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Ontogeny of Flehmen in Sable Antelope, *Hippotragus niger*

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Abstract

Flehmen, a conspicuous posture characterized by eversion of the upper lip, facilitates the transfer of nonvolatile urinary chemicals to the vomeronasal organ and therefore has been implicated in the control of reproduction in ungulates. The ontogeny of urine sampling and flehmen was investigated in semi-free-ranging sable antelope, *Hippotragus niger*, at the National Zoological Park's Conservation and Research Center because behavioural evidence suggests that flehmen is a mechanism of reproductive synchronization among females. During the first year of life, flehmen rates increased with age in both sexes. Flehmen rates of female calves equalled those of adult females by 4 months of age. Male calves first exhibited flehmen at younger ages than did female calves and showed greater increases in flehmen rate during development. Both sexes exhibited flehmen primarily after sampling urine of female conspecifics as it was being voided. During the first 2 months of life, sable antelope preferred to sample urine of other calves, but by 1 year of age adult females were the preferred targets. Females approaching sexual maturity preferred to sample urine from postpartum females (presumably resuming oestrous cycling) rather than from pregnant females, as expected if they were attempting to synchronize oestrus with experienced females. Results are consistent with the hypothesis that flehmen serves to coordinate reproduction among females and further suggest that flehmen may affect reproductive maturation.

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Introduction

Social investigation in many mammalian species is marked by flehmen, a conspicuous posture characterized by eversion of the upper lip (ESTES 1972). In male ungulates, flehmen is a common component of precopulatory behaviour (DAGG & TAUB 1970; ESTES 1972) and typically follows direct sampling of female urine. It is thought that flehmen allows males to evaluate female reproductive status (HART 1983). Although female flehmen has long been considered unimportant (MYKYTOWYCZ 1976; HENDERSON et al. 1980; O'BRIEN 1982; REINHARDT 1983),

recent investigations have suggested that females may utilize flehmen to synchronize oestrus (PFEIFER 1985; BERGER 1992) or parturition (THOMPSON 1991, 1995).

The physiological mechanisms of reproductive synchrony in ungulates are unknown, but the relationship between flehmen and the vomeronasal organ (a component of the accessory olfactory system) suggests that flehmen may mediate social influences on reproductive physiology. Flehmen facilitates transmission of nonvolatile urinary compounds from the oral cavity to the sensory receptors of the vomeronasal organ (HART 1983). The vomeronasal organ impinges upon limbic structures involved in the neuroendocrine regulation of reproduction (reviewed by HALPERN 1987 and WYSOCKI & MEREDITH 1987) and has been hypothesized to mediate a wide variety of reproductive phenomena in rodents, including the acceleration of puberty in females, induction of ovulation and synchronization of oestrus (KEVERNE 1983).

The sable antelope, *Hippotragus niger*, is a particularly appropriate model for investigations of flehmen. In this species, there is strong behavioural evidence that females use flehmen to synchronize the timing of both oestrus and parturition (THOMPSON 1991, 1995). Flehmen frequencies are highest in females during the mating season, just prior to the time of conception. Females perform flehmen in a highly selective manner. Pregnant and postpartum females both preferentially investigate conspecific urine from females experiencing similar reproductive states, as would be expected if pregnant females are attempting to synchronize births and postpartum females are attempting to synchronize oestrus. Females preferentially perform flehmen to multiparous, adult females (which are likely to have achieved and maintained a high degree of reproductive synchrony) rather than to immature or primiparous individuals. The likelihood of any female giving birth near in time to other herdmates is strongly and positively correlated with her pre-partum flehmen rate. Birth synchrony is most pronounced among females that have investigated each other's urine during the months before parturition. The relationship between flehmen frequency and the degree of oestrous synchrony among herdmates is less clear, primarily because behavioural observations alone are insufficient for characterization of the oestrous cycle.

Flehmen frequencies in adult females are rank related (THOMPSON 1991). Dominant females exhibit the highest rates of urine investigation and flehmen and show the greatest degree of birth synchrony within the herd. Subordinates have low flehmen rates and rarely perform flehmen to females ranking above them. The relationship between flehmen and dominance status is somewhat confounded by the strong correlation between dominance rank and age (THOMPSON 1993): low rates of flehmen observed in subordinate females may have resulted from their relative immaturity rather than a lack of motivation or opportunity to investigate urine.

