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# LACTATING NORTH AMERICAN BEAVERS (*CASTOR CANADENSIS*) SHARING DENS IN THE SOUTHWESTERN UNITED STATES

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## LACTATING NORTH AMERICAN BEAVERS (*CASTOR CANADENSIS*) SHARING DENS IN THE SOUTHWESTERN UNITED STATES

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**ABSTRACT**—During a radio-tracking study of North American beavers (*Castor canadensis*) in Arizona, we discovered three adult, lactating beavers using the same bank den at the same time. Two adult females 5.2 km downstream also were using the same den at the same time. For the first case, we documented use of communal dens by lactating adults on seven occasions during 68 days. For the second case, we documented communal use of a den on three occasions during 45 days. To test the hypothesis that these communal females were closely related, we used eight autosomal DNA microsatellites. Two females in the first den were first-order relatives. However, the other communal females were unrelated to the females with which they shared the den. We conclude that communal denning, where multiple females raise one or more litters in the same den, may have occurred in our study area and communal-denning partners are, in some cases, close kin.

**RESUMEN**—Durante un estudio de radiotelemedición del castor (*Castor canadensis*) en Arizona, descubrimos tres hembras adultas en lactancia usando simultáneamente la misma madriguera. Dos hembras adultas localizadas 5.2 kilómetros río abajo también utilizaban la misma madriguera al mismo tiempo. Para el primer caso, documentamos el uso comunal de madrigueras por adultas en lactancia siete veces durante 68 días. En el segundo caso, documentamos el uso por las hembras adultas de la madriguera comunal en tres ocasiones durante 45 días. Para probar la hipótesis que estas hembras comunales estuvieron estrechamente relacionadas, utilizamos ocho microsatélites de ADN autosómicos. Dos hembras en la primera madriguera tenían parentesco de primer orden. Sin embargo, las otras hembras comunales no tenían relación de parentesco entre las que compartían la madriguera. Llegamos a la conclusión que madrigueras comunales, donde varias hembras crían una o más camadas en la misma madriguera, puede haber ocurrido en nuestra área de estudio, y los co-habitantes de madrigueras son, en algunos casos, parientes cercanos.

The typical colony or family unit of the North American beaver (*Castor canadensis*) consists of an adult breeding pair, young of the current year, yearlings born the previous year, and

occasionally, one or more nonbreeding subadults (Bradt, 1938; Aleksyuk, 1968; Svendsen, 1980; Bishir et al., 1983; Hodgdon and Lancia, 1983; Busher, 1987). Reports exist of colonies

containing >2 adult females (Hay, 1958; Payne, 1982) and some studies have documented multiple lactating or pregnant females within a colony or territorial area (Bergerud and Miller, 1977; Busher et al., 1983; Wheatley, 1993; Crawford et al., 2008). Wheatley (1993) reported two pregnant North American beavers within a colony in southern Manitoba, but it was not clear whether both animals were residents of the same lodge. Conditions of the ice and distance from neighboring lodges provided anecdotal evidence that both females inhabited the same lodge. Recently, Crawford et al. (2008) examined genetic relationships within and among colonies to determine if they are a socially monogamous species. Interestingly, colonies did contain unrelated individuals and >50% of litters had been sired by multiple males. However, no published literature has documented multiple lactating or pregnant adults residing in the same lodge or den for an extended period of time. Previous studies have used trapping to provide a snapshot in time regarding composition of colonies, but not actual use of lodge or den. Here we report two cases demonstrating multiple reproductively active females using the same den over an extended period of time.

We investigated the degree of relatedness among females sharing dens using microsatellite DNA markers. Based on similar work by Crawford et al. (2008), we predicted that communal denning occurs in populations and those females sharing dens are closely related. Therefore, we used DNA genotypes to test the hypothesis that pairwise kinship analysis between co-denning females would infer first-order relationships, with the null hypothesis that they were unrelated.

