Olfactory self recognition in two species of snake

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Abstract:

Mark tests, in which an animal uses a mirror to locate and examine an otherwise unnoticeable mark on its own body, are commonly used to assess self-recognition, which may have implications for self-awareness. Recently, several olfactory-reliant species have appeared to pass odor-based versions of the mark test, though it has never been attempted in reptiles. We conducted an odor-based mark test on two species of snakes, Eastern gartersnakes and Ball pythons, with widely divergent ecologies (i.e., terrestrial foragers that communally brumate vs. semi-arboreal ambush predators, that do not). We find that gartersnakes, but not ball pythons, pass the test, and a range of control tests suggest this is based on self-recognition. Gartersnakes are more social than ball pythons, supporting recent suggestions that social species are more likely to self-recognize. These results open the door to examination of the ecology of self-recognition, and suggest that this ability may evolve in response to species-specific ecological challenges, some of which may align with complexity of social structures.

Keywords: mirror self-recognition, self-awareness, odor mark test, gartersnakes, ball pythons

Introduction

There is a lot of debate about what cognitive processes are implied by success on tests of self-recognition (Hayes, 1994; Schwenkler, 2008; Brandl, 2016; Vonk, 2019). The ability to recognize oneself in a mirror may be linked to self-awareness (Gallup, 1982, 1998) or might not require any complex sense of self (Heyes, 1994; Brandl, 2016). Regardless, the capacity of an organism to recognize itself could be adaptive in a number of ways. It may underlie the ability to distinguish between self and non-self, to recognize one's own shelter or territory, or to differentiate between oneself and conspecifics – which may be crucial for some social interactions. Given these benefits, one might expect some capacity for self-recognition to be widespread across taxa. However, empirical results to date have been mixed, with some unexpected species, such as crows, failing to demonstrate self-recognition (Vanhooland et al., 2020) while species often considered less cognitively sophisticated, such as cleaner fish, do pass the tests (Kohda et al., 2018). It has been suggested that the inability to detect self-recognition in some taxa may be the result of testing using paradigms that lack ecological validity (such as relying on mirrors, which animals rarely encounter in the wild) or require additional cognitive capabilities not necessarily related to self-recognition (Hillemacher et al., 2023; Horowitz, 2017).

Mirror Self-Recognition (MSR) research assesses an animal's ability to discriminate cues that originate from themselves from those that do not (Barth et al., 2004). To demonstrate MSR animals must 1) use the mirror to investigate otherwise inaccessible parts of their bodies, and 2) pass the mark test, in which a mark that can only be seen using the mirror is applied to the subject's body, and the subject – on seeing the mark in the mirror – investigates their own body rather than the reflection (Gallup, 1970, 1977). Results from apes that pass both tests have been taken as evidence for self-awareness, often defined as the ability to become the object of one's own attention (Gallup, 1977). Mark tests have been conducted on a wide range of vertebrate species, including Giant pandas, Asian elephants, cleaner fish, and crows (Ma, 2015; Plotnik et al., 2006; Vanhooland et al., 2020; Kohda et al., 2022), though it has been argued that only certain species of great ape consistently pass both tests (Suarez & Gallup 1981; Gallup & Anderson, 2019). It has also been suggested that more social animals are more likely to pass tests of MSR (Krachun et al., 2019). Part of the challenge of conducting mark tests is ensuring that the mark itself does not unintentionally influence behavior, and that the animal is able to use the mirror appropriately (Anderson & Gallup, 2015; de Waal, 2019). The standard testing procedures do not always match the perceptual capabilities of the target species, which has resulted in modifications to the procedure.

Evidence for self-recognition has been sought in species that are not primarily visual, such as dogs (Horowitz, 2017), wolves (Cazzolla Gatti et al., 2021), and reptiles (Alberts, 1992; Angular et al., 2009; Burghardt et al., 2021; Graves & Halpern, 1991; Szabo & Ringler, 2023), using chemosensory-based self-recognition tests. In these paradigms, subjects are presented with the odor of a substance that originated from themselves (e.g., urine) either with or without an olfactory ‘mark’ substance added, as well as odors originating from conspecifics. Subjects that explore their own marked odor in preference to an unmarked odor are considered to have passed the test. While in visual MSR tests subjects must identify the image in the mirror as *being* themselves, in odor-based tasks they must identify the stimuli as having *come from* themselves, despite currently being part of the environment. In addition to the mark test, exploring one’s own odor more than that of a conspecific could be considered a form of ‘mirror’-guided self-exploration, fulfilling the other criterion for MSR. These ecologically driven modifications allow for testing self-recognition in a wider range of species.