I examined the development of flehmen in sable antelope because flehmen is frequently observed in adults and juveniles of both sexes (ESTES & ESTES 1974) and it is thought to have important reproductive consequences for adult females (THOMPSON 1991, 1995). My objectives were: 1. to examine the extent of sex

differences among immature sable antelope, because sexual dimorphism in flehmen is reduced in adults, relative to other ungulate species; 2. to determine whether immature individuals sample urine selectively, as adult females do (THOMPSON 1995); and 3. to determine when immature females begin exhibiting flehmen at rates comparable to that of adult females.

Methods

I observed 39 immature sable antelope (21 males and 18 females) born into two herds at the National Zoological Park's Conservation and Research Center (CRC) near Front Royal, Virginia. Herd size and composition approximated that of free-ranging herds (ESTES & ESTES 1974); herd 1 contained one adult male, nine to eleven adult females and their immature offspring, while herd 2 consisted of one adult male, seven adult females and their offspring. Offspring were generally allowed to remain in their natal herd for approximately 1 yr, then were removed to prevent inbreeding. All animals were individually marked within 24 h of birth with plastic ear tags in unique right–left colour combinations.

I collected data on calves (born during the study year) and yearlings (born the previous year) during 473 h of observation over three seasons (9 Mar.–29 Sep. 1987, 17 May–31 Aug. 1988 and 7 May–5 Nov. 1989). Seventeen males and 14 females were observed from birth until at least 12 wk of age; 14 of these individuals (seven males and seven females) were observed again as yearlings. Four males and four females (born in 1986, prior to the onset of the study) were observed only as yearlings. The mean (± SE) span of data collection for individual calves was 20.1 (± 5.1) wk.

Calves were categorized into five cohorts, based on year of birth and the herd into which they were born. I observed each cohort 1–3 d per wk for periods of 2–14 h. Because individuals within a cohort tend to maintain very close proximity, I was able to watch all individuals in a given cohort simultaneously. Flehmen was operationally defined as all instances in which an animal stood with its muzzle elevated and upper lip everted for a period of at least 5 s. For each instance of flehmen by a yearling or calf, I recorded the following information: 1. the identity of the individual exhibiting flehmen; 2. the identity of the urinating individual that elicited flehmen (the target); and 3. the context (UR: sampling urine as it was voided; GR: investigating urine-soaked substrate; AG: anogenital sniffing; or O: other, e.g. grazing, sniffing air or unknown context). Data on flehmen by adult females (n = 11) in herd 1 were obtained during the 1987 season using the identical sampling regime (THOMPSON 1991, 1995).

When possible, parametric statistics were used to evaluate sex differences and age-related changes in flehmen. Where deviations from the assumptions of parametric statistical tests could not be rectified with data transformation, nonparametric statistics were used. A two-way ANOVA, with sex and cohort as main effects, was used to test for sex differences in the first appearance of flehmen. To examine ontogenetic changes in flehmen, I grouped data into 8-wk segments and calculated flehmen rates (acts/obs. h) for each individual in each time segment. All data points were plotted, but because not all individuals were observed at all ages, a repeated-measures ANOVA could not be performed. Instead, for each individual I regressed flehmen rate against age. I then compared the slopes of regression equations for male and female calves using analysis of variance.

Some, but not all, individuals were observed as both calves and yearlings, therefore neither matched-sample statistics nor independent-sample statistics could be used to examine differences between calves and yearlings within the entire data set. Statistically independent samples of calves and yearlings were obtained by taking a subset of each age class, with each individual represented in only one age class. The data set for calves (n = 23) consisted of observations of individuals born and observed in 1987 (cohort 2) and 1989 (cohorts 4 and 5). The yearling data set (n = 12) consisted of observations of individuals born in 1986 (cohort 1) and 1988 (cohort 3), observed during 1987 and 1989, respectively. For other analyses of sex differences and for comparisons of immatures and adults, analyses were carried out separately for calves and yearlings to avoid pseudoreplication. Flehmen contexts for immature males and females were compared with those observed in adult females using a Kruskall–Wallis test. Subsequent multiple comparisons were conducted with Mann–Whitney U-tests.

