

Differential territorial response toward conspecific and heterospecific scent marks by the Eurasian beaver (*Castor fiber*)

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Abstract: Territorial behavior directed toward conspecific scent marks is common in both the Eurasian (*Castor fiber*) and the North American beaver (*C. canadensis*), but how the two species react to scent marks of the other species is unknown. We tested the hypothesis that *C. fiber* allopatric to *C. canadensis* would discriminate between scent marks of the two species (both males and females) and predicted that it would show a more aggressive response toward conspecific than to heterospecific scent marks. We presented 46 different *C. fiber* colonies with two pairs of experimental scent mounds (ESMs) placed within the territory boundaries during 1999 (N=24) and 2000 (N=22). One pair was marked with anal gland secretion (AGS) and the other with castoreum from the castor sacs. Results showed that *C. fiber* (1) did not spend significantly longer time sniffing conspecific over heterospecific ESMs, (2) spent significantly longer time responding aggressively to conspecific over heterospecific ESMs, and (3) responded significantly more aggressive to conspecific over heterospecific ESMs overnight. Gas chromatographic comparisons of castoreum showed that differences between species accounted for 34% of the total variation in compounds detected, while differences between sexes accounted for 13%. For AGS, 49% and 46% of this variation was explained by differences between species and sex, respectively. These results support our hypothesis that *C. fiber* discriminates between conspecific and heterospecific scent marks. We suggest that the observed discrimination is based on differences between the two species in the chemical composition of AGS and castoreum, and that this difference is brought about by genetic and environmental factors through geographical isolation. *Key words:* *Castor canadensis*, territorial behavior, scent marking, discrimination, allopatric.

Introduction

Chemical signals are extensively used in the advertisement of territorial occupancy (Eisenberg and Kleiman, 1972; Gorman, 1984a; Gosling, 1990; Jannett, 1984) and are often the first line of defence against potential intruders (Jaeger, 1986). If the intruder persists, defence is maintained by aggressive encounters, usually followed by intense reapplication of scent marks by territory holders (e.g. house mice (*Mus domesticus*), Desjardins et al., 1973; tree shrews (*Tupaia belangeri*), Holst and Buerger-Goodwin, 1975; ringtailed lemurs (*Lemur catta*), Jolly, 1966; rabbits (*Oryctolagus cuniculus*), Lockley, 1961; mongooses (*Helogale undulatarufula*), Rasa, 1973; sugar gliders (*Petaurus breviceps*), Stoddart and Bradley, 1994). At territory boundaries and at specialized marking sites where some form of competitive interaction frequently takes place, countermarking (i.e. the deposition of scent on top of or in the immediate vicinity of conspecific scent marks; Ewer, 1968) of unfamiliar scent marks may reinforce the defensive strategy by functioning as competitive advertisement signals (Johnson, 1973; Johnston et al., 1994; Ralls, 1971). Hurst and Rich (1999) argued that when territory owners or dominant individuals are challenged by a competitor attempting to deposit competing scent marks in their scent-marked territory or area of dominance, countermarking of the competitor's scent marks would prove that they have overcome the challenge and successfully excluded the competitor, or otherwise inhibited further challenges. Countermarking also ensures that their own scent marks always remain the most recently deposited. Such behavior is readily seen among conspecifics (e.g. Gosling and Wright, 1994; Ramsay and Giller, 1996; Roper et al., 1993; Rosell et al., 2000), but few studies have examined the prevalence of countermarking between heterospecifics.

In a study on the behavioral importance of scent marking among sympatric wolves (*Canis lupus*) and coyotes (*C. latrans*), Paquet (1991) found that coyotes actively re-marked previously deposited wolf urine, whereas wolves were relatively passive (although over

marking did occur) in their response to coyote scent marks. He concluded that coyotes and probably also wolves regard the other species as trespassing conspecifics (i.e. misdirected intraspecific territorial behavior; Murray, 1971) and respond agonistically by over marking their scent marks. A study by Fornasieri and Roeder (1992) on captive *Lemur fulvus* and *L. macaco* showed that marking rates were considerably lower when individuals were confronted with scent marks of the other species compared to scent marks of unfamiliar conspecifics, indicating that lemurs do not regard heterospecific scent marks to be as high a threat, or as interesting, as those of conspecifics.

Interspecific territoriality might evolve when species with overlapping ecological requirements interact (Simmons, 1951). The greater the degree of overlap between species, the greater the competition for limited resources (Schoener, 1983). Responses to heterospecific scent marks should therefore be profitable in the sense of excluding potential competitors, and by gaining exclusive access to these resources. The ability to adequately respond to heterospecific scent marks should thus be most prevalent among species coexisting within the same area, or in areas of narrow sympatry (Murray, 1971). Among allopatric species the incentive of responding to heterospecific scent marks is thus not present, and Johnston and Robinson (1993) also argued that allopatric species have not been under any selective pressure to respond to heterospecific signals or to recognize particular individuals of another species. However, mammals often respond to scent from allopatric predators and are often repelled by them (e.g. Rosell and Czech, 2000). Dickman and Doncaster (1984) suggested that similar chemicals eliciting avoidance in rodents may commonly occur in the faeces and urine of carnivores (see also Bininda-Emonds et al., 2001). This is supported by observations that rodents often avoid the odors of carnivores with which there has been no evolutionary contact (Nolte et al., 1994; Roberts et al., 2001; Stoddart, 1982a,b). Gorman (1984b) showed that the Orkney race of common voles (*Microtus arvalis orcadensis*) that had

been isolated from mammalian predators for at least 5000 years, strongly avoided stoat (*Mustela erminea*) odor, suggesting an innate rather than learned response. Bowers and Alexander (1967) argued that genetically similar species often share the same olfactory range. Therefore, responses to olfactory signals may also be strong among allopatric congenetics.