Broadly, olfactory self-recognition studies have found that scent-reliant animals will investigate their marked odor more than their unmarked odor (Horowitz et al, 2017; Gatti et al., 2021) or discriminate between self-originating and other-originating skin lipids (Burghardt et al., 2021; Szabo & Ringler, 2023). Burghardt et al. (2021) found that male gartersnakes, but not females, spent more time investigating their own soiled bedding than that of a familiar same-sex conspecific. Although these findings suggest chemical-based self-recognition, these tasks have been criticized for failing to capture key aspects of self-recognition and for lacking important control conditions (Gallup & Anderson, 2018).

Coming across one's own odor in the environment can be a useful cue, suggesting one has marked this territory, crossed one's own path while navigating, or that one may be ill (if the odor is altered). Similar arguments are harder to make for visual MSR, even for studies that have used ecologically relevant marks (Kohda et al., 2019, 2022), as most animals will not encounter mirrors in the wild and will not be familiar with the properties of visual reflection (Barth et al., 2004; Clary & Kelly, 2016). Only recently has there been any consideration of how self-recognition abilities are affected by a species' ecology. For example, it has been suggested that self-recognition skills are more common in more social individuals (Krachun et al., 2019) or species (Gallup, 1985; though this is controversial: see Vanhooland et al., 2023).

Here, we conducted an odor-based self-recognition experiment on two species of snakes, Eastern gartersnakes (*Thamnophis sirtalis sirtalis*) and ball pythons (*Python regius*). These species differ in many aspects of their ecology, such as their hunting and reproductive strategies and their preferred habitats (Luiselli & Angelici, 1998; Luiselli & Akani, 2002; Rossman et al., 1996). Eastern Gartersnakes brumate for several months a year and forage for prey on both land and water, while ball pythons are sub-Saharan African ambush predators that do not brumate.

Importantly, these two species also differ in how often they might encounter conspecifics, and therefore, perhaps, in their need to differentiate their own chemical signature from that of others. Eastern gartersnakes aggregate seasonally at den sites for hibernation and mating (Rossman et al., 1996), and are also social during other times of the year (Skinner & Miller, 2020). In contrast, ball pythons are not known to aggregate in large groups (Gardner et al., 2015). Nonetheless, both species leave traces of their skin lipids in the environment as they move through it, and likely encounter these cues frequently, suggesting they may benefit from the ability to recognize their own chemical signature. Snakes primarily rely on their highly developed vomeronasal system for chemical communication with conspecifics. Here, we use the term ‘scent’ to broadly refer to an animal's chemical signature, including vomeronasal cues (vomodors; Cooper & Burghardt, 1990)

To test their recognition of self and other cues, snakes were individually placed into an arena in which they encountered a pair of scent-soaked cotton pads (see Methods for details). We compared snakes’ exploration of their own scent (which we denote S, for ‘Self’), their own scent with an added scent ‘mark’ odor (SM), the mark alone (M), the scent of a same-sex unrelated familiar conspecific (F), or the scent of a conspecific with a mark added (FM). Following common practice in squamates, we quantified investigation of a stimulus by counting tongue flicks towards the stimulus (Alberts, 1992; Burghardt et al., 2021), as well as by time spent close to the stimulus (Graves & Halpern, 1988; Cote & Clobert, 2007). We distinguished between long and short tongue-flicks, based on the number of tongue-tip oscillations performed before tongue retraction (Daghfous et al., 2012; Gove & Burghardt, 1983). We hypothesized that under these perceptually and ecologically relevant conditions, snakes would demonstrate self-recognition through increased investigation of their own marked scent compared to control scents such as the mark alone, their scent alone, or the scent of a familiar conspecific with the same mark. We did not predict any differences in investigation of the unmarked self-scent and unmarked scents of familiar conspecifics, as snakes would often encounter these scents in their environment, both naturally and in our lab. As chemosensory self-recognition could be beneficial to snakes generally, but could also depend on ecological conditions (such as sociability), we had no specific predictions as to whether or not we would find a difference between the two species.

