Subspecies discrimination in the Scandinavian beaver (*Castor fiber*): combining behavioral and chemical evidence

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Abstract: Geographic isolation is one of several models that has been proposed to explain the evolutionary course of speciation. In this study, we examined how geographical isolation may affect subspecies discrimination in the free-ranging Scandinavian beaver (*Castor fiber fiber* L., 1758) by simulating a territorial intrusion by using scent (castoreum and anal gland secretion) from a con-subspecific (N = 8 for castoreum and N = 7 for anal gland secretion) and a hetero-subspecific (*Castor fiber albicus* Matschie, 1907; N = 2 for both castoreum and anal gland secretion). Direct observations of 33 families during evenings showed that beavers (*i*) sniffed castoreum but not anal gland secretion from *C. f. fiber* significantly longer than from *C. f. albicus* and (*ii*) responded aggressively (i.e., stood on the mound on their hind feet, pawing and (or) overmarking) significantly longer to castoreum but not anal gland secretion from *C. f. fiber* than from *C. f. albicus*. When experimental scent mounds were allowed to remain overnight, the response was significantly stronger to castoreum but not to anal gland secretion from *C. f. fiber* than from *C. f. albicus*. Gas chromatographic comparisons of castoreum and anal gland secretion from the two subspecies supported our behavioral observations for castoreum but not for anal gland secretion. These findings suggest that geographical isolation has developed discriminatory abilities in *C. f. fiber.* We further suggest that the proximate factors involved are of environmental origin.

Résumé : L’isolement géographique est l’un de plusieurs modèles proposés pour expliquer le développement de la spéciation au cours de l’évolution. Notre étude examine comment l’isolement géographique peut affecter la reconnaissance des sous-espèces chez le castor de Scandinavie (*Castor fiber fiber* L., 1758) sauvage par la simulation d’une intrusion territoriale au moyen d’odeurs (castoréum et sécrétions de la glande anale) provenant d’animaux la même sous-espèce (castoréum, N = 8, sécrétions de la glande anale, N = 7) et d’une autre sous-espèce (*Castor fiber albicus* Matschie, 1907; N = 2 pour le castoréum et les sécrétions de la glande anale). Des observations directes de 33 familles en soirée indiquent que les castors (*i*) reniflent le castoréum, mais pas les sécrétions des glandes anales, de *C. f. fiber* significativement plus longtemps que les mêmes substances provenant de *C. f. albicus* et (*ii*) qu’ils réagissent agressivement (position debout sur les pattes arrières sur les monticules, coups de pattes et (ou) marquage excessif) significativement plus longtemps au castoréum, mais non aux sécrétions des glandes anales, de *C. f. fiber*, qu’aux mêmes substances provenant de *C. f. albicus*. Si les monticules portant les odeurs sont laissés toute la nuit, la réaction est significativement plus forte au castoréum, mais non aux sécrétions de la glande anale, de *C. f. fiber* qu’aux mêmes substances provenant de *C. f. albicus*. Des analyses comparatives par chromatographie en phase gazeuse du castoréum et des sécrétions des glandes anales des deux sous-espèces appuient nos observations comportementales dans le cas du castoréum, mais pas dans celui des sécrétions des glandes anales. Ces résultats indiquent que l’isolement géographique a permis le développement de capacités de discrimination chez *C. f. fiber.* Nous croyons que les facteurs proximaux impliqués sont d’origine environnementale.

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Introduction

Geographic isolation is one of several models that has been proposed to explain the evolutionary course of speciation (see Andersson 1994; Bradbury and Vehrencamp 1998; Futuyma 1998). The model emphasizes the importance of geographic barriers as an evolutionary basis for the bisection of a species into two independently evolving populations (Mayr 1963). One submodel under geographic isolation, the vicariant speciation model, occurs when two rather widespread populations are divided by the emergence of an extrinsic barrier, the extinction of intervening populations,

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or migration into a separate region (Mayr 1963; Futuyma 1998). Vicariant speciation is generally supposed to proceed by the operation of natural selection, and by genetic drift, in each of the separated populations, to a greater extent in one than the other (Futuyma 1998). Eventually, interbreeding resulting in viable offspring will no longer be possible between the two populations.