The genus *Castor* consists of two species, the Eurasian beaver (*C. fiber*) (CF) and the North American beaver (*C. canadensis*) (CC), which have different numbers of chromosomes (2N=48 and 40, respectively), following Robertsonian fusion of eight chromosome pairs in CC (Lavrov and Orlov, 1973). The exact geographical origin of the genus is not known, but it appeared in Europe during the Late Miocene and by mid-Pliocene was represented in North America (Ward et al., 1991). Except for introduced populations of CC to Europe in the last century (e.g. Austria, Finland, France, Poland and Russia; see Nolet and Rosell, 1998; Rosell and Pedersen, 1999), the two species have been strictly allopatric since divergence (Novak 1987). The two species are very similar in appearance, behavior and ecology (Djoshkin and Safonov, 1972; Novak, 1987; Wilsson, 1971), and they are reproductively isolated (Ward et al., 1991; Zurowski, 1983). Ward et al. (1991) argued that neither species derived from the other, rather, they were likely derived from isolated populations in which some of the acrocentrics fused independently to produce monobrachial homology. CC and CF have been isolated for at least 9000 years and perhaps as long as 24 000 years (Ward et al., 1991).

Beavers are strictly territorial and defend their territories by scent marking (Houlihan, 1989; Rosell and Nolet, 1997; Rosell et al., 1998; Schulte, 1993;) with castoreum from the castor sacs (Müller-Schwarze, 1992; Rosell and Sundsdal, 2001) and probably with anal gland secretion (AGS) (Rosell and Bergan, 1998). All family members, except kits less than five months old, participate in marking the territory boundaries at scent mounds close to the water's edge (Rosell and Nolet, 1997; Rosell et al., 1998; Svendsen, 1980; Wilsson, 1971).

Aggressive scent marking behavior toward conspecific scent marks has been documented for both species of beavers (e.g. Müller-Schwarze and Heckman, 1980; Rosell et al., 2000). They have also been shown to discriminate between neighboring and unfamiliar conspecifics (Rosell and Bjørkøyli, in press; Schulte, 1998) and between unfamiliar siblings and unfamiliar non-relatives (Sun and Müller-Schwarze, 1997) implying that beavers are capable of discriminating between individuals based on their level of threat. However, no study has so far investigated how CF reacts to scent marks from CC (or vice versa).

We hypothesised that CF would discriminate between scent marks of the two species, i.e. that it would exhibit species discrimination abilities. We predicted that CF would show a more aggressive territorial response toward conspecific than to heterospecific scent marks. This study is one of few attempts that examine how chemical signals and behavioral response to the signals have diverged along with the speciation process. Also, this is of particular interest in the wake of introductions of CC to Eurasia and the impending range concurrence of the two species (Lahti, 1995).

Methods

Experimental area and animals

We conducted the experiment during July–October 1999 and May–August 2000 in a population of free-ranging beavers in Bø, Nome, Sauherad and Seljord municipalities, Telemark County, Norway. The mixed woodland and agricultural countryside contains many brooks, tarns, rivers and lakes. Vegetation is predominated by Norwegian spruce (*Picea abies*), Scots pine (*Pinus sylvestris*) and birch (*Betula pubescens*) with lesser stands of grey alder (*Alnus incana*), aspen (*Populus tremula*), rowan (*Sorbus aucuparia*) and willow (*Salix* spp.). Although some hunting and trapping occurs annually, beaver density appeared to be close to saturation (Rosell and Hovde, 2001; Rosell et al., 1998). A mean colony size of $3.8 \pm$

1.8 SD was found in 19 active colonies during autumn 1995 in Bø municipality (Steifetten and Uren, 1997).

Scent donors

Scent from CF was collected from nine adult males (mean weight (kg) = 16.9 ± 2.4 SD) and six adult females (mean weight (kg) = 16.9 ± 2.2 SD) shot in different colonies within the normal hunting season (1 October–30 April) during 1999 and 2000 in Bø, Nome, Sauherad and Rennebu (Sør-Trøndelag County) municipalities. To reduce the possibility of the test animals having had any previous contact with, or being genetically related to the scent donors, only scent from individuals that were shot >20 km from the experimental site, and in different watersheds, were used in a specific trial.

Scent from CC was collected from nine adult males (mean weight (kg) = 18.3 ± 4.2 SD) and eight adult females (mean weight (kg) = 18.4 ± 3.6 SD) shot within the normal hunting season (20 August–30 April) during 1999 and 2000 in the South Savo Game Management District in central Finland (S. Härkönen pers. comm.).

All beavers were frozen (-20°C) immediately after death until dissection of the animals could be performed. Prior to dissection, each animal was assigned to an age-class based on body weight (Hartman, 1992). The scent organs of CC were brought frozen to Norway. The extraction of castoreum was performed by making a lateral incision through the outer layer of the castor sacs, thereby revealing the castoreum, which in turn could be removed from the pocket lumen (Rosell et al., 2000). AGS, which has a more liquid consistency, was squeezed out by applying external pressure on the anal pocket (Rosell and Sun, 1999). All samples were stored in glass vials and frozen until use. The identification of species and sex was determined by the colour and viscosity of AGS (Rosell and Sun, 1999).

Experimental procedure

To establish whether CF would make the predicted discrimination in favour of its own versus another species, we used a two-sample choice test developed by Sun and Müller-Schwarze (1997). Two pairs of experimental scent mounds (ESMs) (i.e. one pair scented with castoreum and the other pair with AGS) were set up at two different locations on the riverbank/shoreline, preferably in close proximity (5-15 meters) of the active lodge (see Rosell and Bjørkøyli, in press). In cases where this was not feasible due to dense vegetation or rugged terrain we constructed the ESMs in places where beavers were suspected to visit during the night (e.g. fresh foraging sites, canals or paths ascending from the water). The distance between the two pairs varied between trials, but was never less than 10 m. Each pair consisted of one mound with scent from CF and one with scent from CC. The two types of scent were always from the same individual during one trial. Moreover, to avoid a possible bias in response intensity due to physiological differences between the two scent donors, beavers making up a pair were always approximately equal with regard to weight (mean difference (kg) = 2.4 ± 1.3 SD) and to the date on which they were killed (mean difference (days) = 21.7 ± 12.1 SD). To control for side dependencies, counterbalancing of the mounds was performed in a random order for each trial. Male and female scent was never matched during trials.

