JOURNAL OF NATURAL HISTORY, 1980, 14:41–47

Responsiveness of hand-reared roe deer to odours from skin glands

D. M. BROOM and ELIZABETH JOHNSON

Department of Zoology, University of Reading, Reading RG6 2AJ, U.K.

Introduction

Roe deer (*Capreolus capreolus*) have enlarged apocrine and sebaceous glands in at least four regions of the body. These produce secretions, either seasonally or continuously, which may be important in social interaction. A study of the seasonal changes in these specialized regions (Adams and Johnson, in the press) has shown that glands on the forehead, first described by Schumacher (1936), enlarge during the breeding season in roe bucks and regress as the testes regress (Bramley 1970) after the rut. Separation of the chemical components of the secretions by gas chromatography indicates that the components present at the time of the rut are different from those present at other times. During the breeding season roe bucks mark objects in their territory with secretions from the forehead. On the other hand, glands in an area of thickened skin over the metatarsus (the metatarsal gland region) and in the skin between the digits (fore and hindfoot interdigital regions) are enlarged throughout the year in both males and females.

Müller-Schwarze (1971), working with the black-tailed deer *Odocoileus hemionus*, has proposed that the odours from the tarsal gland on the inner aspect of the leg are used in individual recognition whilst those from the metatarsal gland on the outer aspect of the leg act as an alarm pheromone. The odour from the tarsal tuft originates from glandular secretions, urine deposited on the tuft during urination and, probably, from interactions between these substances. The hairs on the tarsal tuft are specialized in structure so that they trap volatile substances (Müller-Schwarze *et al*. 1977). When an extract of the hairs from the tarsal tufts of mule deer *Odocoileus hemionus hemionus* or of black-tailed deer *O. h. columbianus* was sprayed on to the hock of one individual in a group of deer of one subspecies, this hock was sniffed and licked more if the extract was taken from its own subspecies than if it was taken from the other (Müller-Schwarze and Müller-Schwarze 1975). The odour from the tarsal tuft thus allows subspecific recognition.

The present study was undertaken in order to determine the responses of roe deer to the odours of their skin secretions.

Materials and methods

Samples were obtained from live roe deer netted in Southern England in the Forestry Commission's experimental wood at Cheddington, Dorset.

Samples were obtained in March from the following deer:

One mature doe.

One young doe (10 months of age).

One mature buck.

One young buck (10 months of age).

Hairs were removed with scissors at skin level from the forehead and metatarsal

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glandular regions and immediately placed in clean glass vials with close fitting plastic caps.

The hindfoot interdigital region was wiped with a swab of absorbent cotton wool held in plastic forceps. The swabs were similarly enclosed in glass vials.

The samples were stored unopened at $-20°C$ until their use in April. Before use in the olfactory tests, the tubes were warmed to room temperature and the plastic cap rapidly replaced with a close fitting cotton wool bung with a long length of string attached.

The subjects were two hand-reared roe bucks born in Alice Holt Forest, Surrey, at the end of May and brought into captivity shortly after birth. An account of their hand rearing will be reported separately (Johnson and Broom, in prep.). These animals were subsequently used in an investigation of the metabolism of testosterone by the forehead skin (Johnson and Leask 1977).

The two roe buck kids were reared together in a small air-conditioned room for two months. Between 2 and $10\frac{1}{2}$ months of age they were housed together in a caged area of an air-conditioned room, which also contained penned sheep, under conditions of natural day length at 16–17°C. The cage, constructed of weldmesh, measured 7 m × 1·5 m. The roe bucks were fed *ad libitum* on a wide variety of shrub foliage, which was tied in bundles onto the cage mesh; pelleted Diet 18 (Dixon & Sons Ltd.) and drinking water.

