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SOCIAL SIGNALS INVOLVED IN SCENT-MARKING BEHAVIOR BY CHEEK-RUBBING IN ALPINE MARMOTS (*Marmota marmota*)

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Abstract—The Alpine marmot *Marmota marmota* is a territorial rodent. Resident adults regularly scent-mark their territory by cheek-rubbing, mainly on burrow entrances and along boundaries. The purpose of this three part study was to gain further insight into this scent-marking behavior by: (1) observing the response of free-ranging marmots to foreign scent marks, (2) confirming the glandular source of the marking substance by histologic examination of the temporal gland, and (3) identifying biologically active chemical fractions of the marking substance. To allow field tests, we developed a device consisting of a glass tube placed upside down over a stake. Two devices were simultaneously placed at one burrow entrance. On one device, a clean tube was used and, on the other, a tube alternatively coated with either whole natural scent-marking substances or various fractions obtained by solvent extraction or chromatographic separation from whole scent-marking substances. Subsequent observations showed a significant difference in the duration of nose contact and number of cheek-rubbing movements. Resident adult marmots sniffed and marked tubes bearing alien marks significantly more than clean control tubes. Similar differences in behavior were observed with ethanol extracts of whole scent-marking substances. Extracts obtained with pentane and dichloromethane showed no bioactivity, suggesting that highly polar compounds are the active substances in the Alpine marmot. The temporal gland is an exocrine gland located on each side of the head

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with numerous pores opening at the surface of the skin in the cheek area. GC-MS analysis of individually collected samples from these glands showed that over 30 compounds were consistently present. Seven of these compounds were identified. Two fractions were obtained and used together and separately in field tests. Fraction 1 was composed mainly of short-chain alcohols and alkanes, and fraction 2 had a more acid and ester composition. The fact that these two fractions were active together but not separately strongly suggests that the active territorial signal results from a synergistic interaction between several compounds.

Key Words—Alpine marmot, *Marmota marmota*, social rodent, chemical communication, scent-marking behavior, scent gland, bioassay, fractionation, mammalian semiochemical.

INTRODUCTION

The Alpine marmot, *Marmota marmota*, is a large rodent (Rodentia: Sciuridae) found exclusively in grasslands above the timberline (Forter, 1975; Herrero et al., 1994). It is a highly social (Barash, 1989), monogamous (but see Goossens et al., 1998), diurnal species living in permanent family units composed of one adult pair and their offspring from successive litters. Reproduction takes place yearly in April immediately after hibernation, and offspring remain in the family unit until at least 2 years of age (Arnold, 1990a,b; Perrin et al., 1993a,b). Family units are territorial. Resident adults carefully scent-mark their territory by regular cheek rubbing behavior and territories are very stable over time (Bel et al., 1995; Coulon et al., 1994; Lenti-Boero, 1995; Perrin, 1993). In a previous paper (Bel et al., 1995), we showed that: (1) peak scent-marking activity occurs between the breeding period and emergence of offspring from the natal burrow, (2) scent-marking mainly involves the main burrow system and boundaries, and (3) foreign scent marks are removed or replaced. The last two findings strongly suggest that scent-marking plays a major role in territorial defense in this species.

The purpose of the present three-part study was to gain further insights into the scent-marking behavior of *Marmota marmota*. We developed a reliable test to determine the response of marmots to foreign scent marks experimentally placed on their main burrow system. The need for such data in free-ranging mammals has been pointed out (Müller-Schwarze et al., 1986), but few assays have been developed (Stralendorff, 1986). Most studies have involved the North American beaver, *Castor canadensis* (Müller-Schwarze, 1992; Müller-Schwarze et al., 1983, 1986; Müller-Schwarze and Houlihan, 1991; Schulte et al., 1994, 1995; Svendsen and Huntsman, 1988). A few marmot species including *Marmota monax* (Meier, 1991), *M. caudata aurea* (Blumstein and Henderson, 1996), and *M. flaviventris* (Brady, 1997) also have been studied in the field.

We also attempted to determine the glandular source of the marking substance. Previous data limit the number of candidate glands. According to Rausch and Bridgens (1989) and Blumstein and Henderson (1996), all 14 species of the genus *Marmota* have two facial glands, i.e., a temporal (or orbital) gland located bilaterally between the eye and the ear and perioral glands located at both oral angles. In the Alpine marmot, cheek glands were reported by Tiedemann (1816) and described in fuller detail by Schaffer (1940). Koenig (1957) linked scent-marking behavior with the deposition of "cheek gland" secretions from adult males. We tested the activity of secretions collected directly from the temporal gland and assessed glandular structure by histological examination.