The two scent mounds within a pair were always equal in size and shape and the material used was the same for both mounds (e.g. sediments, mud, soil, debris or moss). We used latex gloves at all times during the construction of ESMs to prevent contamination with human odor. The distance between the two scent mounds within a pair was set at 30 cm to ensure that once a beaver responded to one of them, it would also have an equal opportunity to respond to the other. Hence, between-treatment effect could be compared (Sun and Müller-Schwarze, 1997). We used a plastic bottle cap (2.5 cm top diameter) to hold the scent material and to control the evaporation surface area. The cap was placed in the centre of the mound, even

with the surface. For the field bioassay, 0.25 g of AGS or castoreum was applied to each ESM as pilot studies showed that this amount was well above the response threshold of beaver (see also Rosell and Bjørkøyli, in press; Schulte, 1998; Sun and Müller-Schwarze, 1997). For each trial, the application of scent to the ESMs was performed 30–60 min before the beavers emerged from the lodge. Usually the observation period ended when fading daylight prevented further observations, but on a few occasions a powerful searchlight was used to extend the “light period”. This, however, did not affect the behavior of the animals (see Nolet and Rosell, 1994; Rosell and Hovde, 2001). We used a total of 46 different beaver colonies (\geq two adults) as experimental sites (1999: $N=24$; 2000: $N=22$).

Measures of response

We recorded the duration (in seconds) each beaver sniffed (i.e. when beavers while on land and within 5 cm of the ESMs distinctly directed their heads towards one of the mounds and sniffed) and responded aggressively (i.e. when beavers either straddled, pawed and/or over-marked an ESM) towards the ESMs with a dictaphone. Observations of beavers were performed during the evening by using light sensitive binoculars. We included only the first beaver responding to a pair of ESMs in the analyses. Only responses from subadult (12–24 months) and adult (>24 months) individuals were considered. If kits were seen responding first to both sets of ESMs, they were excluded from all analyses since their response resembled playful investigations (e.g. biting and playing with the cap) more than actual responses, and were therefore not considered to be indicative of the discriminatory abilities of the beaver. In such cases the observations were aborted and the ESMs were left overnight. If no beavers were observed during an evening trial, the scent samples were removed and the scent mounds destroyed so that the same colony could be used on a later occasion.

Since beavers live in family units, different members of a family may respond sequentially to a pair of ESMs at different times during the same night (Sun and Müller-Schwarze, 1998a). Schulte (1993) argues that a family response is a better descriptor for beaver response since the end result of a territorial response is based on the collective effort of several family members. Thus, the ESMs responded to during the evening trials were left overnight and measured the following morning. To determine response intensity, each ESM was given a rank index value (0-6) correlated with its level of destruction, 6 indicating top response. An additional value of 1 was given to ESMs in which an over marking had occurred; hence, a maximum score of seven was possible (Rosell and Bjørkøyli, in press).

Gas chromatographic analyses

In an attempt to reveal any chemical correlates of behavioral response, gas chromatographic analyses of AGS and castoreum from both species were performed. Prior to analyses, each sample (0.3g) was added 4 ml of a 3:1 mixture of toluene and methanol, respectively. AGS was completely dissolved. To extract compounds from the more solid castoreum, we also subjected it to high pressure and temperatures. For this purpose a Milestone MLS 1200 Mega microwave oven was used. The extraction program had the following cycle: 5 min at 250W and 100°C, 5 min at 400W and 130°C, and 5 min at 400W and 160°C. All solutions were filtrated and stored in a refrigerator (4°C) until injection.

From each sample 1 μ l was injected into a HP 6890 Series II gas chromatograph equipped with a HP-5 MS 5% phenyl-methyl-siloxane capillary column (30 m x 0.25 mm x 0.25 μ m film thickness) connected to a HP 5973 Series mass selective detector with a split/split-less inlet used in the split-less mode. Helium was used as the carrier gas at a constant flow 0.7 ml/min. The initial oven temperature was set at 130°C, and then increased 4°C/min to 310°C,

which was maintained for 15 min. To avoid that the solvent damaged the detector, a delay of 2 min was set for every run.

Statistical analyses

To check for differences in sniffing intensity, aggressive response and overnight response the Wilcoxon signed-ranks test was used. Tied observations were dropped from the analyses (Siegel and Castellan, 1988). Because no significant difference in any type of response measured was detected between years or between the two sexes, all responses were pooled. All tests were two-tailed with a significance level of 0.05. Statistical analyses were performed with *SPSS 10.0* software.

In order to test whether the gas chromatograms (GC) from the two species (both males and females) differed in the composition of the compounds detected, GC samples were compared using Partial Least Squares (PLS2) regression (Wold et al., 1983). PLS2 is a multivariate calibration method that models the relation between one variable, X, and several Y-variables simultaneously, in search for structure, both between variables and between individuals (Martens and Næs, 1989). As basis for comparison, the total ion current (TIC) for each time unit on the retention scale (82 time units/min) was measured and then calculated on the basis of a correlation matrix. Due to considerable variation in TIC-values between samples of the same species, all samples were first scaled (highest value assigned 1 and lowest value 0) as to minimize the effect of such discrepancy. The statistical software used was *The Unscrambler 7.5*.

Results

Field experiment

Beavers did not spend significantly longer time sniffing castoreum ($T=212$, $N=29$, $P=0.911$) or AGS ($T=280$, $N=29$, $P=0.181$) from CF compared to CC (Fig. 1a). Beavers spent, however, significantly longer time responding aggressively to both castoreum ($T=223$, $N=24$, $P=0.036$) and AGS ($T=223$, $N=23$, $P=0.008$) from CF compared to CC (Fig. 1b). Overnight, beavers responded significantly more aggressively to castoreum ($T=228$, $N=22$, $P<0.001$) and AGS ($T=230$, $N=24$, $P=0.022$) from CF compared to CC (Fig. 1c).