Targets of flehmen were categorized by sex (male or female) and age class (calf, yearling or adult). To analyse ontogenetic changes in flehmen targets, I used only individuals that had targets of each age

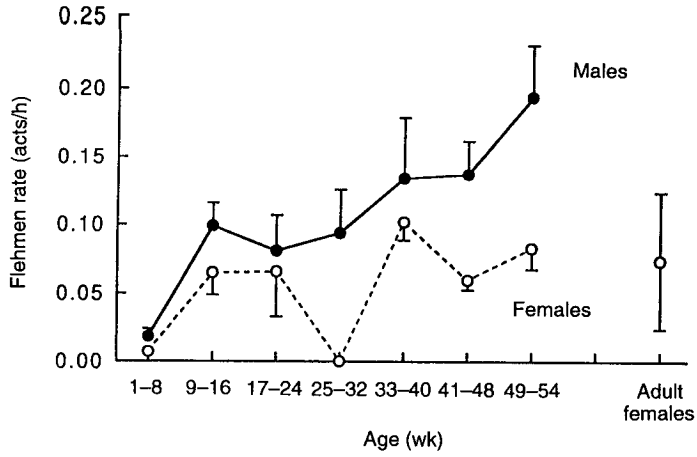

Fig. 1: Mean (± SE) rates of flehmen for male (n = 18) and female (n = 21) sable antelope during ontogeny. Rates for adult females (n = 11) are shown for comparison. Values for adult males were not available

class available. This subset of the data consisted of 19 calves (cohorts 2 and 4) and 12 yearlings (cohorts 1 and 3). Data were tabulated by 16-wk periods. For each individual, I identified the age class that was the most frequent target of flehmen (relative to its representation in the herd). The distribution of preferences of young calves (1–16 wk old) was compared with that of the oldest class of yearlings (49–64 wk old) using a χ^2 test. These two age classes were compared because no individuals were represented more than once.

Results

Sex Differences in Flehmen

I observed a total of 411 instances of flehmen by calves and yearlings ($\bar{X}$ = 10.5 per individual, range = 0–45). When calves and yearlings sampled urine as it was being voided, flehmen virtually always followed (327 of 340 instances, 96.2 %). Of the individuals observed from birth, I observed flehmen in all 17 male calves and 86 % (12 of 14) of female calves. Males were significantly younger than females when first observed performing flehmen (males: $\bar{X}$ = 52.8 d, range = 2–84 d; females: $\bar{X}$ = 75.6 d, range = 37–176 d; main effect for sex: $F = 4.563$, df = 1,21, $p < 0.05$). Cohorts varied in the age at which flehmen was first exhibited ($F = 3.242$, df = 3,21, $p < 0.05$).

For both sexes, flehmen rates increased with age, and mean flehmen rates for males exceeded those of females throughout development (Fig. 1). The slopes of the regression lines for change in flehmen rate over time were significantly greater for males (main effect for sex: $F = 8.666$, df = 1,29, $p = 0.006$). By 9–16 wk of age, flehmen rates of female calves did not differ from those of adult females (t-test, $t = -0.500$, df = 23, $p = 0.622$). Males and females did not differ in the distribution of flehmen responses among targets of different age classes (MANOVA,

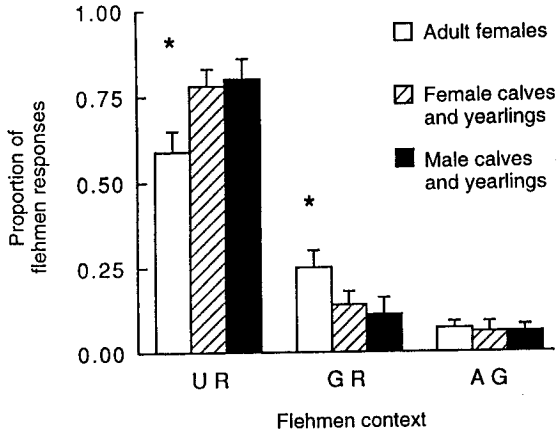

Fig. 2: Distribution of flehmen among different contexts for male calves and yearlings (n = 18), female calves and yearlings (n = 20), and adult females (n = 11). * = $p < 0.05$