In the third part of this study, we tried to identify the active chemical components involved in scent-marking behavior of Alpine marmots. Since mammal secretions are complex mixtures, we tested fractions from the marking substance according to a response-guided approach (see Albone, 1984).

METHODS AND MATERIALS

Study Site and Field Observation

This study was conducted at a mean elevation of 2340 m in La Grande Sassière nature reserve (45°29′N, 6°59′E) of Vanoise National Park in the French Alps where we have been studying the marmot population since 1990 (Allainé et al., 1998; Bel et al., 1995; Coulon et al., 1994; Perrin et al., 1993a,b). A total of 427 live-trapped marmots were individually weighed and sexed by measuring anogenital distance (Zelenka, 1965). Based on body mass and head-to-tail length (Zelenka, 1965), animals were classified into the following four age groups: adults 3 years or older, 2-year-olds, yearlings, and juveniles. Animals were marked by tagging and fur dyeing to allow visual identification (Bel et al., 1995; Perrin, 1993). We observed them at a distance of 150–200 m by using 10 × 50 binoculars and a 20–60× telescope (Optolyth TBS 165).

Bioassays

The bioassays described in this report were performed between 1993 and 1997 on eight marmot family units. The test device consisted of two wooden stakes covered by glass tubes (20 cm long × 2 cm diam.) placed near a main burrow entrance at least two days before data recording to give marmots time to become accustomed to their presence. The glass tube used on one stake (treatment tube) had been placed in another family unit two days before so that it bore a natural alien scent mark. The second tube (control tube) was clean. The treatment and control tubes were randomly placed on the right and left of the entrance. The preinstallation time was shorter than in similar experiments on

the golden marmot, *Marmota caudata aurea* (Blumstein and Henderson, 1996), and the woodchuck, *Marmota monax* (Meier, 1991), in which test devices were placed one week prior to observation. Here, we observed that the characteristic, pungent yellowish mucous scent-marks (Bel et al., 1995) were deposited on clean tubes within two days.

As in previous studies on *Castor canadensis* (Butler and Butler, 1979) and *Odocoileus hemionus columbianus* (Müller-Schwarze, 1971; Crump et al., 1984), tests were performed when scent-marking activity is greatest during daylight hours between mid-April and mid-July (Bel et al., 1995). Tests were generally performed in the morning in early spring (about 09:00 hr), and in the morning and afternoon in summer (about 07:30 hr and 17:00 hr). This scheduling strategy was chosen because *M. marmota* shows a progressive seasonal trend to bimodal rhythm (Perrin et al., 1993a,b). As a precaution, no more than one test was performed per day in a given group in order to prevent a possible confounding effect of habituation.

During tests, attention was focused on individuals approaching within 5 m of either test device. After identification of age, sex, and social status, the duration of sniffing (DS), considered as the total duration of the contact between one animal's nose and each tube during one test, was timed by using a stopwatch, and the total number of cheek-rubs (NCR) performed by one animal on each tube during one test was counted. Data were recorded on an audio tape and in notebooks.

Since scent marks deposited by one resident marmot may affect the subsequent response of the other marmots in the family unit, testing was continued as long as none of the marmots in the family group marked either tube. Whenever a resident marmot scent-marked the treatment and/or control tubes, the test was stopped. Tests in which no marmot approached the experimental device or in which no response occurred after 2 hr were stopped and excluded from analysis.

Isolation and Preparation of Active Compounds

Testing Temporal Gland Secretions. Samples were collected from the temporal glands of nine males (seven adults and two 2-year-olds) and 10 females (seven adults and three 2-year-olds). After anesthetization, the temporal region of the head of each animal was shaved. The shaved area was gently massaged to stimulate glandular secretion, and a clean glass tube was rubbed against the skin about three times. One to four tubes were obtained for each animal. Tubes not immediately used were wrapped in clean aluminum foil to protect from sunlight and contamination and stored at $-5°C$.

Testing Extracts. The compounds deposited on glass tubes were extracted with one of three analytical grade solvents of increasing polarity, i.e., pentane,

dichloromethane, or ethanol. Only one extract with 5 ml solvent was made per tube. After complete evaporation of the solvent, each extract was redeposited on a clean tube and used as the test tube for bioassays. Control test series were also performed to check the response induced by each pure solvent.