[Fig. 1a, b, c near here]

GC comparisons

Although males and females of the two species differed in the chemical composition of castoreum, this difference was obscure for all four groups, in particular between males and females of the same species (Fig. 2a). Of the total variation within all GC-samples, 34% was related to differences between the two species (PC1), while only 13% could be related to differences between sexes (PC2). This demonstrates that conspecific male and female castoreum are more similar in chemical appearance compared to that of the other species.

A more distinctive difference was found when male and female AGS of the two species were compared (Fig. 2b). PC1 and PC2 accounted for 49% and 46% of the total variation, respectively, demonstrating that the chemical composition of AGS shows a considerable difference both between species and sexes.

[Fig. 2a, b near here]

Discussion

The results confirm our hypothesis that CF discriminates between scent marks of the two species. This is supported by the significantly longer time spent responding aggressively, and stronger aggression exerted upon conspecific than heterospecific scent marks. This indicates that CF does not recognize the scent marks of CC to be an equally potential threat as those of CF. Although beavers were indiscriminate when sniffing the ESMs, sniffing can be defined as only the investigation stage within a complete set of multiple responses. The main purpose of a beaver's investigation of an ESM is to identify the sender, and then, based on the information obtained, decide what appropriate actions to take (i.e. signal detection theory; see Bradbury and Vehrencamp, 1998). Thus, similar sniffing durations, or a lack of preference, does not indicate inability to discriminate (Brown, 1979; Gouat et al., 1998; Johnston, 1993), but can be interpreted as a process of decision-making. A similar behavior has also been described for tree shrews where the presentation of heterospecific scent marks elicited intense olfactory investigation, but no equivalent increase in scent marking activity (Holst and Buerger-Goodwin, 1975). If the chemical signal present in castoreum and AGS of each species to some extent matches the chemical template of the other species, this might have led to the undifferentiated sniffing duration because beavers found it difficult to distinguish the two species. As such, sniffing duration is more likely to be a measure of olfactory similarities between the two species than an actual measure of discriminatory abilities.

When congenetic species are separated for any length of time, they may diverge in such a manner that neither species is distinguishable to the other with regard to olfactory signals. Although some chemical constituents may persist in both species, it is not adequate information to evoke a territorial response of similar strength as to a conspecific. As such, CF would regard intrusive scent marks of CC to pose a lesser territorial threat than conspecific scent marks, and would therefore be less likely to spend time and energy countermarking

these scent marks. We can however not rule out the possibility that beavers do recognize some of the chemical constituents of heterospecific scent marks, but without frequent contact they do not respond as aggressively as to conspecific scent marks. Murray (1971) pointed out that interspecific territoriality is a characteristic that is not adaptive and has not been selected for, but might evolve when two species compete for some material resource when they occur in the same habitat (see also e.g. Catchpole, 1978; Greenberg et al., 1996; Griffis and Jaeger, 1998). This implies that a territorial response toward heterospecific scent marks should be based on individual experiences only, and not on autonomically controlled (Paquet, 1991) or innate mechanisms. Thus, the reduced aggression observed toward scent marks of CC might be explained by a lack of stimulation, i.e. both chemical and visual stimulus are needed to evoke a territorial response. Studies of interactions between temporally displaced signals indicate that the first cue (in this case chemical) functions to alert the receiver to the presence of the second cue (visual), increasing the probability of its detection and recognition (Endler, 1992, 1993; Wiley, 1994).

The corresponding results of the two types of aggressive response measured (i.e. direct and overnight responses) indicate that discrimination of heterospecific scent marks is not a specific feature related to the first beaver responding, but is common behavior among most individuals. The fact that beavers live in family units enhances the possibility of more than one family member responding to the same scent marks during the night. This was readily seen during observation trials where several family members successively responded to the same pair of ESMs. Although successive visits would probably increase the cumulative probability of recognition errors, the results in this study show that misdirected territorial aggression is rare, implying that the chemical constituents present in CC scent marks are insufficient to evoke a territorial response.

The GC comparisons of castoreum show that between-sex variation within the same species (13%) is less pronounced than between-species variation (34%). This demonstrates that the composition of compounds present in castoreum differs between the two species, and that the reduced aggression observed toward castoreum of CC may be attributed to this difference. Since castoreum is a mixture of secondary metabolites most likely originating from the beavers diet (Müller-Schwarze, 1992, 1999; Svendsen, 1978), the most obvious explanation to account for the difference in chemical composition would be the differences in the diet between the two species. This would also explain the less pronounced variation found between males and females of the same species, both inhabiting the same habitat containing the same types of food. However, the two species inhabit similar vegetation types (see Nordiska ministerrådet, 1984 for comparison) and probably forage on many of the same plants. Thus, other factors besides diet may be in part responsible for the observed difference (e.g. genetically based components: see Halpin, 1986; bacterial flora: Albone et al., 1977; Walro and Svendsen, 1982).