Our research was conducted on the Salt River in the southwestern portion of Phoenix, Maricopa County, Arizona, downstream of the 91<sup>st</sup> Avenue Wastewater Treatment Plant. The Salt River lies below 1,100–1,200 m elevations in the lower Colorado River Valley subdivision of the Sonoran desertscrub biome (Turner and Brown, 1994). Streamside vegetation traditionally was considered to be cottonwoods (*Populus fremontii*), willows (*Salix gooddingii*), and velvet mesquites (*Prosopis velutina*). Currently, much of the woody vegetation has been replaced by saltcedars (*Tamarix chinensis*). Average annual precipitation varied from 193 mm near the wastewater treatment plant to 180 mm near the western

end of our study area (Turner and Brown, 1994), with the wettest month being March (mean = 27.2 mm) and the driest being June (mean = 2.3 mm; Western Regional Climate Center, <http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?az6486>). Temperatures ranged from an average high of 19°C in January to an average high of 42°C in July. The wastewater treatment plant released a perennial flow of ca. 5 m<sup>3</sup>/s into the Salt River and was managed by the Sub-Regional Operating Group under agreement with the cities of Phoenix, Mesa, Tempe, Scottsdale, and Glendale, Arizona. The riverbed upstream and beyond 11.4 km downstream from the wastewater treatment plant is dry, except during flood events. Irrigation canals flow in and out of this segment of the Salt River.

We captured, immobilized, radio-tagged, and released North American beavers downstream of the wastewater treatment plant during 2004–2006 (Arjo et al., 2008). Females were determined to be lactating and pregnant by presence of enlarged teats and abdominal palpation, respectively. We attached tail-mounted radio-transmitters to 43 adults weighing >10 kg (12 males, 31 females; Rothmeyer et al., 2002; Baker, 2005; DeStefano et al., 2006). At this time, samples of tissue were collected from the tail for DNA analysis. We also collected hair as a secondary source of DNA. We collected tissue and hair from 54 North American beavers that were trapped (this includes the 43 that were radio-tagged) on the Salt River for DNA analysis. Radio-tracking occurred on average once per week during 0700–1130 h. Bank dens (hereafter referred to as dens) were located by homing on radio-tagged individuals. All dens located by this technique were checked every tracking occasion to determine use.

We extracted DNA from tissue with DNeasy Tissue Kits (QIAGEN, Inc., Valencia, California) and from hair using a QIAamp DNA Micro Kit (QIAGEN, Inc., Valencia, California) following protocol of the manufacturer for both types of samples. We amplified eight microsatellite markers according to protocols established by Pelz-Serrano et al. (2009). One of the individuals from the study site was used for development of these markers and all individuals trapped for this study were used for characterization of each marker (Pelz-Serrano et al., 2009). Amplifications were achieved using a Mastercycler EP thermocycler (Eppendorf, Hamburg, Germany)

and genotypes were visualized using an Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems, Inc., Foster City, California) following protocols of the manufacturer. Alleles were scored by employing ABI GeneMapper Software, version 4.0, and scored data was exported from GeneMapper and converted for downstream analyses using GMConvert (Faircloth, 2006). Relevant population-genetic parameters were estimated in ARLEQUIN (Excoffier et al., 2005). We also used the program Kinship 1.2 (Goodnight and Queller, 1999) to estimate relationships of pedigree between pairs of individuals in a population. This program also was used to estimate relatedness ( $R$ ) between individuals. We used this program to test hypotheses of first-order relationships (parent-offspring or full siblings = 0.5 shared pedigree on both maternal and paternal side between pairs of individuals) versus the null hypothesis of unrelated between individuals using a maximum-likelihood framework. Significance of first-order relationships was determined as at least  $P < 0.05$  after 9,000 simulations.

Density of colonies varied spatially and temporally, but averaged 0.7 colonies/km of stream. We discovered nine dens in our study area that received at least some use by radio-tagged individuals. Two of these dens were occupied by multiple adult females for extended periods of time, suggesting that communal denning may be occurring in our study area. In the first den, we observed three lactating adults from one area using the same den at the same time. Beavers five, six, and seven were captured, determined to be lactating, and radio-marked on 1, 8, and 8 July 2004, respectively, ca. 5.6 km downstream of the wastewater treatment plant. All three were using a communal den ( $33^{\circ}22'50''\text{N}$ ,  $112^{\circ}18'37''\text{W}$ ) during a 68-day period. On six tracking occasions, two of the three were located in the same den at the same time. On one tracking occasion, all three were in the same den at the same time. Dates between tracking events, when at least two of the three were located together, were variable, but averaged 8.3 days ( $SE = 4.4$ , range = 1–29 days). Beaver five lost its radio-tag shortly after tagging, but was in the communal den with either beavers six or seven on four of five tracking occasions. Beavers six and seven were in the communal den with each other or beaver five on 6 of 21 tracking occasions during the 68-day period. Communal denning of beavers six and

seven ceased at the end of the 68-day period. Two adult males were trapped in this colony during the 68-day period. The hypothesis of first-order relationships was significant ( $P < 0.001$ ) between beavers five and six, but not between five and seven or six and seven.

