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# Cryptic sociality in rattlesnakes (*Crotalus horridus*) detected by kinship analysis

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**Research on social behaviour has largely concentrated on birds and mammals in visually active, cooperatively breeding groups (although such systems are relatively rare) and focused much less on species that rarely interact other than for mating and parental care. We used microsatellite markers to characterize relatedness among aggregations of timber rattlesnakes (*Crotalus horridus*), a putatively solitary reptile that relies heavily on chemical cues, and found that juveniles and pregnant females preferentially aggregate with kin under certain conditions. The ability to recognize kin and enhance indirect fitness thus might be far more widespread than implied by studies of animals whose behaviour is primarily visually and/or acoustically mediated, and we predict that molecular markers will reveal many additional examples of 'cryptic' sociality.**

**Keywords:** social behaviour; kin recognition; indirect fitness; *Crotalus horridus*; aggregation

## 1. INTRODUCTION

Vertebrates can be obviously social, living in stable, structured groups that exhibit complex cooperative interactions. These groups are often kin-based, since individuals can derive indirect fitness benefits from cooperating with relatives [1,2], and in extreme cases, group members forego reproduction to care for the young of others [3,4]. Much research has focused on these cooperatively breeding groups (reviewed in Komdeur *et al.* [5]), although they comprise only a small percentage of avian and mammalian systems. Conversely, many 'solitary' species might also exhibit kin-structured cryptic sociality, especially those whose interactions are chemically rather than visually or acoustically mediated, but far less work has addressed these interactions (reviewed in Hatchwell *et al.* [6]).

Squamate reptiles are proving ideal for research on incipient sociality. With few exceptions, notably several lizard species [7–10], squamates generally have been viewed as asocial apart from antagonistic interactions and mating behaviours [11]. However, many species aggregate with conspecifics during key stages in their life cycles (e.g. ecdysis, gestation, parturition, hibernation/aestivation) [12,13], and although such aggregations often appear to be based on a common physical resource, some entail mutually beneficial behaviours with conspecifics, such as thermoregulation [14,15]. Because indirect selection might favour those behaviours if directed towards kin, conspecific attraction may be non-random with respect to relatedness and thereby serve as a precursor to the evolution of more complex, overtly social groups. Here, we use microsatellite markers to examine the relatedness of timber rattlesnakes (*Crotalus horridus*) sampled from naturally occurring aggregations.

## 2. METHODS

*Crotalus horridus* uses communal wintering dens (hibernacula) throughout northern portions of its range, and both sexes exhibit high levels of philopatry to the natal hibernaculum [16]. Individuals emerge in spring, spend several days basking at rock outcrops, and then migrate to surrounding areas to forage and mate [16]. In autumn, they move back to hibernacula to overwinter. Females reproduce on average every third year, and when pregnant do not undergo a summer migration; they instead bask at 'rookeries' before giving birth in the autumn [17]. In *C. horridus* and other viperids, females often aggregate at these birthing rookeries [12]. We obtained tissue samples from pregnant females at birthing rookeries and from all individuals at basking areas during emergence and ingress, allowing us to compare relatedness between aggregated individuals and random individuals from the same population that were not aggregated. We did not include litters of neonates. Population sampling and genotyping are as previously described in Clark *et al.* [18].

We used RELATEDNESS v. 5.0 software to calculate pairwise relatedness between all individuals from the same hibernaculum [19], then randomization procedures [20] to compare observed relatedness to an equal number of randomly generated pairs of individuals matched for sex, age and hibernaculum of origin. Randomized datasets were generated 1000 times, and observed samples were considered to be significant if average relatedness was greater than 95 per cent of random samples. For aggregations of individuals other than pregnant females, we first used a two-way ANOVA to determine if either age or sex significantly affected pairwise relatedness, then used randomization procedures to examine relatedness within any sex or age class identified as a significant factor. All mean values are reported as mean $\pm$ s.e. Details for data and material sharing can be found in the electronic supplementary material.

## 3. RESULTS

During the summer gestation period, we collected tissue samples from 29 pregnant females aggregated at 12 birthing sites (figure 1). During spring emergence and autumn ingress, we collected samples from snakes at basking sites associated with 18 hibernacula. Over all hibernacula, 113 of the 419 sampled snakes were aggregated with other individuals, and they included adult and juvenile males and females. Based on male/female and adult/juvenile ratios in our sample, the pairwise sex and age of snakes in aggregations was not different from random pairings of individuals (sex: $\chi^2 = 0.27$, d.f. $= 2$, $p > 0.5$ ; age: $\chi^2 = 0.1$, d.f. $= 2$, $p > 0.5$).

