

## 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<sub>19</sub> 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 5 $\alpha$ -androst-16-en-3-one. Using thinlayer-chromatography, the musk-scent components were identified as 5 $\alpha$ -androst-16-en-3 $\alpha$ -ol (3.5 ng/ml) and 5 $\alpha$ -androst-16-en-3 $\beta$ -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 assumed 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 parturition 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<sub>19</sub> 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<sub>19</sub> 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 androst-enols, 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 5 $\alpha$ -androst-16-en-3-one (5 $\alpha$ -androst-enone) in the 15 saliva samples was 0.48 ng/ml  $\pm$  SD 0.17 (Table 1). The musk scent components were identified as 5 $\alpha$ -androst-16-en-3 $\alpha$ -ol and 5 $\alpha$ -androst-16-en-3 $\beta$ -ol (5 $\alpha$ -androst-enol), both with an average concentration of 3.5 ng/ml of saliva (Table 1). Concentrations in boar saliva of

both the 5 $\alpha$ -androst-enone and the 5 $\alpha$ -androst-enols (Bonneau 1982) were incomparably higher than in moose saliva. Red deer saliva also contained 10-20 times more 5 $\alpha$ -androst-enone 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 androst-enone 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 5 $\alpha$ -androst-enone and 5 $\alpha$ -androst-enol. 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<sub>19</sub> steroids in saliva of bull moose, the wild boar, and red deer stag.

Compound	5 $\alpha$ -androst-enone ng/ml	5 $\alpha$ -androst-enols ng/ml
Saliva		
Bull moose	0.48 $\pm$ SD 0.17	3.5
Red deer stag <sup>1</sup>	50 (10-80)	—
Wild boar <sup>1</sup>	50-300	1000-2930
Kidney fat		
Red deer stag	40-120	—
Blood plasma	0.6-2.2	—

<sup>1</sup>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.

## REFERENCES

- ANDRESEN, O. 1974. Development of a radioimmunoassay for 5 $\alpha$ -androst-16-en-3-one in pig peripheral plasma. *Acta Endocrinol (Kbh)* 76:377-3876.
- ASYORINDE, F., J. W. WHEELER, C. WEMMER, and J. MURTAUGH. 1982. Volatile components of the occipital gland secretion of the Bactrian camel (*Camelus bactrianus*). *J. Chem. Ecol.* 8:177-183.
- BEACH, F. A. 1976. Sexual attractivity, proceptivity and receptivity in female mammals. *Hormones and Behavior*, 7:105-138.
- BOOTH, W. D. 1987. Factors affecting the pheromone composition of voided boar saliva. *J. Reprod. Fert.* 81:427-431.
- BONNEAU, M. 1982. Compounds responsible for boar taint, with special emphasis on androstenone: a review. *Livestock Production Sci.* 9:687-705.
- BUBENIK, A. B. 1987. Behavior of moose (*Alces alces* spp) in North America. *Swedish Wildl. Res. Suppl.* 1:333-366.
- \_\_\_\_\_. 1990. Epigenetical, morphological, physiological and behavioral aspects of evolution of horns, pronghorns and antlers. Pages 3-113 in G. A. Bubenik and A. B. Bubenik, eds. *Horns, pronghorns and antlers*. Springer Publisher, New York.
- \_\_\_\_\_, and H. R. TIMMERMAN. 1982. Spermatogenesis in the taiga-moose of North Central Ontario - A pilot study. *Alces* 18:54-93.
- \_\_\_\_\_, M. DOMBALAGIAN, J. W. WHEELER, and O. WILLIAMS. 1979. The role of tarsal glands in the olfactory communication of the Ontario moose. *Alces* 15:119-147.
- CLAUS, R. 1974. Dosage radioimmunologique du 5- $\alpha$ -androst-16-en-3-one, steroide responsable de l'odeur de verrat, dans le tissu adipeux des porcs. *C. R. Seances Acad. Sci. Ser D*, 278:299-302.
- \_\_\_\_\_, and B. HOFFMANN. 1971. Determination of  $\Delta$  16-steroids in boars. *Acta Endocrinol. (Copenhagen.) Suppl.*, 155:70.
- \_\_\_\_\_, and T. GIMENEZ. 1977. Diurnal rhythm of 5- $\alpha$ -androst-16-en-3-one and testosterone in peripheral plasma of boars. *Acta Endocrinol. (Copenhagen)*, 84:200-206.
- GROWER, D. B., F. A. HARRISSON, R. B. HEAP, and R. L. S. PATTERSON. 1970. The identification of C19  $\Delta$  16-unsaturated steroids and estimation of 17-oxosteroids in boar spermatic vein plasma and urine. *J. Endocrinol.*, 47:357-368.
- JACZEWSKI, Z. 1989. Reproduction in the red deer female and the effect of oestrogens of the antler cycle and behavior. *Acta Phys. Pol.*, 40:85-95.
- JÖCHLE, W. 1975. Current research in coitus-induced ovulation: a review. *J. Reprod. Fert. Suppl.* 22:165-207.
- LENT, P. C. 1974. A review of rutting behavior in moose. *Naturaliste can.*, 101:307-323.
- NISHIMURA, K., K. UTSUMI, M. YUHARA, Y. FUJITANI, and A. IRITANI. 1989. Identification of puberty-accelerating pheromones in male mouse urine. *J. Exper. Zool.* 251, Part 3:300-305.
- PEARCH, G. P., P. E. HUGHES, and W. D. BOOTH. 1988. The involvement of boar submaxillary salivary gland secretions in boar-induced precocious puberty attainment in the gilt. *Anim. Reprod. Sci.* 16:123-125.
- PERRY, G. C., R. L. S. PATTERSON, J. J. H. MACFIE, and C. G. STINSON. 1980. Pig courtship behavior: pheromonal

property of androstene steroids in male submaxillary secretion. *Anim Prod.* 31:191-199.

SADLEIR, R. M. F. S. 1982. Reproduction of female cervids. Pages 123-144 *in* C. M. Wemmer, ed. *Biology and management of the cervidae*. Smithsonian Institution Press, Washington, D.C.