For the second den, we observed two adult females using a communal den during a 45-day period. Beavers 31 and 32 were captured and radio-marked (8 February and 8 June 2006, respectively) ca. 10.8 km downstream of the wastewater treatment plant. Beaver 31 was pregnant and beaver 32 was lactating when captured. The time lag between capture of beaver 31 (8 February 2006) and the observed communal denning (12 June, 26 June, and 27 July 2006) make it unclear as to whether or not both individuals were lactating, pregnant, or raising young in the same den at the same time. On three of three tracking occasions during the 45-day period, radio-tracking revealed these two individuals using the same den ( $33^{\circ}23'03''\text{N}$ ,  $112^{\circ}21'45''\text{W}$ ) at the same time. Dates between tracking events were variable, but averaged 22.5 days ( $SE = 8.5$ , range = 14–31 days). Shortly after the third radio-tracking occasion in which both were using the same den, beaver 32 was never detected again. Males were never trapped in this colony. The hypothesis of first-order relationships was rejected for beavers 31 and 32. However, there was a significant first-order relationship detected between beavers five and 32 ( $P < 0.001$ ) and six and 32 ( $P < 0.05$ ).

Eight loci (Pelz-Serrano et al., 2009) were amplified in each individual with 2–4 alleles/locus and observed heterozygosity of 0.27–0.65 (mean = 0.50). Each locus was in Hardy-Weinberg Equilibrium. Average relatedness calculated among samples was  $R = 0.17$ .

Communal care, or more specifically, communal nesting is common in many populations of rodents and birds (Getz et al., 1992; Manning et al., 1995; McRae, 1996; for a review see Hayes, 2000). Hayes (2000) defined communal nesting as multiple females raising young and sharing parental responsibilities in the same nest. A by-product of communal nesting, especially in rodents, is communal nursing (sharing of milk with offspring that are not their own; Hoogland et al., 1989; Manning et al., 1995; for a review see Roulin, 2002). Our trapping and radio-telemetry data indicated that some North American beavers were lactating or pregnant when sharing

dens. These results suggest that multiple reproductive females gave birth in communal dens. Without direct behavioral observations of these multiple litters in the same den, it is unclear as to whether adults shared parental responsibilities or communal nursing occurred.

For North American beavers, the conventional family unit is comprised of a single adult breeding pair, young of the current year, and yearlings born the previous year. The social organization in our study area has appeared to add multiple reproductive females to the extended family unit. These results concur with what Crawford et al. (2008) have recently reported in that colonies may contain >1 pregnant female. As predicted, we determined that reproductively active females that are first-order relatives will share a den. However, we also determined that non-relative, adult, reproductively active females will share a den. Genetic analysis of communal nesting moorhens (*Gallinula chloropus*) revealed that, in many cases, parents were related (McRae et al., 1996). Den sharing by San Joaquin kit foxes (*Vulpes macrotis mutica*) occurred primarily by members of the same social group (Ralls et al., 2001). One possible explanation of relative and non-relative, adult, reproductively active females sharing a den in this severely human-impacted environment is the habitat-saturation hypothesis. This suggests that when food and space become limited, some species will forgo dispersal and stay at the natal site, leading to individual cooperation and communal nesting (Emlen, 1982; Getz et al., 1992; Wolff, 1994; for a review see Solomon and Getz, 1997). Busher et al. (1983) stated that colonies containing more than one adult male or female may be the result of high densities. Our estimate of population density of 0.7 colonies/km of stream is similar to estimates in New York (0.24–1.14 colonies/km of stream; Müller-Schwarze and Schulte, 1999), New Brunswick (0.34–1.2 colonies/km of stream; Nordstrom, 1972), and Colorado (0.35–0.6 colonies/km of stream; Breck et al., 2001). High-density populations in central and southern Illinois repeatedly exhibited delayed natal dispersal (Crawford et al., 2008). We believe that observed communal denning of adult, reproductively active females in our study area is due, to some degree, to the limited amount of available habitat (i.e., suitable sites for foraging and denning). The riverbed upstream and >11.4 km

downstream from the wastewater treatment plant was essentially dry. Group living among rodents has been linked to sharing burrows, if construction is costly and sites are limited (Ebensperger, 2001). The paucity of available dens and proximate high-quality food in the area could be delaying dispersal of subadults and adults, and in some instances eliminating dispersal. These ecological constraints might explain the communal denning of adult females in southwestern Phoenix, Arizona.

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