Among the traits responsible for reproductive isolation, species-specific signals (visual, tactile, auditory, or olfactory cues) linked to the mate recognition system have by far received the most attention (Andersson 1994). Moreover, studies examining the effects of species divergence have kept the focus on an interspecies level. Little attention has been paid to how the speciation process may affect behavioral responses other than mating preferences, and also how these responses are expressed at intermediate stages. The standard paradigm of speciation (Mayr 1942) states that all species must go through a subspecies stage, making the concept one of evolutionary importance. Such knowledge may provide insight into how a single species bisects both temporally and spatially, and also elucidate which ecological factors are important for speciation to occur.

In essence, a subspecies is a collection of populations occupying a distinct breeding range and diagnosably distinct from other such populations (Mayr and Ashlock 1991). Subspecies discrimination has some empirical support. Smadja and Ganem (2002) found that subspecies (of the house mouse, *Mus musculus* L., 1758) were capable of discriminating between individuals belonging to the same subspecies and those of another subspecies. Moreover, Christophe and Baudoin (1998) also found that house mice discriminated between different populations of the same subspecies. These studies imply, at least for this species, that any segregation in space at any level are sufficient for the development of discriminatory mechanisms. It should be noted, however, that the former study used individuals from parapatric or sympatric populations, which probably would enhance the development of discriminatory abilities in these populations as a result of any detrimental effect of inbreeding depression. Whether the same pattern applies to allopatric populations that are not under the same selective pressure to avoid inbreeding remains to be seen.

In the Eurasian beaver (*Castor fiber* L., 1758), eight subspecies have been acknowledged, although their phylogenetic status is far from clear (Heidecke 1986; Savalyev 1997). For instance, based on craniological features, Lavrov (1983) suggested that *Castor fiber albicus* Matschie, 1907 (CFA) should be recognized as a third species in the genus *Castor* (i.e., as *C. albicus*, Elbe (or the western) beaver, described by P. Matschie in 1907). Currently, most European populations are of mixed subspecific origin (Halley and Rosell 2002), but an exception is the Scandinavian beaver (*Castor fiber fiber* L., 1758) (CFF) in Norway and the Elbe beaver (CFA) in Germany (Halley and Rosell 2002). These two subspecies have remained strictly isolated since the 18th century (Veron 1992). Therefore, it seems reasonable to believe that the process of divergence is the result of geographic isolation.

The beavers' main communication system is olfactory. They are strictly territorial and rely on scent to deter potential intruders (Rosell 2002; Rosell and Bjørkøyli 2002). Scent is used for intraspecific purposes such as individual, kin, family–nonfamily, and mate recognition (Sun and Müller-Schwarze 1999; Rosell 2002; Rosell and Bjørkøyli 2002). It is probably also used in species discrimination (Ø. Steifetten and F. Rosell, unpublished data). However, no study has so far investigated how CFF reacts to scent from CFA (i.e., subspecies discrimination). Because CFF and CFA are adapted to different vegetation zones (i.e., the boreonemoral and the nemoral zones, respectively) (Moen 1999), one might expect that any territorial response to be differentiated. Johnsson et al. (2000) showed that habitat preference increased the level of territorial defence in brown trout (*Salmo trutta* L., 1758). Individuals may acquire a preference for their natal habitat (habitat imprinting) (Klopfer and Ganzhorn 1985), which in turn would shape habitat assessment, and thus, lead to differential discrimination among individuals raised in different habitats. Habitat preference increases the pay-off asymmetry between territory holders and intruders (Alcock 1993). Also, among many species of rodents and other mammals it is well known that diet can influence body odour (e.g., Ferkin et al. 1997). These findings suggest that beavers should be capable of discriminating between individuals based on their level of threat and that territorial behavior through habitat preference may be an important factor as an evolutionary barrier for interspecific interactions. The role of olfactory cues as in ethological isolating mechanisms has already been demonstrated in lemmings, *Dicrostonyx groenlandicus* (Traill, 1823) and *Lemmus trimucronatus* (Richardson, 1825), by Huck and Banks (1980*a*, 1980*b*) and in house mice by Bowers and Alexander (1967) and Cox (1984).