The suggestion that a reduced aggressive response toward scent marks of CC is based on chemical differences between the two species is to a greater extent supported by AGS in which between-species variation accounted for 49%. A possible interpretation for this major difference would be that one of the primary functions of AGS is to signal species identity in order to maintain reproductive isolation. Tinbergen (1953) stated that although closely related species are very often similar in behavior and morphology, there are always some striking differences between mating cues. However, since both species have been separated since bisection, the development of species-specific mating cues has not been required, and therefore has probably also not been selected for. A more plausible interpretation would be that the difference in chemical composition of AGS has gradually evolved as a consequence of genetic drift and/or adaptation to the local environment, following Mayr's (1963)

geographic isolation speciation model. Ovaska (1989) found that in two separated populations of the salamander (*Plethodon vehiculum*), pheromonal divergence could not be explained by premating isolation mechanisms evolved through reinforcement, but suggested that it was brought about by pleiotropic effects associated with other changes evolved in isolation (see also Andersson, 1994; Dempster et al., 1993; Passmore, 1985; Verrel and Arnold, 1989). On the other hand, the profound difference between male and female AGS within the same species (46%) suggests that AGS is used to signal sexual identity (see Rosell and Sun, 1999; Schulte et al., 1995; Sun and Müller-Schwarze, 1999). Its function in territory maintenance, however, is unclear. Compared to castoreum, AGS is probably more costly to produce. Rosell and Sundsdal (2001) found that out of 96 scent marks on snow only four contained compounds from the anal glands. Although no equivalent study has been performed during the ice-free seasons, this indicates that the primary function of AGS is probably not to act as a territory defence signal. Sun and Müller-Schwarze (1998a) recently documented that related individuals of CC shared more features in the chemical AGS profile than did unrelated individuals. Sun and Müller-Schwarze (1998b) further demonstrated that it is possible to use some AGS compounds to classify different families. As such, these studies indicate that AGS is probably used in kin and family recognition.

Future research should focus on the responsive behaviors and territorial interactions between the two species in areas of sympatry. By performing similar experiments in areas in Eurasia where CC has been introduced it will be possible to establish whether or not CF will recognize CC as a potential competitor, and determine the validity of the belief that CC has out competed CF in parts of Finland (Lathi, 1995). It will also be interesting to know how CC reacts to scent marks from CF (under investigation (Schipper et al., unpublished)). Ignorance of the importance of olfactory communication between animals may seriously compromise the existence of endemic species when introducing ecologically similar species (e.g. European

mink (*Mustela lutreola*), Maran et al., 1998; red squirrels (*Sciurus vulgaris*), Wauters et al., 2000).

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References

- Albone ES, Gosden PE, Ware GC, 1977. Bacteria as a source of chemical signals in mammals. In: Chemical signals in vertebrates I (Müller-Schwarze D, Mozell MM, eds). New York: Plenum; 35–43.
- Andersson M, 1994. Sexual selection. New Jersey: Princeton University Press.
- Bininda-Emonds ORP, Decker-Flum DM, Gittleman JL, 2001. The utility of chemical signals as phylogenetic characters: an example from the Felidae. *Biol J Linnean Soc* 72:1-15
- Bowers JM, Alexander BK, 1967. Mice: individual recognition by olfactory cues. *Science* 158:1208–1210.
- Bradbury JW, Vehrencamp SL, 1998. Principles of animal communication. Massachusetts: Sinauer Associates.
- Brown RE, 1979. Mammalian social odors: a critical review. *Adv Study Behav* 10:103–162.
- Catchpole CK, 1978. Interspecific territorialism and competition in *Acrocephalus* warblers as revealed by playback experiments in areas of sympatry and allopatry. *Anim Behav* 26:1072–1080.
- Danilov PI, Kan'shiev VY, 1983. The state of populations and ecological characteristics of European (*Castor fiber* L.) and Canadian (*Castor canadensis* K.) beavers in the northwestern USSR. *Acta Zool Fenn* 174:95–97.
- Dempster ER, Dempster R, Perrin MR, 1993. Behavioural divergence in allopatric and sympatric gerbil species (Rodentia: Gerbillinae). *Ethology* 93:300–314.
- Desjardins C, Maruniak JA, Bronson FH, 1973. Social rank in the house mouse: differentiation revealed by ultra-violet visualisation of urinary marking patterns. *Science* 182:939–941.
- Dickman CR, Doncaster CP, 1984. Responses of small mammals to red fox (*Vulpes vulpes*) odour. *J Zool* 204: 521-531.

- Djoshkin WW, Safonov WG, 1972. Die biber der alten und neuen welt. Wittenberg: A
Ziemsen Verlag.
- Eisenberg JF, Kleiman DG, 1972. Olfactory communication in mammals. *Annu Rev Ecol
Syst* 3:10–32.
- Endler JA, 1992. Signals, signal conditions, and the direction of evolution. *Am Nat* 139
(Suppl.):125-153.
- Endler JA, 1993. Some general comments on the evolution and design of animal
communication systems. *Philo Trans R Soc Lond B Biol Sci* 340:215-225.
- Ewer RF, 1968. *Ethology of mammals*. New York: Plenum.
- Fornasieri I, Roeder JJ, 1992. Behavioral responses to own and other species' scent marks in
Lemur fulvus and *Lemur macaco*. *J Chem Ecol* 18:2069–2082.
- Gorman ML, 1984a. Scent marking and territoriality. *Acta Zool Fenn* 171:49–53.
- Gorman ML, 1984b. The responses of prey to stoat (*Mustela erminea*) scent. *J Zool* 202:419-
423.
- Gosling LM, 1990. Scent marking by resource holders: alternative mechanisms for
advertising the costs of competition. In: *Chemical signals in vertebrates V* (Macdonald
DW, Müller-Schwarze D, Natynczuk SE, eds). Oxford: Oxford University Press; 315–
328.
- Gosling LM, Wright KHM, 1994. Scent marking and resource defence by male coypu
(*Myocaster coypus*). *J Zool* 234:423–436.
- Gouat P, Patris B, Lalande C, 1998. Conspecific and heterospecific behavioural
discrimination of individual odours by mound-building mice. *Anim Physiol* 321:571–
575.
- Greenberg R, Reitsma R, Angon AC, 1996. Interspecific aggression by yellow warblers in a
sun coffee plantation. *Condor* 98:640–642.