When the two roe bucks were ten and a half months old they were confined in a portion of their cage measuring 3 m × 1·5 m and a 90 cm long wind tunnel which was rectangular in cross section (20 cm × 10 cm) was mounted so that its aperture was about 30 cm from the wire-mesh wall of the cage (fig. 1). The fan in the wind tunnel was left running continuously and the position of the air stream which it produced was established by watching the movement of small particles in or near it. A

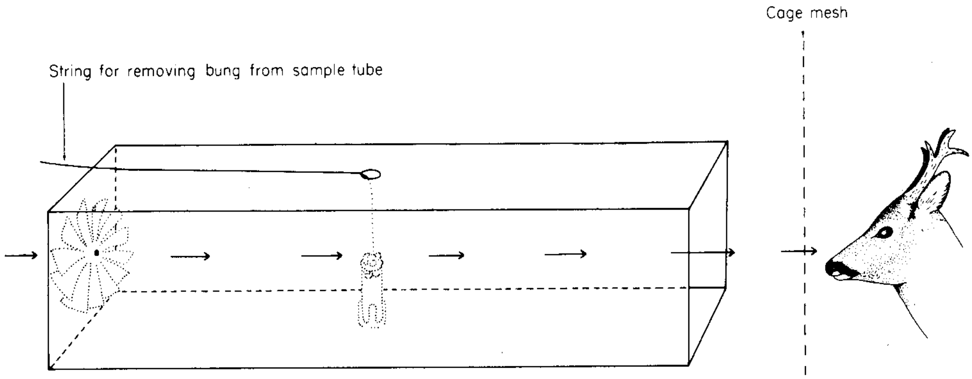

FIG. 1. Diagram to show the position of the wind tunnel, containing a sample tube, in relation to the cage housing the deer.

television camera was mounted above and behind the wind tunnel. The whole of the cage housing the deer could be viewed on a monitor in an adjacent room. The equipment was left in place for one week so that the deer would become habituated to its presence and so that the observer could, by watching the television screen, determine which were the normal patterns of behaviour of undisturbed animals.

In order to test the response of the deer to an olfactory change a tube, whose contents were not known to the experimenter who was carrying out behaviour observations, was taken into the room housing the deer and put in position in the wind tunnel. The end of the string, which was attached to the cotton wool bung in the tube, was held by the observer in the adjacent room. The deer were then left undisturbed for at least five minutes. A videotape of their behaviour was made at this time and during the test period. Bung removal was delayed until one or both of the animals were standing in or near the air stream. Videotaping continued after the removal of the bung until five minutes after any disturbance in behaviour had ceased or for ten minutes, whichever was longer. The tube was then removed and the animals left for at least 30 minutes before being tested again.

The videotape of each test was analysed by playing it several times and comparing the behaviour during the test period with that whilst undisturbed. The observers did not know of the contents of each tube until all behaviour analysis had been completed. Amongst the measures of behaviour used the following require definition:-

Sniff:	movement of nostrils
Lick nose:	the tongue is extended so that the nostril region is moistened.
Head shake:	lateral movement of the head two or three times to left and right.
Jump:	all four feet leave the ground at once.
Stand:	arise from lying position.
Close to air stream:	head within region through which air stream passes but excluding individuals which are clearly attending to a leafy branch or to the food dish.

Results

When the observer in the adjacent room removed the bung from the tube in the air current by pulling the string, the deer sometimes saw the movement. The response, a sudden head orientation or a jump, was shown too rapidly for any odour to have reached the animal. If one animal was lying it sometimes stood immediately after the other animal had jumped. Animals which continued lying throughout the experiment were not included in analysis for they could not have detected anything in the air stream. Sudden head orientations and approach towards the wind tunnel were often shown after all movement of string or bung had ceased. When this movement occurred shortly after bung removal it was difficult to tell whether it was a response to the movement or to an odour in the air stream. Orientation reactions were shown during the control experiments when the tube was empty but the total time apparently responding after bung removal was never more than 20 s (see the table).