Methods

Subjects

Subjects were 36 Eastern gartersnakes (17 male, 19 female) and 18 Ball pythons (6 male, 14 female). Table S1 lists the sources of the snakes and their ages and weights at testing. All snakes of each species were housed in the same room and had previously encountered each other in experiments on social behavior. Gartersnakes were housed in groups of 2-5, in 20 gallon glass aquariums with mesh lids. Their housing room was maintained at 22° C with a 12 hour light cycle (lights on at 7 am). All gartersnakes were fed nightcrawlers with vitamin supplements. Ball pythons were individually housed in a snake rack (ARS-7030, ARS Caging, Indianapolis, IN) in translucent tubs (84 cm x 44.5 cm x 14.5 cm). Their housing room was kept at an ambient 28 °C with humidity ranging from 50-70%. Ball pythons, which are crepuscular/nocturnal, had a reverse 12 hour light cycle (lights on at 7 pm). All ball pythons were fed frozen-thawed rats. Both species had access to belly heat (GS: 30° C; BP: 32° C) provided by heat tape (THGTape, Cornel’s World, Calgary, AB) and shelters (GS: 14 cm x 10.2 cm x 5 cm; BP: 23 cm x 16 cm x 6.5 cm; Cornel’s world) on both the cool and warm sides of their tank. Clean water was provided daily. Gartersnakes had one water dish (11.5 cm x 7.5 cm) whereas ball pythons had two water dishes (11.5 cm x 7.5 cm; placed forward in the enclosure; 15 cm x 15 cm x 6 cm; Ziplock; placed over the heat tape). The enclosures within which snakes of both species were housed were large enough that the snakes could stretch out fully, which has been shown to be important for their welfare (Warwick, Arena & Steedman, 2019). In both species, prey size and frequency of feeding was adjusted based on the size of the snakes. Snakes could all be individually identified by their head markings. One gartersnake died during the course of the experiment and all their data were excluded. All procedures followed the Canadian Council on Animal Care guidelines and were approved by our institutional Animal Care Committee.

Apparatus

Snakes of both species were tested in a 100.5 cm x 30.5 cm x 9.5 cm arena, three walls of which were constructed of PVC. The fourth wall was made of clear plexiglass, as was a lid placed over the top of the arena to prevent escapes (Figure S1). The arena rested on a paper towel substrate that was replaced between each trial, to ensure no odors remained from previous trials. The walls and ceiling of the arena were wiped with an alcohol swab and allowed to dry for 15 minutes between trials. The arena had one small PVC rod (7.5 cm) extending from each short wall, with a metal clip (3.5 cm) attached to it. A folded cotton swab soaked in various scents (see below) was affixed to each clip before each session; the distance between the two stimuli was 78 cm. Snakes were filmed using two cameras (Panasonic HC-V700) placed above and to the side of the arena, though only top-view videos were used in the analysis.

Procedure

Stimulus preparation

To obtain snake scents, snakes' bellies were swabbed with unscented makeup removal cotton pads. Pads had 1 ml of deionized water applied to them via pipette before swabbing. Subjects were first cleaned of any debris or feces by swabbing once down the length of the snake with a pad that was then discarded. Then, an 8 cm long segment of the snakes' ventral scales, anterior to the cloaca, were swabbed rostral to caudal a total of five times. All snakes had a precaudal body length greater than 8 cm. Pads were then placed in a sealed Ziploc bag for 1 hour, folded over to avoid lipids being removed by contact with the bag. Snake skin lipids that adhered to the pad were unlikely to degrade or evaporate between swabbing and their use as stimuli in the experimental trials, as it has been shown that these lipids are mostly non-volatile (Mason et al., 1989) and remain detectable on surfaces that snakes have moved over for at least one week (Wilmes et al., 2012). Snakes that either musked or defecated while being prepped for swabbing were set aside and cleaned and swabbed again later. Snakes were given a minimum of 5 days between swabbings. Immediately before each trial, all pads were cut in half to create two equally-scented stimuli. Stimuli that were to contain marked scents had 0.2 ml of olive oil added to them. Olive oil was chosen as a likely detectable but unfamiliar lipid (olives originated in the Mediterranean; Eastern gartersnakes are from North America and ball pythons are from sub-Saharan Africa; lipids are the chemical cues most likely to be easily identified by snakes [e.g., Mason & Parker, 2010]). The experimenter wore latex gloves when handling snakes or stimuli, and replaced the gloves between handling each stimulus or snake, to avoid their own scent contaminating any part of the procedure.

Trials

After swabbing, snakes were returned to their home cages for one hour before testing began. The appropriate stimuli for the trial to be run were folded once and affixed to the metal clips on either side of the arena, facing towards the arena center. The subject was then placed in the center of the testing arena and allowed to explore freely for 20 minutes while being video recorded. At the end of this time, snakes were returned to their home cages, the arena was cleaned with 70% rubbing alcohol, and the substrate was replaced.