In this study, we examined how geographical isolation may affect subspecies discrimination in the Eurasian beaver. To see whether behavioral discrimination other than mating behavior would occur in a geographically isolated population of CFF, we simulated a territorial intrusion by an allopatric heterospecific (CFA) and a conspecific. We hypothesized that free-ranging CFF would discriminate between scent marks of the two subspecies and predicted that it would show a more aggressive territorial response to scent marks from CFF than from CFA. We also performed chemical analyses of the scent material used to support any possible findings of discrimination. If discrimination was detected, we would also expect to find differences in the chemical profiles of the two subspecies. This study is one of few attempts that examine how chemical signals and behavioral response to the signals may have diverged between subspecies. Also, this is of particular interest in the wake of reintroductions of the Eurasian beaver throughout Eurasia and the impending range concurrence of different subspecies (Halley and Rosell 2002, 2004).

Materials and methods

Study area and study animals

We conducted the experiment during July–August 1998 and June–July 1999 in a population of free-ranging CFF 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* (L.) Karst.), Scots pine (*Pinus sylvestris* L.), and birch (*Betula pubescens* Ehrh.), as well as lesser stands of gray alder (*Alnus incana* (L.) Moench), aspen (*Populus tremula* L.), rowan (*Sorbus aucuparia* L.), and willow (species of *Salix* L.). Although some hunting and trapping occurred annually, CFF density appeared to be close to saturation (Rosell and Hovde 2001; Rosell 2002). A mean (±SD) colony size of 3.8 ± 1.8 was found in 19 active colonies during autumn 1995 in the Bø municipality (Steifetten and Uren 1997). Beavers have been in the area since the 1920s (Olstad 1937).

Scent donors and collection of scent samples

We collected scent from eight adult CFF males (mass (mean ± SD) = 18.6 ± 1.2 kg) shot in different colonies by hunters within the normal hunting season (1 October – 30 April) during 1997 and 1999 in Bø and Seljord municipalities (Parker et al. 2002). Beavers were not shot expressly for the study, but they were a part of the local hunting quota. 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.

We collected scent from two adult CFA males (16 and 22 kg) in the Biesbosch National Park, the Netherlands. During 1988–1991, 42 CFA from the Elbe river in Germany were reintroduced into Biesbosch (Nolet 1995). The population was estimated to be approximately 75 beavers in spring 2000 (Halley and Rosell 2002), and hunting and trapping in the area is prohibited. The first beaver died after surgery from injuries (broken leg) inflicted by a horse on 19 November 1997. The second beaver was killed by a car on 21 May 1999 (V. Dijkstra, personal communication).

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 mass (Parker et al. 2001). We sexed the animals by internal inspection of the sex organs. The scent organs of CFA 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. Anal gland secretion (AGS), which has a more liquid consistency, was squeezed out by applying external pressure on the anal pocket (Rosell and Bjørkøyli 2002). All samples were stored in glass vials and frozen until use. For each field bioassay, castoreum and AGS from the same individual were used, and we tried to use the different samples of CFF and CFA equally among trials. Moreover, to avoid a possible bias in response intensity owing to physiological differences between the two scent donors, beavers making up a pair were always approximately equal with regards to mass (difference (mean ± SD) = 3.0 ± 1.2 kg) and to the date on which they were killed (difference (mean ± SD) = 37.9 ± 8.6 days).

Experimental design

We constructed four types of experimental scent mounds (ESMs) inside each territory: castoreum from CFF (C–CFF) and CFA (C–CFA) and AGS from CFF (A–CFF) and CFA (A–CFA). We placed one C–CFF/C–CFA pair on one side of the lodge and one A–CFF/A–CFA pair on the other, preferably in close proximity (5–15 m) of the active lodge. The distance between the two pairs varied between trials but was never less than 10 m. We placed the ESMs of each pair 30 cm apart and within 50 cm of the water's edge (for details see Fig. 1 in Rosell and Bjørkøyli 2002). The ESMs were constructed where the beavers could easily make a land visit (walk onto land). This made it possible to compare beavers' reponses to C–CFF vs. C–CFA and A–CFF vs. A–CFA (i.e., each family was simultaneously exposed to two different two-sample choice tests; see also Sun and Müller-Schwarze 1997). Placement of the ESMs (C–CFF, C–CFA, A–CFF, A–CFA) were organized randomly by lot on each trial to control for side preference, and each beaver family was tested only once.

