SPECIES AND SUB-SPECIES RECOGNITION IN THE NORTH AMERICAN BEAVER

Anne Marie Peterson, Lixing Sun, and Frank Rosell*

1. INTRODUCTION

Many mammals have complex chemical signals for communicating such information as species, family membership, individuality and physiological state (Müller-Schwarze, 1974). Studies on how closely related species or subspecies recognize each other using chemical signals are of particular significance because, when closely related species are reproducing in the same area, species-specific pheromones can be essential for the formation and maintenance of a precopulatory isolating mechanism among them (Moore, 1965; Doty, 1972; Kotenkova and Naidenko, 1999). Study of recognition between subspecies can provide us vital information about how this mechanism is formed during the speciation process.

Studies of species or subspecies recognition using chemical signals or cues have shown a complicated picture. The subterranean mole rat (*Spalax ehrenbergi*) can discriminate between conspecific and heterospecific individuals (Todrank and Heth, 1996). Tufted capuchins (*Cebus apella*) can recognize three species of New World monkeys but did not show discrimination between two species of Old World macaques (Ueno, 1994). The mule deer (*O. h.. hemionus*) discriminate between its own subspecies and the black-tailed deer (*O. h. columbianus*) through the tarsal gland secretion (Müller-Schwarze, 1974). Male bank voles (*Clethrionomys glareolus*) prefer the scent of females of their own species (Rauschert, 1963), but at the subspecies level, the results vary (Godfrey, 1958; Rauschert, 1963). *Peromyscus maniculatus* males prefer females of their own species as opposed to the congeneric *P. polionotus*. Female *P. m.*, however, equally prefer both species. *P. polionotus.*, on the other hand, did not show any discrimination between the two species (Moore, 1965). Discrimination can depend on the innate capability, contingent physiological conditions of the subject (e.g., Doty, 1972), or on the

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* Anne Marie Peterson and Lixing Sun, Department of Biological Science, Central Washington University, Ellensburg, WA 98926-7537. Frank Rosell, Department of Environmental Sciences and Health Studies, Telemark University College, N-3800 Bøi Telmark, Norway.

experimental procedure designed to detect the discrimination (e.g., Blaustein et al., 1987). To confirm whether species and subspecies recognition happens, the methodological issue should be resolved first. Unfortunately, few studies are available to show that the choice of behavioral patterns is crucial for demonstrating whether there is a discrimination or preference.

There are two allopatric species of beavers in the world, *C. canadensis* and *C. fiber.* From 1935 to 1937, *C. canadensis* was introduced into several European countries (Halley and Rosell, 2002). In Finland, dispersal of *C. canadensis* to Scandinavia is a serious threat to the endemic *C. fiber* populations (Rosell and Sun, 1999). The ability to discriminate subspecies and species affects dispersal pattern, mate choice, and other social interactions of beavers. Therefore a study of this type can help assess the potential ecological and genetic consequences of re-introductions.

Among the 24 subspecies of *C. canadensis*, reintroductions in the past have resulted in different subspecies intermixed or distributed near each other (Hall, 1981). Coexistence of several subspecies artificially brought together may have profound ecological, genetic and evolutionary consequences for the beaver. One fundamental question is whether different subspecies interbreed. This question can be at least partially answered by investigating whether beavers discriminate between subspecies.

In this study, we mimicked beavers' natural scent mound building behavior to examine whether beavers can discriminate between species or subspecies through an olfactory playback experiment. If this discrimination does occur, we predict that beavers should respond more strongly toward individuals of sympatric conspecifics than those of allopatric conspecifics or heterospecifics.

2. METHODS

Beavers live in family units that are usually composed of a mated pair, yearlings, and kits. They occupy and defend territories (Schulte, 1993). Beavers rely heavily on chemical signals for social interaction and recognition. They use anal gland secretion (AGS) and castoreum to communicate many types of information, including family membership, kinship, sex, individuality, and territoriality (Svendsen, 1980; Sun and Müller-Schwarze, 1997; Schulte, 1998). To do these, they build scent mounds on the bank, within 2 meters from the water, in areas of high activity around their territory, and then apply castoreum and/or AGS to the top (Svendsen, 1978, 1980; Rosell and Nolet, 1997; Rosell and Sundsdal, 2001).