Snakes were exposed to five possible stimulus pairs: self-scent (S) vs. self-scent with an added mark (SM), S vs. just the mark (M), S vs. the scent of a familiar same-sex unrelated conspecific (F), F vs. F with an added mark (FM), and SM vs. FM. To avoid a possible preference for related conspecifics (e.g., Lyman-Henley & Burghardt, 1994), snakes acquired from different sources were used as each other's familiar scent source (Table S1). The order of the trials was pseudorandomized across subjects, and all snakes were given at least three weeks between trials. The arrangement of the stimuli within the arena (left vs right side) was pseudorandomized between trials and within individuals. Due to experimental constraints, not all snakes participated in all trials (see Table S1).

Analysis

Videos were coded manually using a custom ethologger program by one coder (TF) blind to the test condition (videos were given codes and the initial segment showing the trial details was removed). Coding involved clicking on the position of the snakes' head. The ethologger registered different types of clicks as indicating mere presence, long tongue-flicks, or short tongue flicks. Long tongue flicks were classified as occurring when snakes oscillated their tongue 4 or more times in a single extension of the tongue; short tongue flicks were all cases where the number of oscillations was 3 or fewer (Gove & Burghardt, 1983). For analysis, tongue-flicks were considered whenever the snake's head was within 10 cm or less of one of the stimuli (see Figure S1). To account for the repeated measures nature of the experiment, Bayesian Wilcoxon signed-rank tests were performed, comparing behaviors (long or short tongue-flicks, or time spent near a stimulus) towards the pair of stimuli in each condition. We report the W statistic and the corresponding Bayes Factor (BF) for each analysis. Preference scores for one stimulus over another were calculated for each condition by dividing the behaviors (time present in a zone or number of tongue flicks while in that zone) emitted to the one stimulus of interest by the total observed of that behavior towards both stimuli. We also analyzed the raw number of tongue flicks directed toward the stimulus of interest (arcsine transformed), the raw total tongue flicks, and the amount of time spent near the stimuli across conditions and species, using a Bayesian linear model (with Species and Condition as fixed factors and Individual as a random effect). We also examined the effect of sex using Bayesian independent-samples T-tests. All analyses were carried out in JASP (JASP team, 2021) and R (v4.2.1, R Core Team, 2022), using the *BayesFactor* package (Morey & Rouder, 2021). The Bayes Factor is the ratio of the likelihoods of two hypotheses (for example, that a group spent more time near one stimulus than the other vs. that they did not): factors larger than one suggest that the tested hypothesis is more likely than the alternative (null) hypothesis. So, a BF of five means that the tested hypothesis is five times more likely than the alternative. As Bayes Factors are both hypothesis test statistics and effect size measures, we report each factor along with a modified effect size label based on those proposed by Kass & Raftery (1995; see also Jeffreys, 1961, for the original adjective series). BF between 1 and 3 provide ‘weak’ evidence for the hypothesis, BF from 3-20 ‘positive’ evidence, 20-150 ‘strong’ evidence, and BF > 150 ‘very strong’ evidence in favor of the hypothesis.

Results

We found very strong evidence that Eastern gartersnakes performed more long tongue-flicks to their own marked scent than to their own (unmodified) scent (Fig. 1A. Self+Mark vs. Self: W = 185, BF = 875), to the mark substance alone (Self+Mark vs. Mark: W = 136, BF =

354), or to the marked scent of a familiar conspecific (Self+Mark vs. Familiar+Mark: W = 154.5, BF = 156). We found weak evidence that snakes attended more to a familiar conspecific's unmodified scent than to their own unmodified scent (Self vs. Familiar: W = 1.5, BF = 2.02), and no evidence of a difference in attention to the scent of a conspecific when it was either marked or unmodified (Familiar+Mark vs. Familiar: W = 48, BF = 0.87).