Testing Fractions. Only ethanol extracts were submitted to further fractionation after initial bioassays, since they were the only ones showing bioactivity. Chromatography-trapping was carried out on a Girdel 300 gas chromatograph equipped with a packed column (3% CP-Sil5 Chromosorb phase) fitted with an effluent splitter set to a split ratio of 8 : 2. Column temperature was programmed from 60°C to 210°C at a rate of 5°C/min (final time, 10 min; total duration, 40 min). Each ethanolic extract (100 μl) composed of individual temporal gland secretion exudates was coinjected along with an internal standard, *n*-dodecane (*n*-C_{12}, MW 170), which has a different retention time from any perceptible secretion peak. Chemicals were trapped in a glass capillary tube cooled in Dry Ice. Three volatile fractions were collected. Fraction 1 eluted 10 sec before the standard and fraction 2 was collected from 10 sec after the standard until the end of the program. The total volatile fraction (duration of elution = 40 min) was also collected and bioassayed. Each fraction was subsequently solubilized in 1 ml ethanol (Wheaton vial) and stored at −5°C pending bioassay.

Gas Chromatography–Mass Spectrometry (GC-MS)

Individual ethanol extracts of temporal gland secretions from known males or females (group, age, social status) were used as analytical samples for GC-MS. Temporal secretions were collected in 4-μl glass capillary tubes and stored in 100 μl of pure ethanol. Preliminary analyses were performed on a HPUX-controlled system consisting of a capillary gas chromatograph HP5890 series II coupled to a quadrupole mass spectrometer HP MS Engine 5989A. The chromatograph was equipped with a CPSil 5WCOT apolar column (Chrompack, 25 m, 0.25 mm ID, 0.12 μm DF). Injection was performed in the split–splitless mode (15 sec; injection port 280°C). Oven temperature was programmed from 35°C (4 min isothermal) to 320°C (5 min isothermal) at a rate of 8°C/min. The mass spectrometer was operated in the electron impact mode (GC-MS-EI, 70 eV) and scanning was performed from 40 to 500 uma at the rate of 1 scan/sec.

Data Analysis

Field trials were replicated in various marmot territories. Under the alternative hypothesis, it was assumed that the treatment tubes had been sniffed longer and marked more extensively than control tubes and thus DS and NCR were tested as quantitative variables by using one-tailed nonparametric Wilcoxon matched-pair signed-rank tests (Siegel, 1956).

Study of Glandular Source

Two full-thickness skin samples including the epidermis and the dermis (1 cm^2) were collected from two free-ranging adult marmots shortly after accidental death. One sample was taken from the temporal zone, the zone supposedly containing the glands secreting the compounds involved in scent-marking (Koenig, 1957; Coulon, personal observation). The second sample was collected near the scapula as a control. Samples were fixed with alcoholic Bouin's fixative and stored pending further study. Histological examination was conducted on 7-μm-thick sections cut after dehydration and embedding in paraffin. Sections were stained with Masson's trichome, a variant of Goldner's stain (Martoja and Martoja-Pierson, 1967).

RESULTS

Bioassay

The results of statistical analysis of DS and NCR data obtained in bioassays are shown in Figure 1a and 1b. The treatment tube always elicited a stronger response than the control tube: resident marmots sniffed tubes with natural alien scent marks longer (Wilcoxon test: $N = 15$, $t = 30$, $P = 0.043$, one-tailed) and rubbed them a greater number of times (Wilcoxon test: $N = 13$, $t = 7.5$, $P = 0.0038$, one-tailed) than control tubes. Therefore, while marmots were equally disposed to scent-mark any tube, previously scent-marked or not (see Bel et al., 1995), they responded significantly more to the marked tube than to the clean one.

Temporal Gland Secretions

Thirty-five tests were performed by using extracts obtained from 21 males and 14 females. Temporal gland secretions induced a strong reaction in resident marmots, as for natural scent marks (Wilcoxon tests, male-female secretions, DS: $N = 33$, $t = 39.5$, $P < 0.0001$ one-tailed; NCR: $N = 27$, $t = 28$, $P < 0.0001$ one-tailed) (Figure 1a and 1b).

Secretions from adult males and females were presented separately in order to study responses according to sex. In each test, we measured the difference of both DS and NCR of the treatment and control tube for each marmot. Resulting variables were then used to assess behavioral responses of those animals that had been presented with a male and a female odor in different field tests. The sex of the odor donor did not influence the responses of males or females (Table 1).

