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Kin recognition in rattlesnakes

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Snakes are often regarded as the least social of all vertebrate groups, but this assumption stems from the fact that they are secretive and difficult to observe in nature, rather than direct evidence. Recent studies have revealed a surprising degree of social complexity in snakes. Here, I examine the ability of captive-raised timber rattlesnakes (*Crotalus horridus*) to recognize siblings by measuring the mean separation distance and frequency of contact between pairs of individuals housed together. The results show that female siblings associate more closely with each other than non-sibling pairs. Previous studies have shown that timber rattlesnakes occupying the same hibernacula have higher relatedness than snakes using neighbouring hibernacula, and frequently form social aggregations. Rattlesnakes exhibit other characteristics consistent with advanced sociality, including group defence, conspecific alarm signals and maternal defence of young. These findings reinforce the notion that, rather than being solitary and asocial, some snake species may form family groups.

Keywords: kin recognition; snakes; *Crotalus horridus*; aggregation; behaviour

1. INTRODUCTION

Although snakes have long been viewed as asocial (Brattstrom 1974; Wilson 1975), many species frequently form conspecific aggregations. These aggregations are sometimes driven by the mutual attraction of individuals to a beneficial habitat, but also occur in the absence of any discernible benefits other than those derived from group living (Gillingham 1987; Gregory *et al.* 1987). Among snakes, crotalines (pitvipers) may be the most social. Gravid female crotalines often aggregate during and after gestation (Graves & Duvall 1995). Individuals of both sexes frequently aggregate when shedding their skins (Gregory *et al.* 1987; Ashton 1999) and sometimes follow the scent trail of conspecifics outside the context of mating (Brown & Maclean 1983; Scudder *et al.* 1988). Female crotalines exhibit parental care of their young, staying with newborns until the natal skin is shed (Greene *et al.* 2002), and in at least two species, females become more defensive after giving birth (Graves 1989; Greene *et al.* 2002). There are also many anecdotes of rattlesnakes exhibiting some degree of gregariousness outside the context of denning and mating (Klauber 1972). Adult timber rattlesnakes, *Crotalus horridus*, are often found under the same cover objects, and exhibit spatially and temporally correlated movements to different habitat structures (R. W. Clark, personal observation).

Presumably, the benefits snakes derive from conspecific aggregation outweigh the automatic costs associated with group living (Alexander 1974). Potential benefits include increased vigilance, enhanced antipredator defence, confusion of predators and the 'dilution effect' (Calvert *et al.* 1979). Rattlesnakes in groups escalate their defensive behaviour more readily than solitary individuals (Duvall *et al.* 1985) and secrete an alarm pheromone when disturbed, signalling the presence of predators to conspecifics (Graves & Duvall 1988). Because there is some cost to group members that engage potential predators, inclusive fitness theory predicts that the benefits of group defence will be greater when groups are composed of kin, rather than unrelated individuals (Hamilton 1964). Although kin recognition occurs across a wide range of vertebrates (Fletcher & Michener 1987), it has not been reported in any snake species.

2. MATERIAL AND METHODS

(a) *Test subjects*

Subjects consisted of 24 *C. horridus* from three litters born in the laboratory to wild-caught females (litter 1: four females, three males; litter 2: three females, six males; litter 3: three females, five males). Adult females were taken from three separate hibernacula in north-central Pennsylvania. To approximate a natural birthing rookery, mothers and neonates of all three litters were housed together in a communal enclosure. Upon shedding their natal skin, neonates were placed individually in 20 gallon aquaria, in which they were maintained for 2.5 years, until this study. Snakes were reared on a diet of pre-killed laboratory mice and maintained in a Cornell University animal holding facility at 22–26 °C under a 12 L : 12 D light cycle.