As can be seen in the table and in fig. 2, the total time that a deer was apparently responding to tube opening was more than 20 s in only five experiments and these were the five in which clippings from a metatarsal gland tuft were in the tube (Mann Whitney U test, metatarsal v empty tube, $p=0{\cdot}004$, two-tailed). The responses of sniffing in the air stream, licking the nose in the air stream, head shaking and rapid ear movement were shown in all three experiments in which clippings from the metatarsal gland of an adult roe deer were in the tube. Sniffing, licking nose and head shaking were each shown in only one of the other twelve experiments and rapid ear movements were not shown during the other twelve experiments (Mann Whitney U test, metatarsal v empty tube, sniffing in air stream, $p=0{\cdot}03$, two-tailed). The

Responses of roe bucks to the opening of either a tube containing skin secretion or an empty tube.

Behaviour	Metatarsal sample					Forehead sample			Interdigital sample			Empty tube					
	Adult			Juvenile		Adult			Adult								
	♂	♀	♀	♂	♀	♂	♀	♀	♂	♀	♀	1	2	3	4	5	6
Total time responding (seconds)	76	149	228	21	45	0	2	0	0	3	17	0	20	11	0	15	17
Jump, stand or turn head suddenly	+	+	+	+	+	−	+	−	−	+	+	−	+	+	−	+	+
Sniff in air stream, number of bouts	3	1	5	0	1	0	0	0	0	0	1	0	0	0	0	0	0
Lick nose in air stream, number of bouts	2	1	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Head shakes	+	+	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−
Rapid ear movements	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−

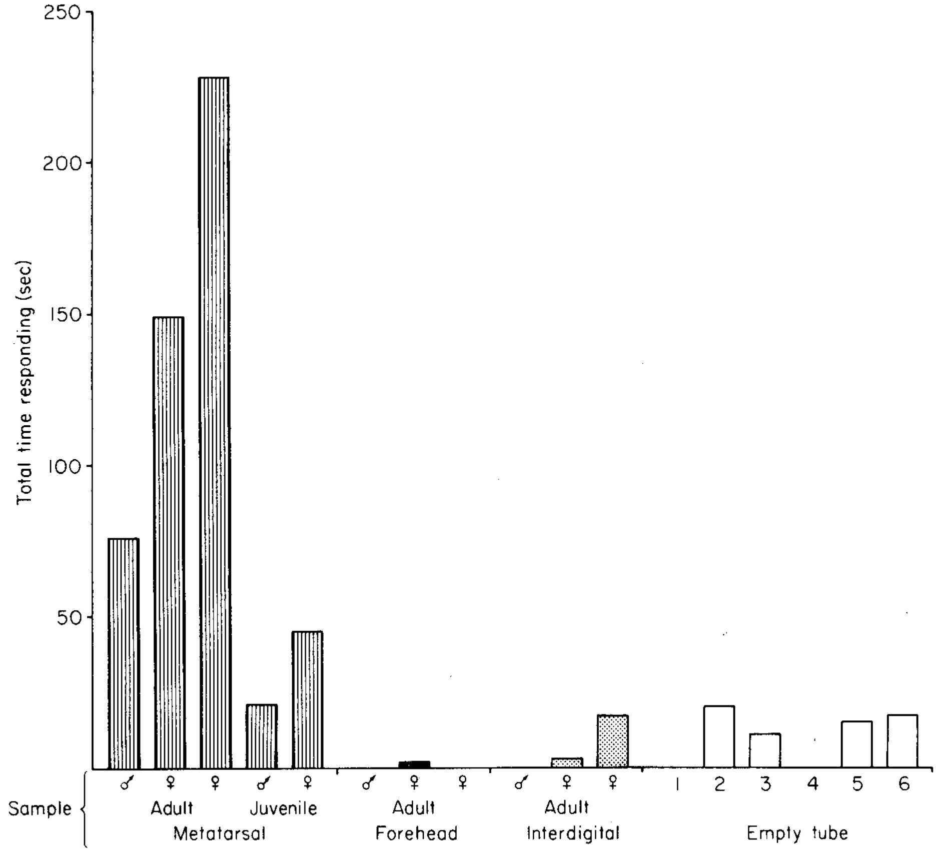

FIG. 2. Each column represents the duration of the response of an individual roe buck to the opening of either a tube containing skin secretion or an empty tube.

durations of the time in the air stream and the total time apparently responding during the experiment were very much greater when adult metatarsal gland clippings were present.