- Griffis MR, Jaeger RG, 1998. Competition leads to an extinction-prone species of salamander: interspecific territoriality in a metapopulation. *Ecology* 79:2494–2502.
- Halpin ZT, 1986. Individual odors among mammals: origins and functions. *Adv Stud Behav* 16:39–70.
- Hartman G, 1992. Age determination of live beaver by dental X-ray. *Wildl Soc Bull* 20:216–220.
- Hill EP, 1982. Beaver (*Castor canadensis*). In: Wild mammals of North America – biology, management, and economics (Chapman JA, Feldhamer GA, eds). Baltimore London: The John Hopkins University Press; 256–281.
- Holst DV, Buergel-Goodwin U, 1975. Chinning by male *Tupaia belangeri*: the effects of scent marks of conspecifics and of other species. *J Comp Physiol* 103:153–171.
- Houlihan PW, 1989. Scent mounding by beaver (*Castor canadensis*): functional and semiochemical aspects (MSc thesis). Syracuse: State University of New York.
- Hurst JL, Rich TJ, 1999. Scent marks as competitive signals of mate quality. In: Advances in chemical signals in vertebrates (Johnston RE, Müller-Schwarze D, Sorensen P, eds). New York: Plenum; 209–226.
- Jaeger RG, 1986. Pheromonal markers as territorial advertisement by terrestrial salamanders. In: Chemical signals in vertebrates IV (Duvall D, Müller-Schwarze D, Silverstein RM, eds). New York: Plenum; 191–203.
- Jannett F, 1984. Scent communication in social dynamics of mammals. *Acta Zool Fenn* 171:43–47.
- Johnson RP, 1973. Scent marking in mammals. *Anim Behav* 21:521–535.
- Johnston RE, 1993. Memory for individual scent in hamsters (*Mesocricetus auratus*) as assessed by habituation methods. *J Comp Psychol* 107:201–207.

- Johnston RE, Chiang G, Tung C, 1994. The information in scent over-marks of golden hamsters. *Anim Behav* 48:323–330.
- Johnston RE, Robinson TA, 1993. Cross-species discrimination of individual odors by hamsters (Muridae: *Mesocricetus auratus*, *Phodopus campbelli*). *Ethology* 94:317–325.
- Jolly A, 1966. Lemur behaviour: a Madagascar field study. Chicago: University of Chicago Press.
- Lahti S, 1995. Bävrens utbredning i Finland från 1980-talet fram till idag. In: Proceedings of the third Nordic Beaver Symposium (Ermala A, Lahti S, eds). Helsinki: Finnish Game and Fisheries Research Institute; 41–43.
- Lavrov LS, Orlov VN, 1973. Karyotity i taksonomija sovremennich bobrov (*Castor*, Castoridae, Mammalia). *Zool Zh* 52:734–742.
- Lockley RM, 1961. Social structure and stress in the rabbit warren. *J Anim Ecol* 30:385–423.
- Maran T, Macdonald DW, Kruuk H, Sidorovich V, Rozhnov VV, 1998. The continuing decline of the European mink, *Mustela lutreola*: evidence for the intra-guild competition hypothesis. *Symp Zool Soc Lond* 71:297–323.
- Martens H, Næs T, 1989. Multivariate calibration. Chichester New York: John Wiley & Sons.
- Mayr E, 1963. Animal species and evolution. Cambridge: Harvard University Press.
- Müller-Schwarze D, 1992. Castoreum of beaver (*Castor canadensis*): function, chemistry and biological activity of its components. In: Chemical signals in vertebrates VI (Doty RL, Müller-Schwarze D, eds). New York: Plenum; 457–464.
- Müller-Schwarze D, 1999. Signal specialization and evolution in mammals. In: Advances in chemical signals in vertebrates (Johnston RE, Müller-Schwarze D, Sorensen P, eds). New York: Plenum; 1–14.
- Müller-Schwarze D, Heckman S, 1980. The social role of scent marking in beaver (*Castor canadensis*). *J Chem Ecol* 6:81–95.

- Murray BG Jr, 1971. The ecological consequences of interspecific territorial behavior in birds. *Ecology* 52:414–423.
- Nolet BA, Rosell F, 1994. Territoriality and time budgets in beavers during sequential settlement. *Can J Zool* 72:1227–1237.
- Nolet BA, Rosell F, 1998. Comeback of the beaver *Castor fiber*: an overview of old and new conservation problems. *Biol Conserv* 83:165–173.
- Nolte DL, Mason JR, Epple G, Aronov E, Campbell DL, 1994. Why are predator urines aversive to prey? *J Chem Ecol* 20:1505-1516.
- Nordiska ministerrådet, 1984. Vegetationstyper i Norden. Stockholm: Nordiska ministerrådet.
- Norusis MJ, 1993. SPSS Base System User's Guide Release 6.0. Chicago: SPSS, Inc.
- Novak M, 1987. Beaver. In: Wild furbearer management and conservation in North America (Novak M, Baker JA, Obbard ME, Malloch B, eds). Ontario: Ministry of Natural Resources; 283–312.
- Ovaska K, 1989. Pheromonal divergence between populations of the salamander *Plethodon vehiculum* in British Columbia. *Copeia* 3:770–775.
- Paquet PC, 1991. Scent-marking behavior of sympatric wolves (*Canis lupus*) and coyotes (*C. latrans*) in Riding Mountain National Park. *Can J Zool* 69:1721–1727.
- Passmore NI, 1985. Sibling species, the acoustic environment and the anuran specific-mate recognition system. In: Species and speciation (Vrba ES, ed). Pretoria: Transvaal Museum; 125–127.
- Ralls K, 1971. Mammalian scent marking. *Science* 171:443–449.
- Ramsay NF, Giller PS, 1996. Scent-marking in ring-tailed lemurs: responses to the introduction of “foreign” scent in the home range. *Primates* 37:13–23.
- Rasa OAE, 1973. Marking behaviour and its social significance in the African dwarf mongoose, *Helogale undulatarufula*. *Z Tierpsychol* 32:293–318.