calves: $F = 0.892$, $df = 2,21$, $p = 0.425$; yearlings: $F = 1.434$, $df = 2,9$, $p = 0.288$). Calves and yearlings performed flehmen in all contexts previously described for adult females (THOMPSON 1991). There were no sex differences in the relative frequencies of UR, GR and AG flehmen, but immatures of both sexes differed significantly from adult females ($K = 7.255$, $df = 2$, $p = 0.027$; Fig. 2). Adult females exhibited proportionately less UR flehmen ($U = 64$, $df = 1$, $p = 0.037$ and $U = 44$, $df = 1$, $p = 0.019$ for comparison of adults with male and female immatures, respectively) and more GR flehmen ($U = 180$, $df = 1$, $p = 0.007$ and $U = 138$, $df = 1$, $p = 0.034$, respectively) than did calves and yearlings.

Targets of Flehmen

The vast majority (94 %) of UR, GR and AG flehmen responses of both calves and yearlings were directed to female targets. Calves and yearlings did not perform flehmen to their mothers more frequently than to other adult females (sign test, calves: $p = 0.93$; yearlings: $p = 0.61$). Of the 15 acts of flehmen directed at male targets, 11 were performed to calves, 3 to yearlings and 1 to the herd bull. Only flehmen responses directed to female targets were considered in subsequent analyses.

Ontogenetic changes in flehmen targets were pronounced (Fig. 3). During the first 16 wk of life, calves were most likely to sample the urine of other calves. The number of individuals preferring peers decreased and the number preferring adults increased during development. By approximately 1 yr of age, adult females were the preferred targets of flehmen, and the distribution of preferred targets was significantly different from that of young calves ($\chi^2 = 22.12$, $df = 2$, $p < 0.001$).

To maximize their chances of synchronizing reproduction with experienced females, yearling females (those entering oestrus for the first time) should pref-

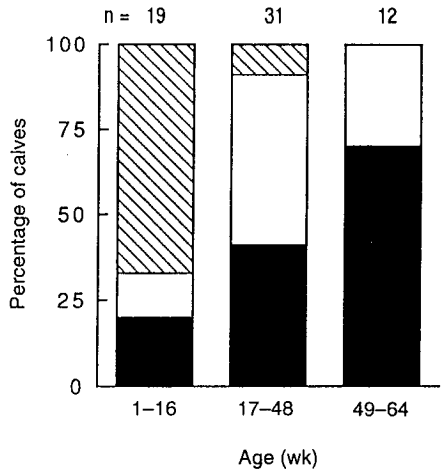

Fig. 3: Preferred targets of flehmen (relative to their abundance in study herds) of immature sable antelope. Only female targets were considered. Targets were classified as calves (0–32 wk of age, cross-hatched bars), yearlings (33–64 wk of age, open bars) or adults (> 2 yr of age, solid bars)

erentially perform flehmen to adult females that are oestrous cycling. Yearlings did indeed discriminate among adult females of differing reproductive status. During the birth season, when both pregnant and postpartum (and therefore potentially oestrous cycling) females were available as flehmen targets, 80 % of all acts of flehmen were directed at postpartum targets. All yearling females performed flehmen more frequently to postpartum females than to pregnant females (Wilcoxon matched-pairs signed-ranks test, $T^+ = 21$, $n = 6$, $p = 0.016$).

Discussion

Flehmen was observed in immature sable antelope of both sexes and was similar to adult female flehmen in form and context. Flehmen was first exhibited during the first few months of life, long before sexual maturity, which occurs at 1–2 yr of age (WILSON & HIRST 1977). Immatures performed flehmen in much the same manner as adults, primarily in response to urination by female conspecifics. Sampling urine as it was being voided was the primary context for flehmen, followed by investigation of freshly voided urine on the substrate. These are the most common contexts for flehmen in adult sable antelope also (THOMPSON 1991).