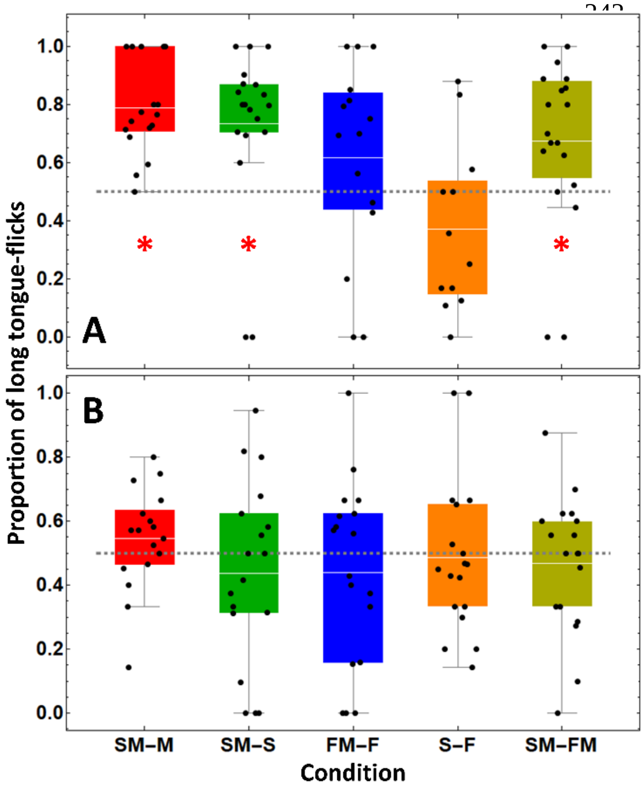

Figure 1. Proportion of long tongue-flicks directed to the stimulus of interest for each test condition for gartersnakes (**A**) and ball pythons (**B**). In each x-axis label, the stimulus of interest is listed first. Black dots show all the raw data; white lines inside the bars give means; the bars extend from the 25th to the 75th quantiles, and error bars show 95% confidence intervals. Red asterisks indicate conditions in which the evidence indicated a strong preference for the stimulus of interest (Bayes Factors > 100); there was no evidence for a preference in either direction in the other conditions (BF < 3). S = self, M = mark, SM = self + mark, F = familiar conspecific, FM = familiar conspecific + mark.

These effects were only detectable in gartersnakes' long tongue-flicks. We found no differences between conditions in short tongue-flicks (Fig. S2A. Self+Mark vs. Self: W = 121.5, BF = 0.58; Self+Mark vs. Mark: W = 73, BF = 0.26; Self+Mark vs. Familiar+Mark: W = 94, BF = 0.25; Self vs. Familiar: W = 5, BF = 1.18; Familiar+Mark vs. Familiar: W = 34, BF = 0.32) or in the time spent on either side of the arena (Fig. S1B. Self+Mark vs. Self: W = 99, BF = 0.24; Self+Mark vs. Mark: W = 91, BF = 0.36; Self+Mark vs. Familiar+Mark: W = 96, BF = 0.24; Self vs. Familiar: W = 7, BF = 0.75; Familiar+Mark vs. Familiar: W = 39, BF = 0.38).

Unlike gartersnakes, we found no evidence of a difference between conditions in ball pythons' long tongue-flicks (Fig. 1B. Self+Mark vs. Self: W = 66.5, BF = 0.24; Self+Mark vs. Mark: W = 89.5, BF = 0.38; Self+Mark vs. Familiar+Mark: W = 55, BF = 0.24; Self vs. Familiar: W = 46, BF = 0.75; Familiar+Mark vs. Familiar: W = 82.5, BF = 0.25), short tongue-flicks (Fig. S3A. Self+Mark vs. Self: W = 74, BF = 0.28; Self+Mark vs. Mark: W = 41, BF = 1.55; Self+Mark vs. Familiar+Mark: W = 43.5, BF = 0.77; Self vs. Familiar: W = 71, BF = 0.37; Familiar+Mark vs. Familiar: W = 74.5, BF = 0.26), or time spent on either side of the arena (except positive evidence of a preference for Mark over Self+Mark: W = 29, BF = 4.63; note that this effect is in the opposite direction to that observed in gartersnakes; Fig. S3B. Self+Mark vs. Self: W = 99, BF = 0.26; Self+Mark vs. Familiar+Mark: W = 56, BF = 0.48; Self vs. Familiar: W = 74, BF = 0.34; Familiar+Mark vs. Familiar: W = 57, BF = 0.41).

Differences in long tongue flicks to the stimulus of interest across species were not driven by individual differences alone, as we found very strong evidence that a model including species differences in preference was superior to a model including only individual identity (BF = 227.92). As the previous tests only compared the relative numbers of tongue-flicks to the two stimuli, we additionally compared the total number of long and short tongue flicks, as well as the total time spent near either stimulus, across conditions and species (to assess whether there were any differences in activity levels, for example, across conditions or species). We found positive evidence that gartersnakes generally performed more long tongue flicks than ball pythons, independent of condition (BF = 5.27.; the full effects table is given in Table S2A), but the number of long tongue flicks did not vary across conditions (BF = 0.35). Further analysis indicated that this species difference was the result of more long tongue flicks by gartersnakes when near the stimulus of interest in each pairing (BF = 103.43), but not when near the alternate stimulus (BF = 0.41). Gartersnakes did not perform more short tongue flicks than ball pythons

