ARE SEX-PHEROMONES INVOLVED IN MOOSE BREEDING BEHAVIOR

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ABSTRACT: Evidence is presented that saliva of bull moose (*Alces alces gigas*) contains 16-unsaturated C_{19} steroids. These pheromones have been identified in red deer (*Cervus elaphus hippelaphus*) and wild boar (*Sus scrofa*) and operate in the later as a potent primer stimulating estrus and copulation readiness of the sow. Saliva samples collected from mature bull moose contained a mean concentration 0.48 ng/ml ($n = 15$, SD = 0.17) of 5α-androst-16-en-3-one. Using thinlayer-chromatography, the musk-scent components were identified as 5α-androst-16-en-3α-ol (3.5 ng/ml) and 5α-androst-16-en-3ß-ol (3.5 ng/ml). Bull moose produce signalling pheromones in concentrations 10-20 times lower than those of the boar. Additional research is required to determine the role of these compounds in rut synchronization and induced estrus.

ALCES VOL. 26 (1990) pp. 104-107

Moose, like many other ruminants are spontaneous ovulators (Sadleir 1982). The annual breeding season, which begins earlier in males than females, occurs prior to the autumn equinox in late-September and early-October. Generally, it is assume that the timing of estrus in spontaneous ovulators (Jöchle 1973, Sadleir 1982) is an endogenous process, triggered by photoperiod. Estrus timing can be advantageous when the sex ratio is skewed (Bubenik and Timmerman 1982), and in northern latitudes where a short breeding season, results in parturation during a period with optimum conditions for offspring development (Bubenik, 1990).

The three phases of breeding, the attractive, proceptive, and receptive (Beach 1976) can be accelerated, delayed, or eventually induced by the presence of sexually active males or females (Ayorinde *et al* 1982, Pierce 1988, Jaczewski 1989, Nishimura *et al.* 1989). Rut synchronization results from many stimulating cues (visual, auditory, olfactory) during the breeding season (Bubenik 1987). Olfactory cues include sex pheromones contained in urine and saliva. The former are carried on the tarsal gland tufts on which both sexes urinate (Bubenik *et al.* 1979), whereas the latter are thought to be produced by the bull and smeared from his chin and bell to the cow by chinning (Lent 1974, Bubenik 1987).

A special group of steroids secreted by the boar testis are the 16-unsaturated C_{19} steroids (Grower *et al.* 1970, Claus and Hoffmann 1971, Andresen 1974) which have no hormonal activity but are released with the saliva before mating. They function as pheromones by stimulating the standing reflex in the sow and stimulating estrus in the gilt. These steroids play a positive role in synchronizing and promoting male and female courtship behavior in swine (Perry *et al.* 1980).

Based upon pilot studies conducted with red deer it could be shown that secretion timing and concentration of pheromones of the androstenone group were correlated with age and sexual performance of the stag (Claus and Bubenik pers. comm.). Based on these preliminary findings, Bubenik suggested that moose may also produce these pheromones, which could partially explain the cows behavior toward bull urine in the rutting pit. The purpose of this study was to analyze saliva of bull moose to determine if sex pheromones of the androstenone group were present.

METHODS

Fifteen saliva samples were collected on cotton swabs from 6 mature bull moose (ages 2-7 yrs) during the major period of the rut (5 Sep - 10 Oct). Samples were stored frozen and shipped to Germany unfrozen via airfreight (about 48 hrs transit) for analysis. Samples were analyzed for the 16-unsaturated C_{19} steroids following procedures described by Claus (1974). Sample preparation included extraction with hexane followed by a solvent distribution against 90% methanol. Aliquot portions of the methanol containing the steroid were dried and measured in the assay system. Alternatively, for more specific determinations of the corresponding androstenols, the extracts were transferred to thin-layer plates and chromatographed. The radio-immunoassay was carried out after individual elution. Values were corrected for procedural losses.

RESULTS AND DISCUSSION

The mean concentration of 5α-androst-16-en-3-one (5α-androstenone) in the 15 saliva samples was 0.48 ng/ml ± SD 0.17 (Table 1). The musk scent components were identified as 5α-androst-16-en-3α-ol and 5α-androst-16-en-3ß-ol (5α-androstenol), both with an average concentration of 3.5 ng/ml of saliva (Table 1). Concentrations in boar saliva of both the 5α-androstenone and the 5α-androstenols (Bonneau 1982) were incomparably higher than in moose saliva. Red deer saliva also contained 10-20 times more 5α-androstenone than moose saliva (Table 1).

The absolute concentration of pheromones in our samples likely were low. Collection of saliva on cotton swabs was difficult and consequently only small quantities were obtained making quantification difficult. Also, because pheromones are not stable compounds (Booth 1987), shipping time likely resulted in losses. Similarly, it has been established that there is considerable diurnal and seasonal variation in androstenone concentrations (Claus and Gimenez 1977, Bonneau 1982). Additional studies are underway to more closely quantify the actual concentrations.

Our results do confirm Bubenik's suspicion that moose produce both 5α-androstenone and 5α-androstenol. These powerful sex pheromones may play a significant role in rut synchronization and estrus in moose. These preliminary findings suggest that we must view the roll of the bull in the mating process. We can no longer simply consider the male a sperm donor who breeds with a cow when her physiological status dictates copulation. There is a high likelihood that the bull moose induces estrus in the female.

Table 1. Concentrations of 16-unsaturated C_{19} steroids in saliva of bull moose, the wild boar, and red deer stag.

Compound	5α-androstenone ng/ml	5α-androstenols ng/ml
Saliva		
Bull moose	0.48 ± SD 0.17	3.5
Red deer stag[1]	50 (10-80)	—
Wild boar[1]	50-300	1000-2930
Kidney fat		
Red deer stag	40-120	—
Blood plasma	0.6-2.2	—

[1]Red deer stag data from (Claus and Bubenik pers. comm.) and wild boar data from Bonneau (1982).

ACKNOWLEDGEMENTS

We thank D. C. Johnson and P. DelVecchio for sample collection. K. H. Hundertmark and S. Morgan reviewed the manuscript.

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