- Roberts SC, Gosling LM, Thornton EA, McClung J, 2001. Scent-marking by male mice under the risk of predation. *Behav Ecol* 12:698-705.
- Roper TJ, Conrard L, Butler J, Christian SE, Ostler J, Schmid TK, 1993. Territorial marking with faeces in badgers (*Meles meles*): a comparison of boundary and hinterland latrine use. *Behaviour* 127:289–307.
- Rosell F, Bergan F, 1998. Free-ranging Eurasian beavers, *Castor fiber*, deposit anal gland secretion when scent marking. *Can Field-Nat* 112:532–535.
- Rosell F, Bergan F, Parker H, 1998. Scent-marking in the Eurasian beaver (*Castor fiber*) as a means of territory defense. *J Chem Ecol* 24:207–219.
- Rosell F, Bjørkøyli T, In press. A test of the dear enemy phenomenon in the Eurasian beaver (*Castor fiber*). *Anim Behav*.
- Rosell F, Czech A, 2000. Responses of foraging Eurasian beavers *Castor fiber* to predator odours. *Wildl Biol* 6:13–21.
- Rosell F, Hovde B, 2001. Methods of aquatic and terrestrial netting to capture Eurasian beavers. *Wildl Soc Bull* 29:269–274.
- Rosell F, Johansen G, Parker H, 2000. Eurasian beavers (*Castor fiber*) behavioral response to simulated territorial intruders. *Can J Zool* 78:931–935.
- Rosell F, Nolet BA, 1997. Factors affecting scent-marking behavior in the Eurasian beaver (*Castor fiber*). *J Chem Ecol* 23:673–689.
- Rosell F, Pedersen KV, 1999. *Bever*. Oslo: Landbruksforlaget.
- Rosell F, Sun L, 1999. Use of anal gland secretion to distinguish the two beaver species *Castor canadensis* and *C. fiber*. *Wildl Biol* 5:119–123.
- Rosell F, Sundsdal LJ, 2001. Odorant source used in Eurasian beaver territory marking. *J Chem Ecol* 27:2471-2491.
- Schoener TW, 1983. Field experiments on interspecific competition. *Am Nat* 122:240–285.

- Schulte BA, 1993. Chemical communication and ecology of the North American beaver (*Castor canadensis*) (PhD dissertation). Syracuse: State University of New York.
- Schulte BA, 1998. Scent marking and responses to male castor fluid by beavers. *J Mammal* 79:191–203.
- Schulte BA, Müller-Schwarze D, Sun L, 1995. Using anal gland secretion to determine sex in beaver. *J Wildl Manage* 59:614–618.
- Siegel S, Castellan NJ Jr, 1988. Nonparametric statistics for the behavioral sciences. New York: McGraw-Hill.
- Simmons KEL, 1951. Interspecific territorialism. *Ibis* 93:407–413.
- Steifetten Ø, Uren G, 1997. Dagens beverforvaltning: et komparativt studie av tellende areal og tellende vannlengde som tildelingsgrunnlag for jakt og fangst av bever (BSc thesis). Bø i Telemark: Telemark University College.
- Stoddart DM, 1982a. Demonstrations of prey olfactory discrimination by the short-tailed vole, *Microtus agrestis*. *Anim Behav* 20:293-294.
- Stoddart DM, 1982b. Does trap odour influence estimation of population size of the short-tailed vole, *Microtus agrestis*? *J Anim Ecol* 30:375-386.
- Stoddart DM, Bradley AJ, 1994. Plasma testosterone concentration, body weight, social dominance and scent-marking in male marsupial sugar gliders. *J Zool* 232:595–601.
- Sun L, Müller-Schwarze D, 1997. Sibling recognition in the beaver: a field test for phenotype matching. *Anim Behav* 54:493–502.
- Sun L, Müller-Schwarze D, 1998a. Anal gland secretion codes for relatedness in the beaver, *Castor canadensis*. *Ethology* 104:917–927 .
- Sun L, Müller-Schwarze D, 1998b. Anal gland secretion codes for family membership in the beaver. *Behav Ecol Sociobiol* 44:199–208.

- Sun L, Müller-Schwarze D, 1999. Chemical signals in the beaver. One species, two secretions, many functions? In: *Advances in Chemical Signals in Vertebrates* (Johnston RE, Müller-Schwarze D, Sorensen PW, eds). New York: Kluwer Academic/Plenum Publishers; 281-288.
- Svendsen GE, 1978. Castor and anal glands of the beaver *Castor canadensis*. *J Mammal* 59:618–620.
- Svendsen GE, 1980. Patterns of scent-mounding in a population of beaver *Castor canadensis*. *J Chem Ecol* 6:133–148.
- Tinbergen N, 1953. *Social behaviour in animals*. London: Methuen & Co.
- Verrel PA, Arnold SJ, 1989. Behavioral observations of sexual isolation among allopatric populations of the mountain dusky salamander, *Desmognathus ochrophaeus*. *Evolution* 43:745–755.
- Vorburger C, Ribi G, 1999. Aggression and competition for shelter between a native and an introduced crayfish in Europe. *Freshw Biol* 42:111–119.
- Walro JM, Svendsen GE, 1982. Castor sacs and anal glands of the North American beaver (*Castor canadensis*): their histology, development, and relationship to scent communication. *J Chem Ecol* 5:809–819.
- Ward OG, Graphodatsky AS, Wurster-Hill DH, Eremina VR, Park JP, Yu Q, 1991. Cytogenetics of beavers: a case of speciation by monobrachial centric fusions. *Genome* 34:324–328.
- Wauters LA, Lurz PWW, Gurnell J, 2000. Interspecific effects of grey squirrels (*Sciurus carolinensis*) on the space use and population demography of red squirrels (*Sciurus vulgaris*) in conifer plantations. *Ecol Res* 15:271–284.

- Wiley RH, 1994. Errors, exaggeration, and deception in animal communication. In: Behavioral mechanisms in evolutionary ecology (Real LA, ed.). Chicago: University of Chicago Press; 157-189.
- Wilsson L, 1971. Observations and experiments on the ethology of the European beaver *Castor fiber* L. *Viltrevy* 8:115–266.
- Wold S, Martens H, Wold H, 1983. The multivariate calibration problem in chemistry solved by the PLS method. In: Lecture notes in mathematics, vol. 973 (Kågstrøm B, Ruhe A, eds). Berlin Heidelberg: Springer; 286–293.
- Zurowski W, 1983. Worldwide beaver symposium, Helsinki 1982: opening remarks. *Acta Zool Fenn* 174:85-86.