The two scent mounds within a pair were always as equal as possible in size and shape, and the material used was the same for both mounds (e.g., sediments, mud, soil, debris, or moss). We wore clean latex gloves at all times to prevent contamination with human odor and scraped a handful of mud and debris from the bottom of the stream or from the land when constructing the ESMs. We used a canoe or walked along the bank to the site where the ESMs were constructed. Each ESM was apporximately 15 cm wide and 10 cm high. The 30-cm distance between the two scent mounds ensured that once a beaver responded to one of them, it would also have an equal opportunity to respond to the other; therefore, between-treatment effect could be compared (Sun and Müller-Schwarze 1997).

We used a plastic bottle cap (2.5 cm top diameter, 1.2 cm high) in each ESM to hold 0.25 g of scent material and to control the evaporation surface area (Schulte 1998). The bottle cap was placed in the center of the ESM with the surface of the top even with the surface of the mound. For each trial, scent was set out 30–60 min before the beavers emerged from the lodge (1800–2000) (Rosell and Bjørkøyli 2002). The observation period usually ended when fading daylight prevented further observations (around 2200–2300), but on a few occasions a powerful searchlight was used to extend the "light period". This, however, did not affect the behaviour of the animals (see Nolet and Rosell 1994; Rosell and Hovde 2001). If no beavers were observed during an evening trial, we usually terminated the trial, removed the ESMs, and tried again on another evening. We used a total of 33 different beaver colonies (≥2 adults) as experimental sites (1998: N = 26; 1999: N = 7).

Measures of response

Direct observations

An observer with binoculars downwind on the opposite bank recorded on a dictaphone the duration in seconds of the two response patterns to ESMs (to C–CFF and C–CFA and (or) to A–CFF and A–CFA): (1) sniffing (on land and directed towards and within approximately 5 cm of the ESM) and (2) the "aggressive response", which involved standing on the ESM on its hind feet, pawing and (or) overmarking (putting a pile of mud either at the side or on top of the ESM and then marking it with castoreum and (or) AGS) (Rosell and Bjørkøyli 2002). Sniff duration was used as a measure of the time required by beavers to identify the scents. The "aggressive response" duration indicated how strong an agonistic behavior the ESMs triggered. We included only the responses of the first beaver's first land visit to the ESM, from the moment the beaver walked onto land within a radius of approximately 0.5 m from the ESMs to when it returned to the water, in our analyses because physical damage to the scent mounds (pawed, flattened, or obliterated) may cause some carry-over biases in the following responses by the same or other beavers (Sun and Müller-Schwarze 1997). Only responses from subadult (12–24 months) and adult (>24 months) beavers were considered. If kits were seen responding first to both sets of ESMs, they were excluded from all analyses because their response resembled playful investigations (e.g., biting and playing with the cap) more than actual responses, and therefore, were 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.

Overnight activity

We also ranked the overnight response by checking the ESMs the following morning. Since beavers live in family units, different members of a family may respond to a pair of ESMs sequentially at different times during the same night (Schulte 1993; Rosell and Bjørkøyli 2002). Therefore, we checked and ranked the response results overnight to characterize the intensity of the collective beaver family response. To determine response intensity, each ESM was given a rank index value (0–6) correlated with its level of destruction, with 6 indicating the top response. When beavers scent-marked over ESMs and (or) close by on self-constructed scent mounds (which could occur independent of ESM status), we gave the respective ESM an additional index value of 1; hence, the maximum score could be 7 (for details see Table 1 in Rosell and Bjørkøyli 2002). After measuring the response intensity of the ESMs the following morning, they were completely removed. Activity at the ESMs that could be attributed to other mammal species such as mink (*Mustela vison* Schreber, 1777) was not observed.