Overall, individuals from the same hibernaculum did not exhibit significant pairwise relatedness (average $r = -0.04 \pm 0.03$). However, average pairwise relatedness between aggregated pregnant females was greater than random ($r = 0.09 \pm 0.05$, $p = 0.02$). In aggregations with more than two females, at least two always shared relatedness ($r > 0.15$), and the average relatedness of only the most related pair was $0.21 \pm 0.05$ ($p < 0.001$; figure 2). Related female pairs included both same-age females and those that differed by 5–10

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Figure 1. Aggregated pregnant female *Crotalus horridus* at summer birthing rookery (photo Polly Smith-Blackwell).

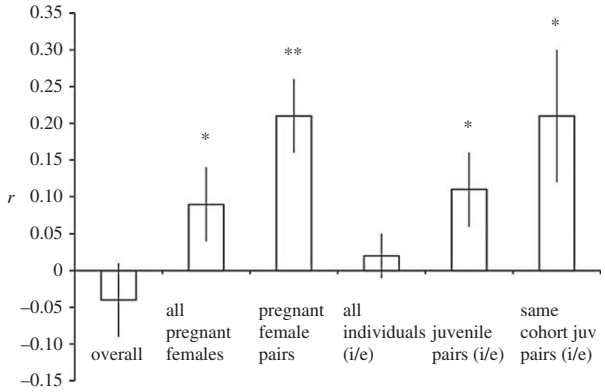


Figure 2. Average pairwise relatedness between aggregated timber rattlesnakes. Overall denotes average pairwise relatedness ($r$) across all snakes, all hibernacula; all pregnant females represent all pairs of females aggregated at birthing rookeries; pregnant female pairs denote most highly related pairs in aggregations with more than two pregnant females; all individuals denote all individuals aggregated during ingress to and emergence from (abbreviated as i/e) hibernacula; juveniles pairs denote juveniles aggregated during i/e; same cohort juv pairs represent same-age juvenile pairs aggregated during i/e. Significant values denoted by $*p < 0.05$, $**p < 0.001$.

years in age, indicating aggregations occur between relatives of different generations.

Average pairwise relatedness of aggregated snakes during emergence and ingress was not different from random ($r = 0.02 \pm 0.03$, $p = 0.26$; figure 2). A two-way ANOVA found that age, but not sex, was a significant factor ($p = 0.006$), with no significant interaction effects. Average relatedness was not significant for dyads with either two adults or one adult and one juvenile ($r = 0.07 \pm 0.04$, $p = 0.81$; $r = 0.01 \pm 0.05$, $p = 0.33$), but greater than random for dyads with both juveniles ($r = 0.11 \pm 0.05$, $p = 0.01$). Of the 29 juvenile dyads, 17 were same-age and 12 were from different cohorts. The average relatedness of the juveniles from the same cohort was greater than random ($r = 0.21 \pm 0.09$, $p = 0.01$), whereas the average relatedness of juveniles from different cohorts was not ($r = 0.01 \pm 0.06$, $p = 0.28$), indicating that high relatedness among juvenile pairs is probably driven by continued association of littermates (figure 2).

## 4. DISCUSSION

Juvenile and pregnant timber rattlesnakes in the field preferentially aggregate with kin, but other aggregations show no kin preference. Past studies have implicated individual functional benefits from aggregation in crotaline species [12], and our results suggest further benefits may accrue when aggregating with kin. For example, females often thermoregulate in open habitats during gestation, exposing themselves to diverse mammalian and avian predators [21]. When confronted with a predator, rattlesnakes may exhibit group defence, possibly coordinated with an alarm pheromone [22], and individuals may be more likely to engage predators if there are relatives nearby that could benefit from such behaviour.

Individuals may also benefit from kin aggregation through physiological processes. For example, newborn sidewinder rattlesnakes maintain optimal temperatures in a thermally extreme environment through a dynamic 'balling' behaviour, performed in the presence of their mother [23]. Although the inter-individual dynamics of group thermoregulation in snakes have not been examined in detail, cooperation may be necessary to ensure access to critical physical locations within the group. If so, these processes may be more efficient when group members are related and thereby can result in indirect as well as direct benefits. Moreover, benefits relating to thermoregulation and hydration may be more important for smaller snakes, because of increased surface area to volume ratios, and to pregnant females, which need higher and less variable temperatures during gestation [24]. Thus, snakes may exhibit stronger tendencies to aggregate with kin at times when such aggregation can be most beneficial.

This study is the latest in a series of investigations that highlight the complex social lives of timber rattlesnakes. Newborn individuals use conspecific chemical cues to locate overwintering sites [25,26], and the survival rate of neonates separated from their mothers after birth was half that of neonates left with their mothers [27]; individuals in captivity preferentially associate with kin as well as use conspecific chemical cues to guide decisions about foraging and habitat use [28,29]. These long-lived reptiles evidently have subtle but complex social lives that have long been underappreciated. This may be true of snakes in general, as recent research has revealed parental care, kin recognition and cryptic sociality in several other species [30–33]. Molecular genetic approaches will probably continue to reveal that many secretive species exhibit more complex social lives than previously expected.

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