The two subspecies of *C. canadensis* used in this study are far apart, and there is no record indicating that either has been introduced to the other. To collect secretion samples from *C. canadensis*, we trapped both subspecies of the beaver using Hancock live traps baited with aspen (*Populus tremuloides*) in Allegany State Park, New York, and Ellensburg, Washington, between 1995 and 1998. Beavers were sexed based on AGS color (Schulte et al., 1995) by the presence or absence of the os penis (Osborn, 1955). They were aged based on their size and weight (Schulte, 1993). Samples from *C. f.* were collected from beavers killed during the hunting season of 1997 in Bø municipality, Norway. All samples were immediately stored at -20°C until use. Past research has shown that the chemicals found in AGS and castoreum remain intact using the above procedure for collection and storage (Sun and Müller-Schwarze, 1997).

To make a species or subspecies scent, rather than individual scent, we first mixed AGS or castoreum from several adults of the same species or subspecies. Specifically, we blended AGS from 14 males and eight females to make the solutions for this study. Sixteen males and seven females provided castoreum. Each individual contributed approximately an equal amount (in volume) to each mixture of the combined secretion (either AGC or castoreum). Next, we took 0.3 ml of either blended AGS or blended castoreum and dissolved it into 6 ml of methylene chloride (= 1:20 volume ratio). Then, 0.25 ml of this solution was applied to the cork for each treatment on a given evening in the field. Sun and Müller-Schwarze (1997) found that these concentrations are far above the response threshold and can elicit observable territorial response in the beaver.

We mimicked beavers' natural scent mounding behavior for the field playback bioassay with a randomized block ANOVA design with three levels of treatment, blocking on secretion type, AGS or castoreum. The three treatment levels were secretions from 1) *C. c. leucodontus*, 2) *C. c. acadicus*, and 3) *C. fiber*. The dependent (measured) variable was response frequency over a 6-night trial session. The subjects of the experiment were eight *C. c. leucodontus* families at Ellensburg, Washington. Each beaver family was considered to represent one subject. This is because overnight response does now allow us to discriminate responses from different family members. For the same subspecies, the donors and recipients were at least 20 km away so as to avoid possible previous contacts between them.

During the playback, we used the procedure of Sun and Müller-Schwarze (1997) and built three experimental scent mounds (ESMs) for each secretion type each night before beavers emerge from the lodge. With latex gloves, we built ESMs (30 cm apart, 20 cm high, 20 cm wide and 30 cm from the shoreline) to mimic beavers' natural scent mounds. A cork (top diameter: 8 cm) was inserted into each ESM. We then applied 0.25 ml of one of the three treatments in random order to the cork of each ESM. A total of six scent mounds were constructed at each site each night, a group of three scented with AGS and another group of three scented with castoreum. The two sets of ESMs were separated by at least 10 m. Because beavers in our study area rarely emerge before dark, it was difficult to observe them directly. Instead, we recorded the beavers' overnight responses to the ESMs. During a trial (6 consecutive nights), we recorded the state of the ESMs on the next day after response, eliminated all residue from the previous ESMs, rebuilt new sets of ESMs and applied fresh samples to the cork every day. We used the same response patterns (e.g. sniffing, pawing, etc.) as described by Sun and Müller-Schwarze (1997).

Scent mound construction in beavers shows a seasonal pattern. Because it is most intense from April to June, and gradually tapers off (Svendsen 1980), our study started in June to avoid this seasonal effect in beaver mound construction and response. A total of 108 nights of data were collected from June to November in 1998. Five of the families were tested with two to three (6-night) trials and three of the families were used in one trial. Because of this inconsistency, we included only data from the first trial for each family. Because there were many nights that beavers did not respond, we only include data with a minimal response frequency of 40% as measured by the category "ESM Responded" for data analysis to avoid these blanks. Families that were used in more than one trial were given at least three weeks off between trials to avoid possible habituation from repeated use (Sun and Müller-Schwarze, 1997).