Gas-chromatographic analyses

In an attempt to reveal any chemical correlates of behavioral response, gas-chromatographic analyses of AGS and castoreum from both subspecies were performed. Prior to analyses, 4 mL of a 3:1 mixture of toluene and methanol was added to each sample (0.3 g). 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 250 W and 100 °C, 5 min at 400 W and 130 °C, and 5 min at 400 W and 160 °C. All solutions were filtrated and stored in a refrigerator (4 °C) until injection (Rosell and Sundsdal 2001).

From each sample, 1 μL was injected into a HP 6890 series II gas chromatograph equipped with a HP-5 MS 5% phenyl-methyl-siloxane capillary column (30 m long × 0.25 mm diameter × 0.25 μm 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 of 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 prevent the solvent damaging the detector, a delay of 2 min was set for every run (Rosell and Sundsdal 2001).

Data analysis

The data did not fit the assumptions of distribution and homogeneity of variance for parametric analysis (Sokal and Rohlf 1995); therefore, we used nonparametric statistics in accordance with Siegel and Castellan (1988). We used Wilcoxon's matched-pairs signed-ranks test to check for differences in sniffing intensity, aggressive response, and overnight response. Tied observations were dropped from the analyses (Siegel and Castellan 1988). We combined the data from the 2 years because no significant differences in any of the measures of response were found for the different ESMs. All tests were two-tailed and a probability level ≤0.05 was considered significant. Statistical analyses were performed with SPSS version 10.0 (SPSS Inc. 1999).

To test whether the gas chromatograms (GC) from the two subspecies differed in the composition of the compounds detected, a discriminating variable $\mathbf{y}$ was defined as 1 for *C. f. fiber* and 0 for *C. f. albicus*. Regressand variables $\mathbf{X}$ were defined as GC peaks for the samples involved. 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. As a result of considerable variation in TIC values between samples of the same subspecies, all samples were first scaled (highest value assigned 1 and lowest value 0) to minimize the effect of such discrepancy.

The linear discriminant model $\mathbf{y} = b_0 + \mathbf{Xb} + \mathbf{f}$ was used where b_0 is the offset, $\mathbf{b}$ is the regression coefficient, and $\mathbf{f}$ is the Y residuals. Since the various GC peaks (X variables) were strongly intercorrelated and the number of X variables were greater than the number of samples, a regression method developed in the field of Chemometrics was used (partial least squares regression (PLSR); Wold et al. 1983). The PLSR handles rank-deficient data sets and utilizes the natural intercorrelations between the GC peaks as a stabilizing advantage (rather than a "collinearity problem" like conventional full-rank ordinary least squares regression).