Sex differences were observed in the age at which flehmen was first exhibited and in the rate at which flehmen increased in frequency during ontogeny, with males exhibiting flehmen earlier in life and at greater frequencies than females. Other aspects of flehmen, including the sex and age class of preferred targets and the proportion of responses in differing contexts, did not differ between the sexes. Although flehmen in sable antelope was sexually dimorphic in frequency from infancy, sex differences were far less pronounced than reported for immature

domestic cattle (REINHARDT 1983). Domestic pony foals showed flehmen rates comparable to those of sable antelope calves, but the magnitude of sex differences did not increase during ontogeny (CROWELL-DAVIS & HOUPT 1985). Rather, flehmen rates for fillies and colts converged during early ontogeny, with rates for males decreasing linearly over the first 20 wk of life while rates for females remained relatively constant. Thus, the only quantitative studies of flehmen ontogeny in ungulates produced three widely differing results: one study showed flehmen to be almost exclusively exhibited by males, one showed convergent flehmen rates in males and females, and one showed divergence among immature males and females. Further studies of flehmen ontogeny in non-domesticated species are necessary to interpret the significance of these interspecific differences.

Ontogenetic changes in flehmen contexts and targets were also evident. Immature sable antelope of both sexes were more likely to sample urine as it was being voided and less likely to sample urine on the substrate than were adult females. These differences may reflect greater selectivity to specific types of stimuli or differing access to freshly voided urine. Low-ranking adult females appear to be inhibited from gaining access to freshly voided urine because of intrasexual aggression, but access to urine on the substrate is unrelated to rank (THOMPSON 1991). Immatures may be free of rank-related constraints on urine sampling. Adults did not behave aggressively towards calves and yearlings attempting to sample their urine, allowing them unrestricted access to fresh urine.

At the time calves first began exhibiting flehmen, other calves were the most frequent targets. Adult-like preferences for sampling the urine of adult females developed by 1 yr of age. This ontogenetic shift in flehmen targets may result from differences in proximity among animals of different age classes. Flehmen is positively related to proximity in adult females (THOMPSON 1995). Spatial relationships between calves and adults have not been studied, but if calves are in closer proximity to other calves than to adult females, then high rates of urine sampling among calves would be expected. Alternatively, changes in preferred flehmen targets may reflect increased selectivity for reproductively experienced female targets.

The functional significance of flehmen in immature animals deserves further study. For female sable antelope reaching sexual maturity, flehmen may play a role in the induction of first oestrus and may allow them to synchronize their reproduction with that of experienced females. Exposure to urine and/or conspecific odours is known to accelerate the timing of puberty in female rodents (VANDENBERGH 1969; STODDART 1980) and domestic ungulates (KIRKWOOD et al. 1983; VANDENBERGH & IZARD 1983; BOOTH 1984). In the CRC population, females first conceive at 1 yr of age. Yearling females in this study resembled adult females in the frequency, contexts and targets of flehmen and performed flehmen in a manner consistent with a strategy of achieving oestrous synchrony with experienced adults.

Males reaching sexual maturity may utilize flehmen for identification of sexually receptive females. The appearance of flehmen in young male cattle coincides with the onset of sexual interest in oestrous females and with pubertal

increases in testosterone (REINHARDT 1983). Pubertal increases in testosterone may likewise explain the rapid increase in flehmen by young male sable antelope. Subadult males (1.5–2 yr old) are behaviourally inhibited from mating in the presence of adult males, but are physiologically capable of fertilizing females (WILSON & HIRST 1977). Frequent monitoring of female conspecifics may allow young, subordinate males occasional mating opportunities.

Flehmen may be an important mechanism for mediating social influences on reproduction in sable antelope and other ungulate species (PFEIFER 1985; THOMPSON 1991, 1995; BERGER 1992). The role of the nasal chemical senses in influencing reproductive maturation in rodents has been intensively studied; these studies have implicated the vomeronasal system as the primary pathway by which social cues affect reproduction (KEVERNE 1983). Ungulates (and sable antelope in particular) are an especially promising model for further investigations of the role of the vomeronasal system in mediating reproduction because exposure to urinary chemosignals can be quantified using flehmen.

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