(b) *Kin aggregation experiments*

Experiments were conducted between 1 February 2003 and 21 March 2003. Because female timber rattlesnakes may be more gregarious than males, I tested kin associations between males and females separately. Trials were conducted using four treatments: pairs of female kin, pairs of female non-kin, pairs of male kin and pairs of male non-kin. The design of these trials was limited in part by the small number of available snakes. There were 10 females from three different litters available, leading to a possibility of 12 different unique pairings of female kin. Because these pairings involve using the same individuals in multiple trials, all treatments were balanced after this fashion. The same individuals were used in the same number of trials when pairing female non-kin, and 10 randomly selected males from the three families were used in pairings of male kin and non-kin. Thus, each treatment consisted of 12 unique pairings of individuals, with some individuals being used in more than one pair in a balanced design. Prior to the study, all individuals were coded with small paint marks on their dorsum. The observer was blind as to the familial identity of the individuals. At the beginning of a trial, two individuals were introduced simultaneously into an open-topped arena (75 cm × 75 cm × 120 cm) lined with clean construction paper. The position of each individual was recorded during daylight hours four times daily, at 3 h intervals for 3 days. Each individual was recorded as being in either an active (uncoiled) or resting (coiled) position, and the minimum distance between the individuals was estimated to the nearest 5 cm. The sides of the arenas were marked at 5 cm intervals to aid in this estimation. After each trial, the enclosures were cleaned with water and detergent. A minimum of 2 days elapsed between trials.

(c) *Statistical analysis*

All values are mean ± s.e. To control for the same individuals being used in more than one pair, data were analysed using a mixed-model ANOVA (Proc Mixed procedure in SAS) specifying individual snake identity as a random factor. For measures of frequency of contact, proportional data were used as the dependent variable. *Post hoc* comparisons between means were made with the Tukey procedure. Where appropriate, continuous data were square-root transformed, and proportional data were arcsine transformed.

3. RESULTS

The mean distance between two individuals in a pair was significantly less for female siblings (6 ± 1 cm) than

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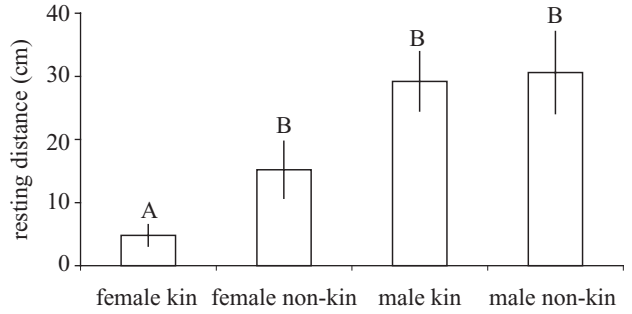

Figure 1. Mean ± s.e. distance between resting individuals in pairs of timber rattlesnakes housed together. A < B, $p < 0.05$, $T > 2.92$.

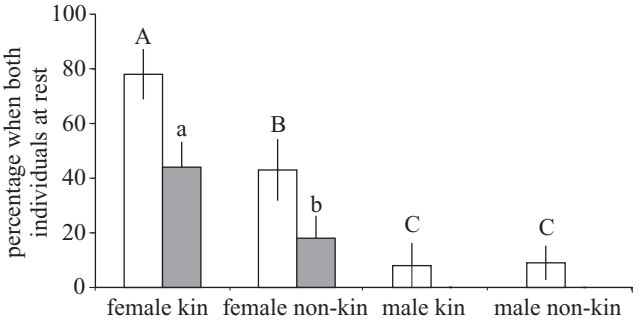

Figure 2. Proportion (mean ± s.e.) of observations where both individuals in a pair were at rest and touching (open bars), or entwined around each other (shaded bars). A > B, $p < 0.03$, $T > 2.94$; B > C, $p < 0.02$, $T > 3.09$; a > b, $p < 0.04$, $T > 2.22$.