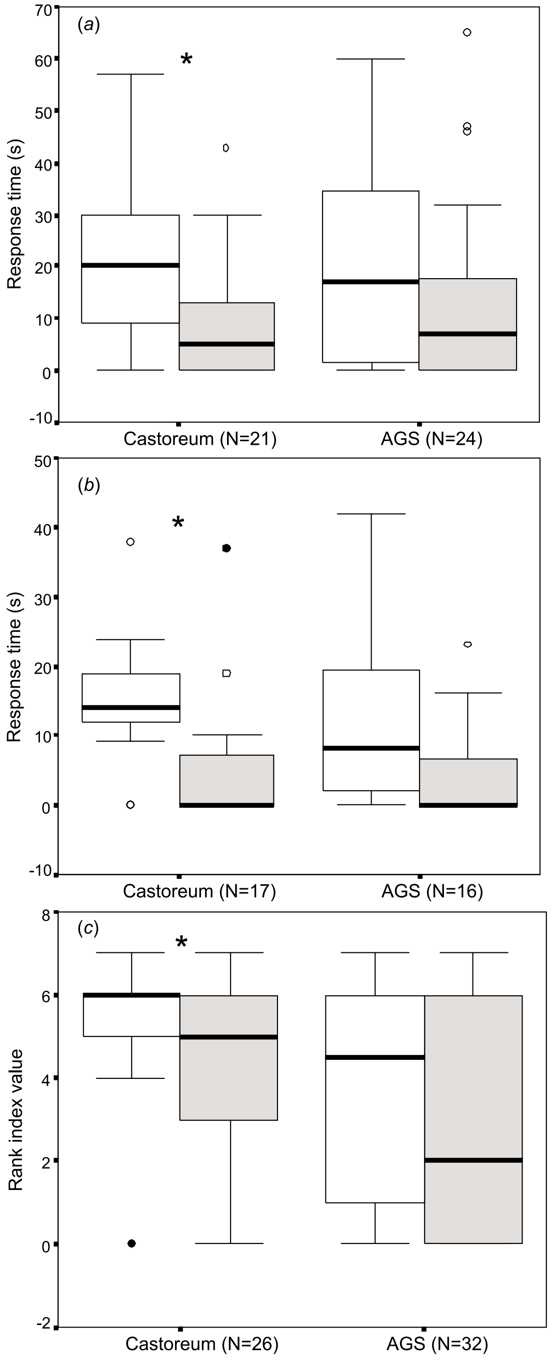
The PLSR is a bilinear method that extracts a small number of PLS components (PCs) $\mathbf{T}$ from $\mathbf{X}$; each orthogonal PC explains as much as possible the covariance between $\mathbf{X}$ and $\mathbf{y}$. These latent variables $\mathbf{T}$ are then used for bilinear modelling of both $\mathbf{X}$ and $\mathbf{Y}$: $\mathbf{X} = \bar{\mathbf{x}} + \mathbf{TP}' + \mathbf{E}$ where $\bar{\mathbf{x}}$ is the mean, $\mathbf{P}'$ is the X-loading matrix, and $\mathbf{E}$ is the X residuals and $\mathbf{y} = \bar{\mathbf{y}} + \mathbf{Tq}' + \mathbf{f}$ where $\bar{\mathbf{y}}$ is the mean, $\mathbf{q}'$ is the Y-loading matrix, and $\mathbf{f}$ is the Y residuals. The Unscrambler version 7.6 (CAMO Technologies 2000) was used for the PLSR analysis.

Results

Field experiments

Beavers spent significantly more time sniffing castoreum from CFF than from CFA (Wilcoxon's matched-pairs signed-ranks test, $T = 191$, $N = 21$, $P = 0.007$) but showed no difference for AGS ($T = 186$, $N = 24$, $P = 0.313$; Fig. 1*a*).

Fig. 1. Responses of beavers to CFF (open bar) and CFA (shaded bar) scent mounds experimentally marked with castoreum or anal gland secretion (AGS) and presented as the time Scandinavian beavers, *Castor fiber*, (*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 boxplot indicates the median; the lower and upper ends of the boxplot indicates the 25% and 75% values, respectively; and the two whiskers indicate the distance from the end of the boxplot to the largest and smallest observed values that are less than 1.5 box lengths from either end of the box. ○, outliers (1.5–3 box lengths from the end of the box); ●, extreme values (more than 3 box lengths from the end of the box); *N*, number of colonies; $^*P < 0.05$.



Beavers also spent significantly more time responding aggressively to castoreum from CFF than from CFA ($T = 131$, $N = 17$, $P = 0.008$) but showed no difference for AGS ($T = 99.5$, $N = 16$, $P = 0.107$; Fig. 1*b*). Overnight, beavers showed a significantly stronger response to castoreum from CFF than from CFA ($T = 264$, $N = 26$, $P = 0.021$) but showed no difference for AGS ($T = 321.5$, $N = 32$, $P = 0.287$; Fig. 1*c*).

GC Comparisons

CFF and CFA differed substantially in the chemical composition of castoreum (Fig. 2*a*). A full cross-validation analysis showed that three PCs were significant in explaining the variance within the data. PC1, PC2, and PC3 accounted for 84%, 7%, and 1% of the variance, respectively, indicating a higher dissimilarity between than within the two subspecies. It is worth noting that to a lesser extent the same dissimilarity was maintained when AGS was compared (Fig. 2*b*). Here, two PCs were significant, with PC1 and PC2 accounting for 58% and 22% of the variance, respectively.