Other observations

Prior to and during these experiments the 11-month-old bucks interacted competitively and one, called the dominant, always won. Both showed some marking behaviour, rubbing the forehead and antlers on branches. This behaviour continued after antler removal when the roe bucks were separately housed at $11\frac{1}{2}$ months. At 14 months (July) marking reached a peak, a rancid odour was detected and a red resinous secretion was produced by the forehead glands. When the secretion from the forehead of the dominant buck was smelled on the hands of the experimenters by the other buck, this individual responded by crying and rushing around the stable in which it was housed. The smell of the forehead secretions of the submissive buck caused the dominant buck to lower his head and paw the ground.

Discussion

The major result of this experiment was the observation of the clear responses of the young roe bucks to the odour from the metatarsal glands of strange individuals. These responses were not shown in control experiments when there was no odour.

There was also an indication of greater responsiveness to odour from the metatarsal glands of adults than from those of juveniles. The responses, which involved remaining in the airstream, sniffing, licking the nose, head shakes and rapid ear movements, did not indicate that the odour was aversive. Prior (1968) has described roe deer in the field licking the nose when a strange odour was present. Thus, it seems likely that when hairs from the metatarsal tuft were in the airstream, the deer were attending to and investigating the odour. Roe deer might show such investigatory responses to any strange odour. It has been suggested that the secretions from the metatarsal gland in roe deer may allow individual recognition but there is no previous experimental evidence for this.

Our observations that odours from the metatarsal glands of other roe deer are investigated but elicit no flight response support this hypothesis. It is possible that the odours serve some other function but it would seem likely that the metatarsal gland of roe deer is similar in function to the tarsal gland of black-tailed deer *O. hemionus* (Müller-Schwarze 1971). There is much evidence, from many species, of investigatory behaviour by individuals which are in the presence of the smell of a strange conspecific (review by Johnson 1973). For example, Mykytowycz (1965) reported that rabbits mark their territory extensively with the submandibular gland and that individuals entering a stranger's territory adopt an alert posture. Solomon and Glickman (1977) showed that hamsters will spend much more time investigating the bedding of strange hamsters than that of familiar individuals or clean bedding.

The lack of responsiveness of the roe bucks to the odour from the forehead gland is probably due to the inactivity of the gland at the time of collection in March. Our observations in July of the forehead gland secretions, their smell and their drastic effect on buck behaviour support the idea (Forestry Commission 1970) that these secretions are used to mark territory and repel intruders.

Prior (1968) has suggested that the interdigital glands may produce a trail-marking pheromone. Our experiments provide no evidence for this but it is possible that the samples collected on the swab did not contain any appreciable quantity of the secretion.

These preliminary experiments have shown that it would be possible to use such behavioural observations to determine which chemical components of gland secretions act as pheromones. The technique is a feasible laboratory alternative to the methods for pheromone bioassay in the field reviewed by Müller-Schwarze (1977).

Summary

Odours from skin glands of wild roe deer were blown into the home cage of two $10\frac{1}{2}$-month-old roe deer whilst the behaviour was videotaped. Clear investigatory responses but no avoidance were shown when the metatarsal gland odour was presented. The function of these glands probably concerns individual recognition rather than communication of alarm. Observations of the forehead glands in July and of the effect of their odour provide further evidence for their importance as a means of warning and repelling intruders in a territory.

Acknowledgments

We thank the Forestry Commission for obtaining the roe kids used in this study, the late Mr T. Pearson together with other animal technicians for help with the care of the deer, and Mr E. Snowden for help with the videotape recording.

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