(BF = 0.54, Table S2B), and there was no effect of condition (BF = 0.04). We found no difference in the amount of time spent near the stimuli across species (BF = 0.01) or conditions (BF < 0.01). Finally, we compared all our measures across sexes (in each species separately). We found only weak evidence for differences between the sexes in short tongue flicks and time spent near the stimuli in ball pythons (Table S3B), and no differences between sexes in gartersnakes (Table S3A). In summary, gartersnakes but not ball pythons directed more long tongue flicks to the stimulus of interest (i.e., stimuli that suggest self-recognition) in all conditions. Both ball pythons and gartersnakes spent a similar amount of time near the stimuli and performed a similar number of short tongue flicks. This suggests that species differences in movement or overall tongue-flicking frequency alone cannot explain our results.

Discussion

We tested two species of snake with very different ecologies on their ability to self recognize using a modified olfactory mark test. One species was the Eastern gartersnake, an active terrestrial and semi-aquatic forager that aggregates for brumation, mating, and gestation; the other was the ball python, which is an ambush predator – possibly semi-arboreal – from Sub-Saharan Africa that is not known to be social outside of mating and does not brumate. The mark test involved modifying the snake's scent by adding an olive oil 'mark'. We found that gartersnakes, but not ball pythons, performed more tongue flicks towards their own marked scent than to their unmarked odor, to the mark alone, or to the marked scent of a familiar conspecific. These results provide evidence that gartersnakes are able to recognize their own scent and can tell when it has been modified and, importantly, that they are motivated to explore these modified scents.

Several alternative hypotheses must be considered that might explain some of our results without requiring that snakes can self-recognize. First, snakes might simply be innately interested in some scents more than others. However, our results cannot be attributed to an interest in the mark substance itself, as the snakes also investigated their own marked scent more than the mark substance alone. Moreover, our results are not attributable to an increased interest in more complex stimuli, as gartersnakes also showed a preference for their own marked scent over that of a marked conspecific (both of which are equally complex). This result also suggests that long tongue flicks are not simply used to explore more complex stimuli. Additionally, as snakes did not spend significantly more time in any particular zone, differences in tongue flicks do not result from unequal exploration of the arena.

Second, snakes might be choosing scents to explore on the basis of familiarity, avoiding either more or less familiar scents. However, gartersnakes showed only a very slight tendency to explore the unmodified scent of a familiar conspecific more than their own unmodified scent (as did some snakes in Burghardt et al., 2021), and did not discriminate between marked and unmarked conspecific scents. The latter result is in stark contrast to gartersnakes' discrimination between their own marked and unmarked scent. The contrasting responses across the Self+Mark vs. Self and the Familiar+Mark vs. Familiar conditions show that gartersnakes react differently to modifications of their own scent than they do to modifications of the scent of a familiar conspecific. These results suggest that snakes do not simply avoid either familiar or unfamiliar scents. This question could have been further explored if we had added a condition in which snakes were presented with the odor of an unfamiliar conspecific. While such a condition was not included in the present study, this potential limitation would be an interesting topic for future study.

Third, it has been suggested that effects similar to our data might result from dishabituation (Gallup & Anderson, 2018). On this view, snakes are habituated to their own scent and the addition of the mark dishabituates them, making them explore that stimulus more. This would be analogous to apes reducing mirror-mediated self-exploration due to having habituated to the images, and only resuming when the image changed due to the addition of the mark, something that does indeed happen if animals are exposed to a mirror for long enough (Shorland et al., 2020). Though dishabituation could explain a preference for one's own marked scent over one's own unmarked scent, gartersnakes also showed a preference for their own marked scent over the marked scent of a familiar conspecific, neither of which they are likely to have been habituated to (or both of which they should be approximately equally habituated to).

Fourth, it has been suggested that an interest in one's own marked scent – a stimulus not previously encountered but which shares features with one's own familiar scent – could result from expectancy violation (e.g., Baragli et al., 2021). A key condition of the olfactory self-recognition paradigm that addresses this question is the direct comparison between an organism's modified (marked) scent and the modified (marked) scent of a conspecific. In this condition, both scents violate the expectation of what 'snake' smells like, and gartersnakes' preference for exploring their own marked scent over the conspecific's suggests their behavior is not driven by a general expectancy violation not related to self-perception.

