5	2.71 / 34	2.26 / 17	2.71 / 14	2.66 / 6	3.5 / 1
> 4.5	6.76 / 15	7.5 / 9	7.5 / 6	6.94 / 16	7.3 / 10
Total	3.84 / 49	4.07 / 26	4.15 / 20	5.77 / 22	6.95 / 11
		3.97 / 75		5.40 / 53	

Table 2. Mean maternal age and number (/N) for the two population phases and mean number of embryos (/N) for the two age groups. NS = not significant.

| | Mean maternal age | | |
	Low-density	Increase	P
Sex			
♀	4.67 / 23	3.31 / 26	0.036
♂	4.5 / 12	3.71 / 14	NS
♀♀	4.5 / 7	4.03 / 15	NS
♂♀	5.78 / 7	5.76 / 15	NS
♂♂	6.25 / 7	7.36 / 7	NS
Total	4.89 / 51	4.37 / 77	NS
Embryos	4.614 / 35	3.45 / 40	0.044
Singletons	5.5 / 16	5.36 / 37	NS
Twins	4.89 / 51	4.37 / 77	NS
Age of mother in years			
1.5-3.5	1.22 / 27	1.33 / 45	NS
> 4.5	1.42 / 22	1.69 / 32	0.043
Total	1.31 / 51	1.48 / 77	0.062

density phase this trend was not significant. Also, the difference of maternal age in the low density and increase phases was present in 'cheaper' litters (♀-♀♀, 'cheaper' according to Williams's model); in more expensive litters (♂♀-♂♂) there was no marked difference in maternal age. Furthermore, the difference in mean maternal age was greater (and more significant) in the 'cheapest' litters (♀) and the difference was smaller (and near-significant) in litters of medium cost (♂, ♀♀).

A difference in maternal age between the phases in relation to litter size was significant in singletons, but not in twins. The age difference between cows with singletons and cows with twins was significant (P = 0.001) in the increase phase, but not in the low-density phase.

The difference in the average litter size in relation to population phase was significant in prime-aged females (> 4.5 years old), but not in young females (1.5-3.5 years old).

Table 3. General distribution of samples according to the two maternal age classes (1.5-3.5 and > 4.5 years), litter size (singletons or twins) and sex ratio.

| | Age in years /N | |
	1.5-3.5	> 4.5
Singletons	70.8% / 51	42.8% / 24
Singletons	58.5% / 75	
♀	47.2% / 34	26.8% / 15
♀	38.2% / 49	
♂	23.6% / 17	16.1% / 9
♂	20.3% / 26	
Twins	29.1% / 21	57.2% / 32
Twins	41.4% / 53	
♀♀	18.4% / 14	10.7% / 6
♀♀	15.6% / 20	
♂♀	8.3% / 6	28.6% / 16
♂♀	17.1% / 22	
♂♂	1.3% / 1	5.1% / 10
♂♂	8.6% / 11	
Total	51.4% / 93	48.6% / 88
♀	73.1% / 68	48.9% / 43
♀	61.3% / 111	
♂	26.9% / 25	51.1% / 45
♂	38.7% / 70	

The distribution of the sample in relation to maternal age class, litter size and sex ratio is shown in Table 3.

The influence of terminal investment on sex ratio and the twinning rate was not included in my study due to the small number of old animals in the sample.

Discussion

As they grow older, female moose increase their reproductive investment as shown by litter size and sex ratio. If age indexes condition, then there is clear agreement with Williams's (1979) model, which is supported also by the difference in mean ages of mothers of singletons and twins. Yet the distribution of maternal age in the low-density phase differs from Williams's model as there is no significant difference in the mean ages of mothers of singletons and twins. There is, however, a strong agreement with the model in the increase phase. This is probably a result of a larger participation of young females (whose condition differs more from the population median condition) in breeding during the increase phase.

It can be presumed that Williams's model (and the Trivers-Willard theory on which this model is based) is expressed even more strongly in the population peak phase, when environmental capacity is reached, and there is stronger female-female competition for resources. It can also be expected that local resource competition starts to affect sex ratio in the peak phase when there is intense competition between members of the more sedentary sex. The moose, like other large herbivores, may experience the effects of population density only when density is near environmental capacity (Fowler 1987). It has also been pointed out that with the rise in density, general fertility also increases due to the decrease in non-fertilised females in the population (Matveyev & Bakunin 1994). With extremely high population densities and low food resources fertility decreases, fewer cows are impregnated, the number of twins decreases, yearlings do not participate in reproduction, there is more embryo resorbence and the postnatal mortality of calves is higher (Matveyev & Bakunin 1994).

As pointed out by Sutherland (1996), younger individuals breed if the density is reduced, but not at high levels of density. It is clear that young females participate more in breeding during the increase phase, and the mean litter size of older females enlarges significantly.

One may assume that in the low-density phase, when there are sufficient resources and little competition, grown females are in extremely good condition and are

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