Characterization of Chemosignals

Only ethanol extracts induced significant responses. Marmots sniffed tubes marked with an ethanol extract longer (DS Wilcoxon test: $N = 28$, $t = 125.5$, P

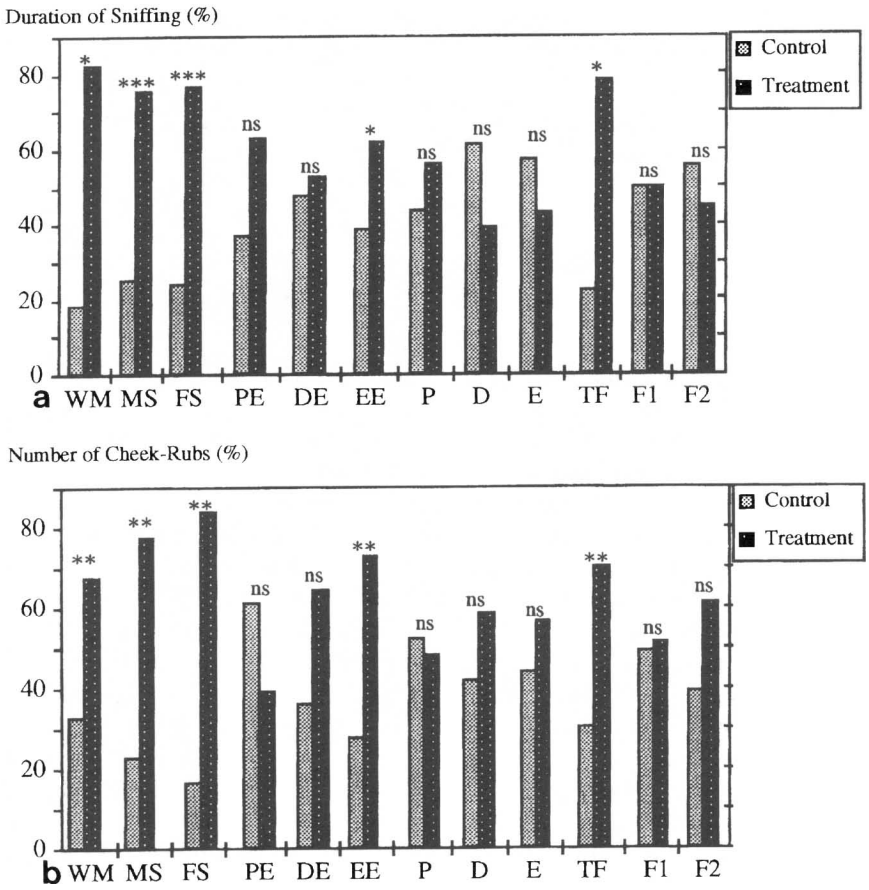

FIG. 1. (a) Relative duration of sniffing (DS) on each tube for different series of tests. Each series of tests is represented by the nature of treatment tube (number of tests): WM (N = 10): whole marks; MS (N = 21): male secretion; FS (N = 14): female secretion; PE (N = 30): pentane extract; DE (N = 13): dichloromethane extract; EE (N = 58): ethanol extract; P (N = 7): pentane; D (N = 10): dichloromethane; E (N = 12): ethanol; TF (N = 18): total fraction; F1 (N = 9): first volatile fraction; F2 (N = 10): second volatile fraction. (b) Relative number of cheek rubs (NCR) on each tube for different series of tests. Each series of tests is represented by the nature of treatment tube; abbreviations as in (a); numbers of tests are given in parentheses. WM (N = 50), MS (N = 21), FS (N = 14), PE (N = 34), DE (N = 21), EE (N = 58), P (N = 7), D (N = 10), E (N = 12), TF (N = 18), F1 (N = 9), F2 (N = 10). Results of Wilcoxon tests are shown (ns: not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; one-tailed).

TABLE 1. PUTATIVE DISCRIMINATION OF TEMPORAL SCENTS ACCORDING TO SEX OF ODOR DONORS AND VARIABILITY AMONG MALE AND FEMALE RESPONSES

	Intensity of marking		Duration of sniffing	
Reactions of females towards male vs female scent-marks[a]	$N = 6$; $t = 5$; $Z = -1.166$ $P = 0.2436$	NS	$N = 5$; $t = 4$; $Z = -0.944$ $P = 0.3452$	NS
Reactions of males towards male vs female scent-marks[a]	$N = 5$; $t = 6.5$; $Z = -0.272$ $P = 0.7855$	NS	$N = 6$; $t = 8.5$; $Z = -0.420$ $P = 0.6741$	NS

[a]Two-tailed Wilcoxon matched-pairs signed ranks test; here, there are N paired responses of given individuals towards both male and female scents. Z and P values are corrected for ties.