As these alternative explanations cannot explain our results, our findings add to prior research showing that many reptiles are sensitive to differences between their own and conspecific scents (male rock lizards: Mangiacotti et al., 2020; desert iguanas: Alberts, 1992; timber and prairie rattlesnakes: Chiszar et al., 1991; Fitzgerald's tree iguana: Aguilar et al., 2009). A sensitivity to conspecific discrimination is, however, different from genuine self-recognition. Scent recognition experiments often present subjects with their own scent and that of a conspecific and measure the number of tongue flicks directed toward each stimulus (Alberts, 1992; Burghardt et al., 2021). Although it seems likely that different responses in such situations represent genuine self-recognition, it is difficult to know whether individuals respond to their own scent because they recognize it as their own, because they recognize a familiar component of the scent (e.g., relatedness or diet), or due to habituation. This issue is controlled for in olfactory self-recognition mark tests, such as the one used in this study, as an individual's own scent is present in both the marked and control stimuli.

Similar to findings from chemical mark tests done with wolves (Cazzolla Gatti et al., 2020) and dogs (Horowitz, 2017), our results suggest that gartersnakes can recognize their own scent. In particular, our results are concordant with Horowitz's (2017) results on olfactory self-recognition in dogs, although we found little discrimination between unmarked self- and other-scents. Two previous studies demonstrating self vs. other discrimination in Eastern gartersnakes have also suggested that these snakes can recognize their own chemical signature. Halpin (1990) demonstrated that gartersnakes tongue flick more to a conspecific scent than to their own, and Burghardt et al. (2021) showed that male snakes, but not females, tongue flick more to their own scent than the scent of a conspecific on the same diet. Similar to these studies, we found that gartersnakes could differentiate between self and familiar scents. However, they only did so when both scents were marked. We note that, in both the cited studies and our own, the conspecific scent used differed in familiarity and genetic similarity to the test subject: unfamiliar but possibly related (Halpin, 1990), unfamiliar and related (Burghardt et al., 2021), and familiar but unrelated (the current study). Given these different results across studies, future research should consider using a wider range of scent stimuli that differ across both familiarity and relatedness (e.g., Cazzolla Gatti et al., 2020). We note that gartersnakes showed discrimination between the stimuli only in their long tongue-flicks (flicks consisting of 4+ oscillations of the tongue). There was no effect on short tongue-flicks or on the time spent near each stimulus. It has been previously demonstrated that gartersnakes perform longer tongue-flicks when exploring than in other contexts, which may partially explain this finding (Gove & Burghardt, 1983).

In contrast to the self-odor discrimination demonstrated by gartersnakes, we found that pythons did not explore their own marked scent more than any control scents, by any measure. Though we cannot tell whether this effect results from pythons' inability to discriminate between the scents or from a lack of motivation to explore them differentially, the contrast with gartersnakes' behavior in the identical task is intriguing, considering the ecological differences between the species. Snakes have a highly sensitive vomeronasal system, which they employ to detect predators, prey, and conspecifics (Halpern, 1987; Halpern & Martinez-Marcos, 2003). Gartersnakes can use chemosensory information to recognize individuals, and identify kin, sex, size, and even the diet of other snakes (Lyman-Henley & Burghardt, 1994; Yeager & Burghardt, 1991; LeMaster & Mason, 2002). They also follow scent trails left by others over large distances to locate hibernation sites (Constanzo, 1989). Gartersnakes would therefore often encounter their own scent in the environment and self-recognition would be of value to them when foraging or when navigating between their preferred sheltering locations. Ball pythons, who do not aggregate and are ambush predators (Luiselli & Angelici, 1998) – spending less time foraging – may not benefit as much from the ability to recognize when they have crossed their own path.

Alternatively, the failure of ball pythons to pass our tests may be due to a lack of motivation to differentially respond to the stimuli (Clary et al., 2020). Some mammals, for example, can use their vomeronasal systems to detect illness or parasite loads in conspecifics (Boillat et al., 2015). Identifying potential illness, by detecting a change in the scents they leave behind in the world, could be important to the gregarious gartersnakes, as illness may spread quickly through their groups (Schmid-Hempel, 2017). In this way, gartersnakes may be motivated to differentially respond to contaminated scents in order to avoid certain conspecifics, which might help limit pathogen spread (as in fungus-infected ants that avoid their colonies; Stockmaier et al., 2021). In contrast, scent-altering sickness may be of less interest to ball pythons, as contagion is less likely in their diffuse social systems.