Frequency of response was calculated by adding up the number of times a particular response category occurred in each night of the 6-night trial and dividing by six. Because the data were proportional values, they were arcsine transformed to meet the normality and equal variance prerequisites in our ANOVA analysis (Zar, 1996) using the Minitab software (McKenzie and Goldman, 1999). The pre-decided level of significance was 0.05 for all statistical tests. For representation in the figures, all response frequency data were transformed from arcsine back to percentages.

3. RESULTS

Beavers did not respond significantly differently to different types of secretion (AGS and castoreum) in different months for four of the five sites. For the exception site, there was a significant interaction between secretion type and month ($F_{1,12}=15.98$, $P<0.001$). A simple effects analysis indicated that the beavers at this site responded significantly more to AGS than castoreum in August (73 ± 1% compared to 0 ± 1%, $F_{1,4}=248.80$, $P=0.0001$), but responded significantly more to castoreum in June and July (June: 61.25 ± 1.20 %, July: 62.0 ± 1.20 %, $F_{2,6}=45.34$, $P<0.001$). Also, there was no significant difference in response to different taxa and over time for all response categories for the sites used in more than one trial. Because there was little evidence to indicate that seasonality played a significant role in beavers' response, we combined all results in the following analyses.

The overall response follows the expected trend of greatest frequency for the weaker patterns and lower frequency for the stronger territorial responses. Beavers showed no significant difference in response frequency to secretions from their own subspecies, a different subspecies, or *C. fiber* in any response category, based on either AGS (Figure 1) or castoreum (Figure 2). Response to *C. fiber* was consistently higher than to either of the subspecies, but the difference was not significant. However, in six of the ten response patterns, beavers responded significantly stronger to castoreum than to AGS ($F_{1,24}=5.47$, $P=0.028$ for ESM Removed; $F_{1,24}=7.80$, $P=0.010$ for ESM Flattened; $F_{1,24}=11.36$, $P=0.003$ for ESM Obliterated; $F_{1,24}=4.80$, $P=0.038$ for Cork Touched; $F_{1,24}=5.77$, $P=0.024$ for Cork Removed). For Cork Dug Out, the secretion effect was significant ($F_{1,24}=4.59$, $P=0.043$), but there was an interaction between treatment and secretion ($F_{1,24}=3.78$, $P=0.037$).

4. DISCUSSION

Our results rejected our prediction that beavers would respond more strongly to conspecifics versus heterospecifics and to the same subspecies versus different subspecies. Thus, we do not have evidence that beavers are able to recognize species and subspecies by AGS or castoreum. The two beaver species had been separate from the Oligocene until the introduction of the North American species in Europe in 1935 (Rosell and Sun, 1999). It appears that they have been allopatric for so long that there has been no selection force for a differential response toward their own species. Even between-species mating was observed in captivity, though no hybrid offspring were born (Lavrov and Orlov, 1973). In the wild, however, there has been no report that the two species are interbreeding or any hybrid has been produced in Finland where the two species came

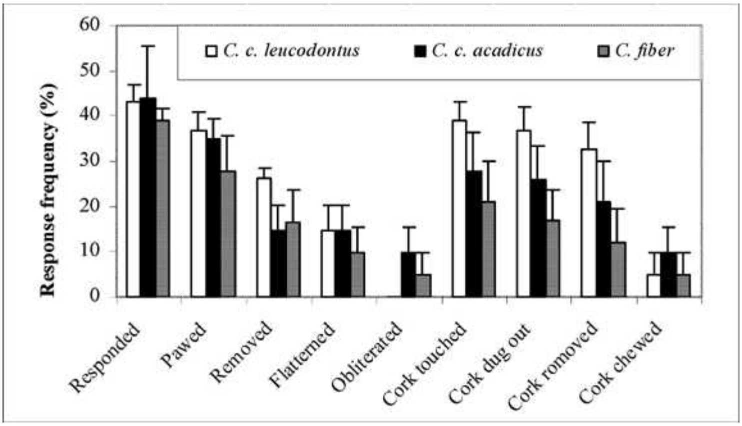

Figure 1. Overnight response of *C. c. leucodontus* to anal gland secretions (ACG) of *C. c. leucodontus*, *C. c. acadicus*, and *C. fiber*. Bars are standard errors.