= 0.0385, one-tailed) and marked them more extensively (NCR Wilcoxon test: $N = 11$, $t = 3$, $P = 0.0035$, one-tailed) than control tubes (Figure 1a and 1b). Blank tests with pure solvents ruled out the solvent as the cause of the biological activity of extracts, with results as follows: Wilcoxon tests; DS: pure ethanol: $N = 9$, $t = 13$, $P = 0.1292$, one-tailed; pure dichloromethane: $N = 8$, t 11, $P = 0.1623$, one-tailed; pure pentane: $N = 5$, $t = 5.5$, $P = 0.2904$, one-tailed; NCR: pure ethanol: $N = 4$, $t = 2$, $P = 0.1284$, one-tailed; pure dichloromethane: $N = 5$, $t = 5$, $P = 0.2398$, one-tailed; pure pentane: $N = 6$, $t = 6.5$, $P = 0.1976$, one-tailed).

No significant response was induced by either of the two fractions obtained by fractionation-trapping of the ethanol extract (for DS Wilcoxon tests: Fraction 1: $N = 8$, $t = 17$, $P = 0.4441$, one-tailed; fraction 2: $N = 7$, $t = 8$, $P = 0.153$, one-tailed; for NCR Wilcoxon tests: Fraction 1: $N = 6$, $t = 10$, $P = 0.4578$, one-tailed; fraction 2: $N = 4$, $t = 3$, $P = 0.2286$, one-tailed). However, a significant response was observed from the total fraction (DS: $N = 15$, $t = 28.5$, $P = 0.0366$, one-tailed, NCR: $N = 10$, $t = 2.5$, $P = 0.0051$, one-tailed).

The chemical composition of 24 whole ethanol samples from 18 males and 6 females was analyzed by GC-MS. Major qualitative and quantitative differences were observed. These differences appeared to be unrelated to sex. The 30 most consistently observed peaks, found in more than 50% of individual samples, were selected for further study. A typical total ion chromatogram (TIC) from an adult male is shown in Figure 2. The two fractions used in the bioassay (fraction 1 and fraction 2) showed different GC peaks. Fraction 1 was composed

FIG. 2. (Opposite) Alcoholic extract from temporal secretions of one adule male Alpine marmot: representative total ion chromatography (TIC), analyzed by GC-MS. Preliminary determination could be performed by matching mass spectrum of each peak with those of Wiley and NBS libraries. Fractions 1 and 2, only mixed together once, were biologically active. Fraction 1 is mainly represented by short-chain alcohols (a) and alkanes (h). The second part of the TIC (fraction 2) is characterized by numerous acids (c) and esters (e). Numbers 1–7 refer to precisely identified peaks (see Table 2).