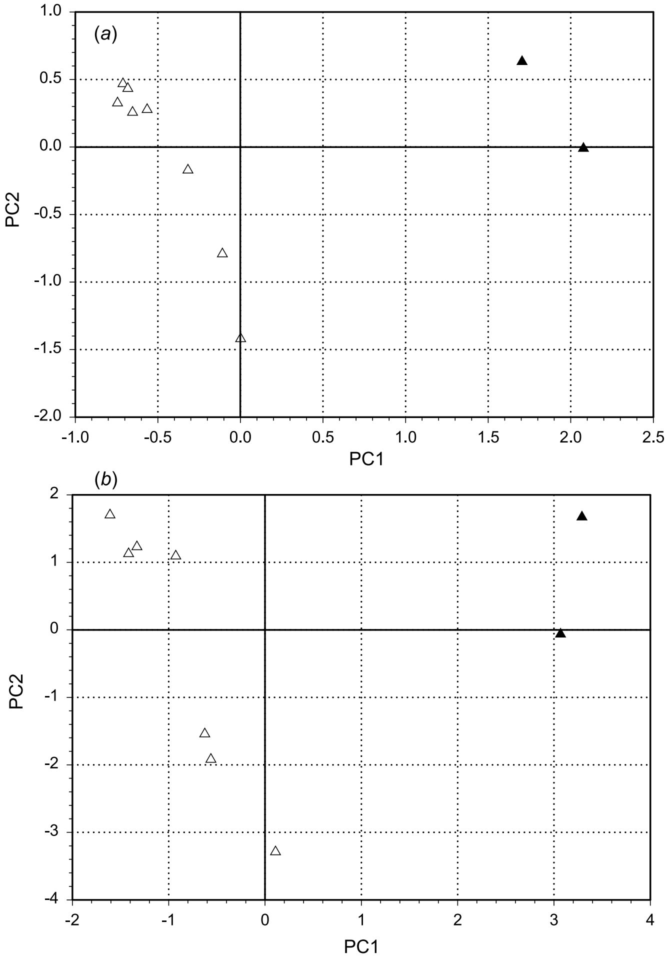
Discussion

Discrimination of castoreum

As predicted, the field results showed that CFF responded significantly longer and more strongly to castoreum from its own subspecies than from that of CFA. This suggests that the chemical signals from the two subspecies are different, which was also supported by our chemical analyses. As a consequence, CFF might not have recognized the scent marks of CFA to be an equally potential threat, or as interesting, as those of CFF.

The castor sac is a pocket lined with a layer of nonsecretory epithelium and is used to store what is believed to be a mixture of secondary metabolites from the urine (collectively called castoreum) (Walro and Svendsen 1982; Sun and Müller-Schwarze 1999). There is evidence that numerous castoreum compounds are derived from plants which beavers eat (Müller-Schwarze 1992). Therefore, an explanation to account for the observed difference in chemical composition would be the differences in the diet between the two subspecies. This would also explain the less pronounced variation found between males of the same subspecies, both inhabiting the same habitat containing the same types of food. We also found a more heterogeneous nature of the habitat in Telemark (O. Bozsér and F. Rosell, unpublished data) than in Biesbosch (Nolet et al. 1994). Nolet et al. (1995) found that in Biesbosch, in contrast to other food studies on the Eurasian beaver, CFA nearly exclusively ate woody plants (i.e., willows) all year round (see also Recker 1997; Heidecke 1988). In south Norway, however, roots of waterplants and green parts of herbs form a large part of the CFF diet during spring and summer (Histøl 1989; F. Rosell, unpublished data). Although some chemical constituents may persist in both subspecies, it is not likely to be adequate enough information to evoke a territorial response of similar strength. As such, CFF would regard intrusive castoreum scent marks of CFA to pose a lesser territorial threat than conspecific scent marks, and therefore, would be less likely to spend time and energy countermarking these scent marks. Castoreum contains information about territoriality (Sun and Müller-Schwarze 1999; Rosell 2002), and scent marks from