into contact (Nolet and Rosell, 1998). Failure to show discrimination between the two subspecies found in our study may be another piece of evidence that there is little selection force favoring discrimination of individuals of allopatric populations. Beaver do not show behavioral isolation whether speciated completely (*C. canadensis* versus *C.*

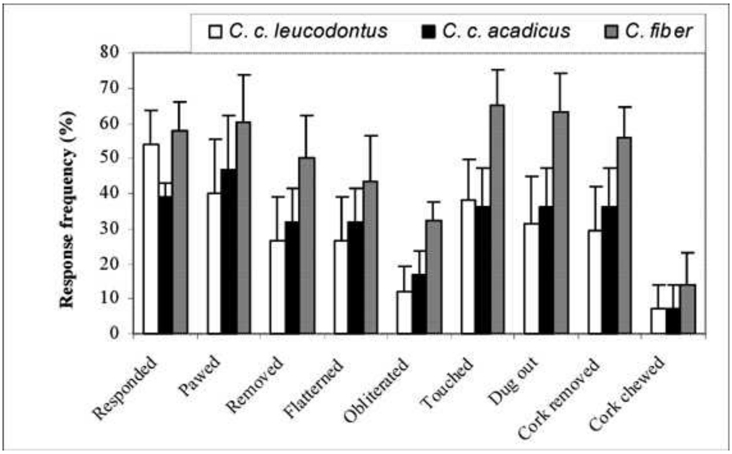

Figure 2. Overnight response of *C. c. lecodontus* to castoreum of *C. c. leucodontus*, *C. c. acadicus*, and *C. fiber*. Bars are standard errors.

fiber) or incompletely (*C. c. leucodontus* versus *C. c. acadicus*). These results further support the allopatric hypothesis (Mayr, 1970) for the origin of behavioral isolating mechanisms, which states that premating isolating mechanisms arise as by-products of genetic divergence in geographically isolated populations.

In our study, the seasonality of beavers' scent mounding behavior did not significantly affect the response of the subject. This is most likely due to the fact that we started the experiment in June and successfully avoided the peak of the scent mounding activity. Hence, between-trial habituation did not occur, although within-trial habituation may be likely, especially for castoreum (Sun and Müller-Schwarze, 1998a). Thus, it is legitimate to use data from the first night of each trial in the analysis for the main effects of taxon and secretion type.

There are two possibilities that could result in the failure of showing differential response to the two species and two subspecies. One is that *C. c. leucodontus* cannot recognize different species and subspecies of beavers. The other is that they recognize species and/or subspecies but they do not show explicit discrimination in the behavioral categories that were used in our study. Castoreum is derived from food (Müller-Schwarze 1992). When diet changes, it will necessarily result in a difference in the chemical constitution of the castoreum. This would provide information as fine as those from neighbors versus those from non-neighbors for beavers to discriminate (Schulte, 1998; Rosell and Bjørkøyli, 2002). Therefore, there is no reason to believe that beavers cannot recognize the difference between sympatric conspecific individuals and allopatric conspecific or heterospecific individuals where the differences in castoreum compounds are much larger. For AGS, the similarity in the chemical composition is positively related to the genetic relatedness (Sun and Müller-Schwarze, 1998b). Beavers are able to detect slight differences for kin recognition among individuals of the same population, in addition to sex (Sun and Müller-Schwarze, 1999). When the chemical composition of AGS is more different between subspecies, let alone between species (Rosell, 2002), it is highly unlikely that beavers are unable to detect the difference between species, or subspecies while they are able to recognize information as detailed as an individual's scent. Thus, a more convincing argument is that *C. c. leucodontus* are able to recognize different species and subspecies, but this study failed to detect the discrimination. The overnight response used in our study may not be sensitive enough to show beavers' differential responses. Lack of observable discrimination does not mean lack of recognition. Thus, our study demonstrates that not all behavioral patterns can be used in choice tests to show discrimination.

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