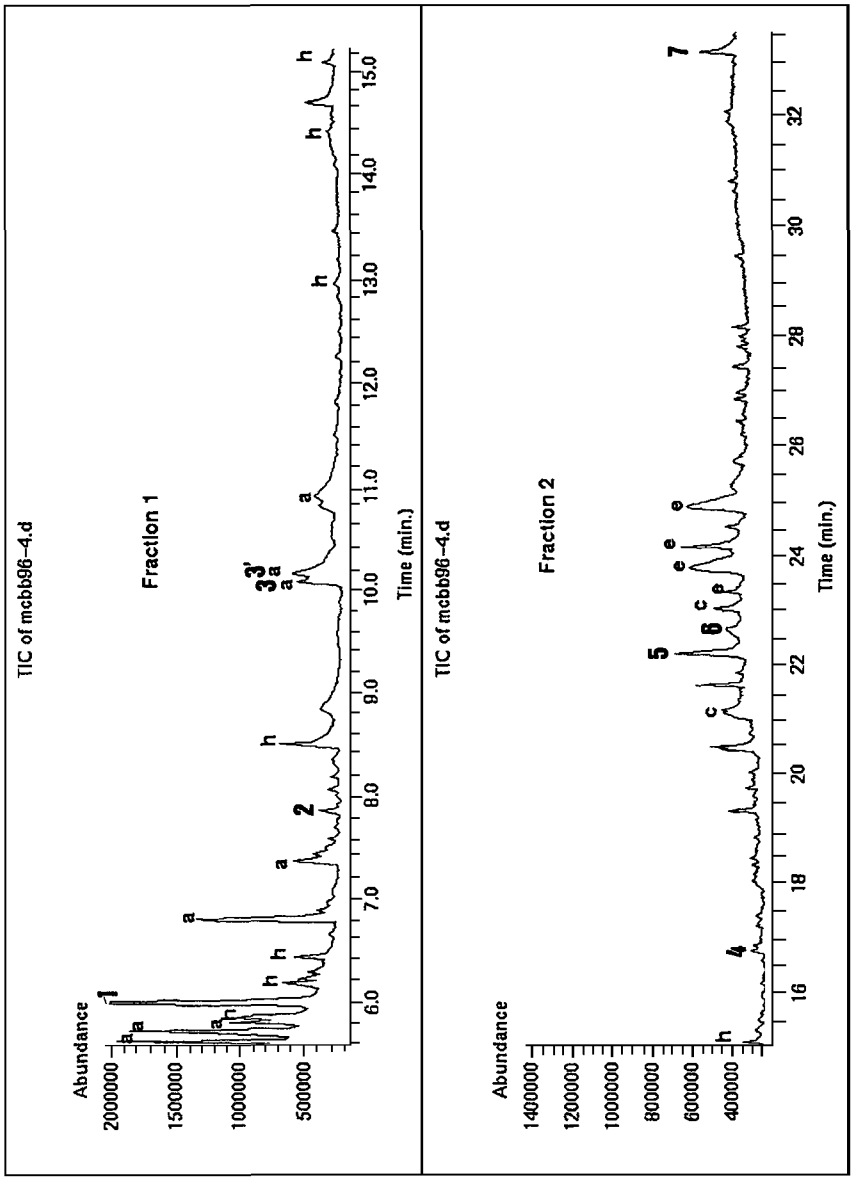
TIC of mcbb96-4.d
Fraction 1
Abundance
Time (min.)
TIC of mcbb96-4.d
Fraction 2
Abundance
Time (min.)

TABLE 2. PRELIMINARY CHEMICAL DETERMINATION OF INDIVIDUAL ETHANOLIC EXTRACTS

Peak	Ret. time (min)	Compound	Formula	Diagnostic ions EI-GC-MS	Reference
1	6	4-Hydroxy-4-methyl-2-pentanone	$C_6H_{12}O_2$	43,59,101 (MW 116)	Hefetz and Lloyd (1993), Finidori-Logli et al. (1996)
2	7.8	Benzenemethanethiol	C_7H_8S	45,65,91,124 (MW 124)	83% Wiley
3	10.08	Nonadien-1-ol	$C_9H_{16}O$	41,55,83,122,140 (MW 140)	70% Wiley
3′	10.16	Nonadien-1-ol (isomer)	$C_9H_{16}O$	41,55,83,122,140 (MW 140)	70% Wiley
4	16.8	2-Ethylamino-2-(2-thienyl)-cyclohexanone	$C_{12}H_{17}NOS$	97,110,123,152,166,195 (MW 223)	87% Wiley
5	22.3	Palmitic Acid	$C_{16}H_{32}O_2$	41,43,57,73,129,185,213,256 (MW 256)	78% Wiley
6	22.6	Musk Ambrette	$C_{12}H_{16}N_2O_5$	77,91,115,146,253,268 (MW 268)	60% Wiley
7	33.2	Cholesterol	$C_{27}H_{46}O$	(MW 386)	95% Wiley

[a]These peaks are also referenced by their number in the total ion chromatogram (Figure 2).

FIG. 3. Temporal gland of an adult Alpine marmot. Schematic distribution of duct openings on the skin surface of a live-trapped adult (the arrow indicates a drop of secretion oozing out of one of the duct openings).

mainly of short-chain alcohols and alkanes, while fraction 2 displayed more acid and ester composition. As listed in Table 2, seven compounds were identified.

The Marking Gland

Macroscopic Findings. Secretory activity seemed to be age-related with no secretion observed in juvenile marmots, while several large drops were obtained from mature animals. However this difference was not tested statistically. The drawing in Figure 3 shows the number and location of pores in sexually mature marmots. More than 30 secretory pores were observed on either side of the face. Pore density was greater in the area between the eye and ear than in the vicinity of the cheek bone. Few, if any, variations of this pattern were observed.

Microscopic Studies. As shown in Figure 4, the temporal gland is an exocrine gland located in the dermis. It is composed of a cluster of specialized interconnected, tubular lobules surrounded by connective tissue and supported by muscle. Secretions drain into a network of interconnecting ducts leading into primary ducts, which end as pores on the surface of the skin. These pores are not associated with hair.