Figure legends

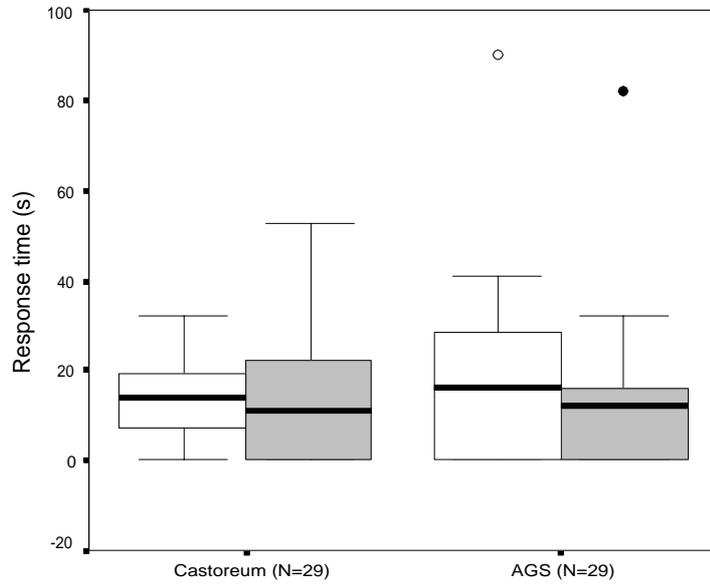
Figure 1

Responses of *C. fiber* to conspecific (□) and heterospecific (■) scent mounds experimentally marked with castoreum or anal gland secretion (AGS) and presented as the time (in seconds) beavers (a) sniffed and (b) responded aggressively (straddling, pawing and/or over marking), and (c) the ranked level of aggression measured overnight (see text). The line in the box indicates the median, the lower and upper ends of the box the 25% and 75% values, respectively, and the two whiskers the distance from the end of the box to the largest and smallest observed values that are less than 1.5 box lengths from either end of the box. Outliers (1.5 to 3 box lengths from the end of the box) are indicated with an open circle and extreme values (more than 3 box lengths from the end of the box) with a filled circle (Norusis, 1993). N=number of colonies. *P<0.05.

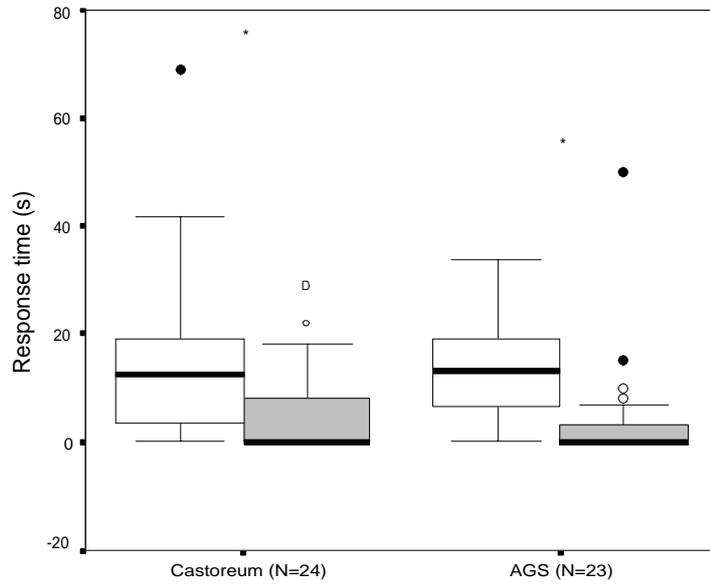
Figure 2

Partial Least Square (PLS2) score plots showing the position of each gas chromatogram of *C. fiber* (▽: Male; ○: Female) and *C. canadensis* (▼: Male; ●: Female) on the first two components for (a) castoreum (*C. fiber*: N=8 males and N=5 females; *C. canadensis*: N=9 males and N=5 females) and (b) anal gland secretion (*C. fiber*: N=6 males and N=5 females; *C. canadensis*: N= 7 males and N=5 females).

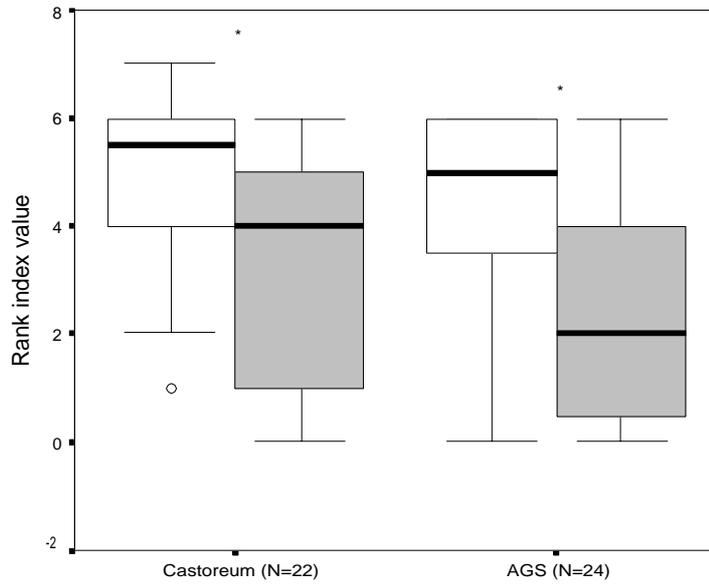
[Steiffetten and Rosell; Figure 1a]



[Steiffetten and Rosell; Figure 1b]



[Steifetten and Rosell; Figure 1c]



[Steifetten and Rosell; Figure 2b]