for female non-siblings (14 ± 3 cm), male siblings (22 ± 4 cm), or male non-siblings (20 ± 4 cm). To provide a better approximation of gregariousness, I also examined the mean distance between individuals when both were in a resting (coiled) position, and the proportion of observation in which the two individuals were touching each other, or in the entwined position. Female siblings at rest were significantly closer to each other (5 ± 2 cm) than were resting female non-siblings (15 ± 5 cm), resting male siblings (29 ± 5 cm) and resting male non-siblings (31 ± 6 cm) (figure 1). When both individuals in a pair were resting, female siblings were in contact significantly more often than female non-siblings (78 ± 9% versus 43 ± 11%), and female non-siblings were in contact significantly more often than male siblings (8 ± 8%) and male non-siblings (9 ± 6%) (figure 2). When both individuals in a pair were resting, female siblings were also observed entwined around each other significantly more often than female non-siblings (44 ± 9% versus 18 ± 8%). Males were never seen in the entwined position.

4. DISCUSSION

Females from the same litter associate with each other more closely than females from different litters. This increased gregariousness among related individuals is evidence that timber rattlesnakes are able to discriminate kin from non-kin, even after being raised for more than 2 years in isolation. Despite the fact that many species have been shown to aggregate with consepecifics (Gillingham 1987; Gregory *et al.* 1987), this is the first evidence, to my knowledge, of kin discrimination in a snake species. The close association exhibited by females indicates that there may be some benefit to being close to conspecifics that is enhanced when they are related. Although rattlesnakes have well-developed defences in the form of their venomous bite and acoustic aposematism, they are still killed by a variety of predators (Klauber 1972; Greene 1988). Predation on venomous snakes probably requires careful, focused attacks. The presence of more than one snake in the same area exhibiting antipredator displays would provide a significant interruption to such attacks. However, there is always a potential cost to a snake that abandons crypsis and reveals itself to a predator. This cost will be less if group members that benefit from the behaviour are relatives (Hamilton 1964).

There was no difference in the amount of association between male siblings and male non-siblings. Given that males can be found in aggregations with conspecifics at certain times of the year (R. W. Clark, personal observation), the lack of gregariousness in this study may be because captive maintenance results in a reproductively active state that induces an intolerance for the presence of other males (Aldridge & Brown 1995). When housed with females in pilot trials, males in this study actively directed courtship behaviour towards females, indicating reproductive readiness.

Among other squamate reptiles, several lizard species have been shown to exhibit kin recognition, including green iguanas (*Iguana iguana*), common lizards (*Lacerta vivipara*) and three Australian Scincid species (*Egernia stokesii*, *E. striolata* and *Tiliqua rugosa*) (reviewed in Bull *et al.* 2001). It is unclear how widespread kin recognition is among snakes. Even though garter snakes frequently aggregate (reviewed in Graves & Duvall 1995), research on Butler's garter snake (*Thamnophis butleri*) showed no effect of kinship on aggregation (Lyman-Henley & Burghardt 1994). However, timber rattlesnakes from the same hibernacula are more closely related to each other than to individuals at neighbouring hibernacula, even though their active summer ranges may overlap (Bushar *et al.* 1998). Such genetic structuring is not present in the sympatric black rat snake (*Elaphe o. obsolete*), which also uses communal hibernacula (Lougheed *et al.* 1999). Timber rattlesnakes sharing a hibernacula also share basking sites where aggregations occur (Bushar *et al.* 1998), making it likely that these basking aggregations occur between relatives. Additionally, female timber rattlesnakes provide parental care for their newborn young (Greene *et al.* 2002) and leave chemical trails that are used by neonates to locate winter hibernacula (Brown & Maclean 1983; Reinert & Zappalorti 1988).

In conclusion, this study supports the hypothesis that certain snake species may live in family groups (Greene *et al.* 2002), and key characteristics of other taxa regarded as social (kin recognition, group defence and parental care; Alcock 2001) also occur in timber rattlesnakes. Other snake species may also have much richer social behaviour than previously suspected.

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