DISCUSSION

Bioassay

As in a previous study (Bel et al., 1995), strong overmarking behavior was observed. Marmots sniffed tubes bearing alien scent marks longer and marked them more extensively than clean tubes. Since these trials were performed with free-living animals, there were no captivity-related confounding effects from

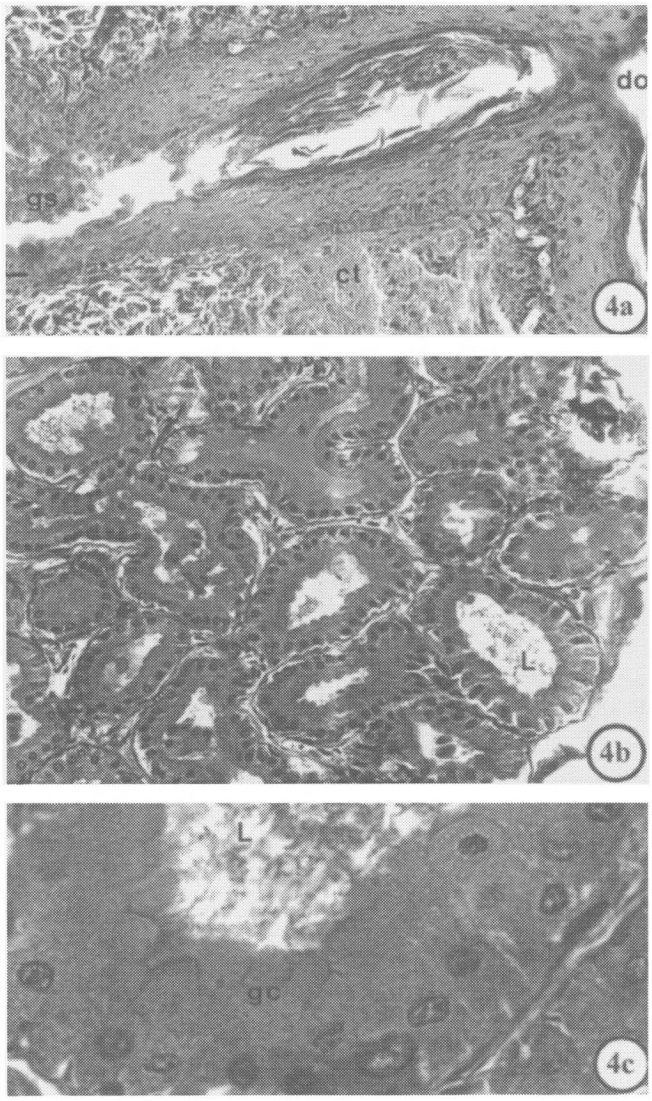

FIG. 4. Temporal scent gland of an adult Alpine marmot. (4a) Transverse section of a glandular duct opening onto the skin surface (do), surrounded by connective tissue (ct) and containing glandular secretion (gs); scale: 100 μm. (4b) Transverse section of a multilobular exocrine skin gland; scale: 100 μm. (4c) Detail of one lobule composed of glandular cells (gc) converging into a central lumen (L); scale: 25 μm.

diet, social system, or territory. Moreover, when meeting foreign marks from conspecifics on their territories, resident marmots also tended to remove or to replace them by their own scents. Thus, this bioassay method provides a simple, reliable method to assess the biological activity of extracts and fractions. In contrast, occurrence of marking was not a discriminant variable (see Bel et al., 1995), probably because marmots are likely to mark any new object found on their territories.

Stralendorff (1986) reported that behavioral responses such as sniffing of chemical signals from conspecifics were rarely specific in mammals and considered olfactory bioassays unsuitable for detection of pheromonelike signals in mammals. However sniffing behavior is often the only reliable means of investigation available for numerous studies of the mammalian olfactory communication system. In our experiments, sniffing behavior together with scent-marking behavior proved to be a powerful tool for assessing the biological activity of a substance. Similarly, in other marmot species, including *M. caligata* (Barash, 1989), *M. caudata aurea* (Blumstein and Henderson, 1996), *M. flaviventris* (Brady, 1997), and *M. monax* (Meier, 1991), sniffing has been used to investigate abilities to discriminate scents from individuals of different sex, familiarity, and/or territorial status.

Although marmots probably sniff odors at a distance, the duration of nose contact with the tubes provides an easy, precise, reproducible criterion. Another advantage is that by comparing duration of nose contact of each marmot towards both tubes, each animal can be used as its own control. In this way an index of the differential reaction of the resident marmot can be determined regardless of the amount of secretion that is deposited. This approach is not sensitive to interindividual or temporary variations in scent-producing ability.