Fig. 2. Partial least square (PLS) score plots showing the position of each gas chromatogram of *Castor fiber fiber* (△) and *Castor fiber albicus* (▲) on the first two PLS components (PC) for (*a*) castoreum (*C. f. fiber*: $N = 8$; *C. f. albicus*: $N = 2$) and (*b*) anal gland secretion (*C. f. fiber*: $N = 7$; *C. f. albicus*: $N = 2$).



strangers containing similar compounds may indicate a higher territorial threat and willingness to take over the territory. Therefore, a higher territorial response may be given to strangers with similar habitat preferences (i.e., to beavers that live in the same environment and who feed on the same plant species). CFF may have the cognitive ability to use habitat characteristics to adjust their territorial aggression.

Discrimination of AGS

Contrary to expectations, our results showed that CFF did not respond significantly longer and more strongly to AGS from its own subspecies than from that of CFA. Why such discrepancy between castoreum and AGS? Compared with the castor sacs, the anal gland is a holocrine secretory gland that is probably not affected by diet (Svendsen 1978). From such a point of view, we would expect that the chemical profiles of AGS from the two subspecies to be more similar. However, our chemical analyses showed a clear difference, although this was less pronounced than for castoreum. One possible explanation is that although the overall difference in chemical composition is large, one or more key constituents may have prevailed in such amount that it was enough to elicit a behavioral response. These key constituents may be species specific, and they are more likely to be found in AGS than castoreum because of the resilience of AGS to environmental influence. Also, since beavers are able to detect slight differences in AGS between close kin and support has been found for the dear enemy phenomenon (Sun and Müller-Schwarze 1999; Rosell and Bjørkøyli 2002), it is highly unlikely that beavers are not able to detect differences between subspecies. Therefore, it is necessary to be cautious when interpreting results that indicate no behavioral discrimination between stimuli (see Stoddard 1996).

Evolution of the discrimination system

In the light of the above considerations, we speculate that the patterns of divergence, as evidenced for both castoreum and AGS, may characterize both subspecies across their entire range. Thus, divergence may have taken place during their independent evolution in allopatry (Mayr 1963). The limit of their present geographical range (Halley and Rosell 2002) suggests that they have developed in different environments through natural selection and supports adaptation as the cause of divergence (i.e., adaptive radiation). One of the few examples of reproductive isolation in animals that is ascribable to ecological selection is found among finches (species of *Geospiza* Gould, 1837) of the Galápagos Islands in which divergence in the size and shape of the bill represents adaptations to different diets (Grant 1986). Interestingly, the divergence in the size and shape of the cranium of the two subspecies of beaver may also suggest adaptation to different diets (i.e., adaptations to different ecological conditions). Lavrov (1983) found that CFA differed from CFF in 17 craniological features out of 28, which constitutes a 60.7% dissimilarity. These cranium differences may have evolved by natural selection related to habitat use, since they have consistently evolved by parallel evolution in different localities. Thus, "parallel speciation" (i.e., independent evolution of the "same" reproductively isolated forms in different localities) suggest that ecological selection can cause speciation in beavers as is shown for other animals (e.g., Schluter and Nagel 1995; Ogden and Thorpe 2002).

Further research should try to elucidate if other differences (e.g., behavior, physiology, and morphology) between the two subspecies exist, and therefore, studies should also be carried out in the Netherlands, as well as other parts of its range. If maintaining completely unmixed stocks of each of the presently described subspecies is to be a serious management goal, attention should also be given to other subspecies and their responses to chemical signals. Recent population bottlenecks are known to have sharply reduced genetic variability in CFF (Ellegren et al. 1993; Halley and Rosell 2002). Also, the level of genetic variation between the two subspecies is extremly low (less than 2%), suggesting that the current populations are too young to have undergone sizeable genetic differentiation and that the colonization of the present range is extremely recent, most probably occurring after the last glaciation (Ducroz et al. 2003). Therefore, whether CFA should be accepted as a third species in the genus *Castor* remains to be assessed. However, we now know that CFA is morphologically (Lavrov 1983) and chemically (this study) distinct from allopatrically distributed populations of CFF.

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