Scent overmarking could serve several purposes in social and territorial species. One possibility is that scent marking is part of a "scent matching" mechanism that provides the basis of territorial maintenance with mutual advantages for the territory owner and intruder (Gosling, 1982). Alternatively, as in the golden hamster *Mesocricetus auratus*) (Johnston et al., 1995) or in the meadow vole *Microtus pennsylvanicus* (Johnston et al., 1997a,b), overmarking could be used by the sexual partner of the group to assess the individual's quality, and counter-marking could strongly influence mate-choice decisions. In the Alpine marmot, overmarking could be used to communicate information to both potential intruders and members of the group, such as the codominant sexual partner.

Marking Gland

Our histological findings support previous data in other marmot species (Rausch and Bridgens, 1989). The results strongly indicate that the temporal or "orbital" glands (Blumstein and Henderson, 1996) are the major source of secre-

tions used for scent-marking in Alpine marmots and suggest that cheek-rubbing is the main method of application. Bioassays determined that secretions from the temporal gland conveyed chemical information between conspecifics. Like golden marmots (Blumstein and Henderson, 1996) and woodchucks (Rausch and Rausch, 1971), adult Alpine marmots exhibit a zone of sparse fur coverage between the eye and ear on both sides of the head. This feature is probably the result of repeated rubbing against rocks, stones and the ground. Microscopic observation confirmed the presence of an apocrine gland in this zone.

Other products have been implicated in scent-marking behavior in mammals. Saliva plays a role in intraspecific communication in domestic cats (Feldman, 1994) and ground-dwelling squirrels (Steiner, 1974). Our results showed that saliva was not necessary to induce significant responses in Alpine marmots. The lack of any perioral secretion (J. L. Clément, personal observation), as well as of any particular smell in the perioral area, suggests that no functional perioral gland exists in this species. Feces and urine left in the territory also may convey chemical information between conspecifics, but we did not study those behaviors. Our results (Bel et al., 1995; this study) suggest cheek-rubbing to be the main marking mode.

Fractionation

This is the first time that fractionation has been used to study chemical communication in a marmot species. For this purpose we devised special techniques to allow field collection and analysis of pure and concentrated material. To minimize the effects of aging of scent-marks, which has been reported in some mammals (Clark, 1982; Johnston et al., 1995), all field trials were performed by using either fresh samples or samples that had been frozen immediately after collection.

Pentane, dichloromethane, and ethanol were used for extraction. Some compounds in the three extracts might be the same but only the ethanol extract was biologically active in eliciting a response similar to that of natural secretions. Pentane extracts showed no biological activity, and dichloromethane extracts induced only a slight, nonsignificant response. These findings suggest that the most polar fraction contains most of the active compounds. Although not commonly used for GC analysis, ethanol allowed successful solubilization of active compounds in the North American beaver (Svendsen and Huntsman, 1988; Schulte et al., 1995).

Neither of the two subfractions (fractions 1 and 2) exhibited bioactivity. There are two possible explanations for this result. The first may be that separation resulted in a selective loss of material. Molecules interacting specifically with active compounds could have been altered or too heavy to elute (e.g., proteins). The second explanation may be that detection of chemosignals by Alpine

marmots requires a threshold concentration. This requirement has been noted in many other species. Our results suggest that the active signal may be composed of a mixture of different molecules interacting synergistically (see Müller-Schwarze et al., 1986). Fractionation probably separated molecules that must interact to form a functional complex.

Most studies have concluded that chemosignals in mammals are highly complex and many compounds that interact to form functional mixtures have been identified (Stralendorff, 1987; Houlihan, 1989; Jemiolo et al., 1989; Müller-Schwarze and Houlihan, 1991; Schulte et al., 1994). Findings have generally been in agreement with the "social odor hypothesis," which states that mixtures are more active than single compounds and larger mixtures more active than smaller ones.

Albone (1984) stated that the "response-guided" strategy "is of only limited applicability in mammals," because chemosignals are complex mixtures. However, response-guided studies on semiochemicals have been successful in the North American beaver (Müller-Schwarze and Houlihan, 1991; Müller-Schwarze, 1992; Schulte et al., 1994, 1995), mice (Jemiolo et al., 1992, 1994; Singer et al., 1993), tree shrews (Stralendorff, 1987), and black-tailed deer (Müller-Schwarze, 1971; Crump et al., 1984). Further experiments with the fractionation-bioassay procedure are needed to identify the compounds underlying chemical communication in Alpine marmots.

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