Inherent differences between visual and olfactory MSR tests should be considered when interpreting an animal's passing or failing the test. Passing visual MSR tests requires subjects to identify that the image in the mirror is of their own bodies. This ability can be broken down conceptually into two separate skills: 1) a concept of self/not-self that allows subjects to distinguish cues that emanate from themselves (in any relevant modality; Platek et al., 2004) from those that do not, and 2) the understanding that mirrors reflect images, a form of physical cognition. As suggested by Heyes (1994), it seems likely that most or all vertebrates possess a basic proprioceptive sense of self. The second skill is presumably acquired during pre-exposure to the mirror, which even chimps require to show MSR (Gallup, 1970). On a minimalist view of visual MSR, the process that occurs during exposure to a mirror consists of expanding the self/not-self concept to encompass the images viewed in the mirror. During the mark test, subjects identify that something has changed in the reflected image, and react to it as they would to a mark on a visually accessible body part. Importantly, this explanation suggests that some of the species that fail to pass MSR tests (Suarez & Gallup, 1981; Jin et al., 2015; Vanhooland et al., 2023) might not be capable of the physical cognition required (learning how reflections work), rather than lacking the ability to distinguish self from not-self. This question could be further explored in species that are capable of learning to use mirrors instrumentally, to locate otherwise hidden objects in their environment (pigs: Broome et al., 2009; dogs: Howell et al., 2013; parrots: Pepperberg et al., 1995; elephants: Povinelli, 1989). Gartersnakes – along with other species that pass the mark test – may have a flexible self-recognition capability, which allows for the concept to be expanded to encompass an external stimulus (as in humans, who can ‘embody’ a fake limb; Botvinick & Cohen, 1998), whether that stimulus is viewed in a mirror or sniffed in the environment. In contrast, one possible reason for ball pythons not passing the test is that they may have a more rigid concept of self/not-self that does not extend to environmental stimuli. In the current study, we compared two species that both interact with their environments primarily via scent. The olfactory version of the mark test does not require subjects to understand the physics of mirrors, but it is a test of their ability to expand their self-recognition to external cues. In this sense, it captures many of the same kinds of self-recognition capabilities as the visual mirror test.

There have been several sharp criticisms of chemical-based self-recognition paradigms, suggesting that they do not provide evidence for self-recognition comparable to visual MSR experiments (Gallup & Anderson, 2018, 2019). We have addressed some of these criticisms in considering alternate explanations of our results above. In addition, it has been suggested that animals encountering their own marked scent should engage in an exploration of their own bodies, analogously to visual mirror-mediated inspections (Gallup & Anderson, 2019). However, if the goal of the investigation is to identify – rather than remove – the change in one’s own scent, we should expect animals to preferentially examine the external cue. This may be especially true when animals can do little to affect the perceived change in their scent. Additionally, the scents animals leave behind in the environment are not necessarily the same as those of their own bodies (e.g., urine) and exploring one’s own body may not help in identifying the change that has occurred. We believe it is possible for scent-based tests to provide evidence that is comparable to that obtained by visual MSR paradigms, and that it is possible for animals to pass both types of tests without having sophisticated concepts of selfhood or the more pronounced kind of conscious self-awareness that chimps are said to possess.

Self-recognition can undoubtedly result from the operation of any of several cognitive processes that vary in complexity from the basic ability of an immune system to differentiate its own components from foreign pathogens (Gallup, Anderson, & Platek, 2011), to expectancy violation (one’s own scent being not as expected), to self-consciousness, which has been argued to entail a sense of continuity over time, personal agency, and identity (Gallup, 1998). In this study, gartersnakes but not ball pythons demonstrated greater interest in their own scent when marked compared to a number of key control conditions. The kind of self-recognition exhibited by these snakes likely falls somewhere between the above extremes. We avoid attributing higher-order abilities such as self-awareness or consciousness to snakes, particularly as we do not believe that the results of MSR tests on their own provide evidence for these skills.

Our findings strengthen the argument that some reptiles can self-recognize (Burghardt et al., 2021; Chiszar et al., 1991; Aguilar et al., 2009; Alberts, 1992) and that using ecologically relevant stimuli may improve our ability to detect self-recognition across taxa (Burghardt et al., 2021; Horowitz et al., 2017). We found that the more social of the two species we tested showed self-recognition and the less social species did not, adding weight to recent arguments that self-recognition may be tied to social skills (Krachun et al., 2009). We note that squamates, which inhabit a wide range of habitats and have diverse social systems, are a fruitful taxon in which to further explore this idea (Doody et al., 2021). We do not intend to suggest that sociality is the only means of developing self-recognition, but that a more developed capacity to self-recognize may offer an adaptive benefit to animals in complex social systems. Conversely, it may simply be true that self-recognition is easier to observe in social species, who could be more motivated to interact with the stimuli (visual or olfactory). Additionally, our comparative analysis adds to an exponentially growing body of literature that challenges the tendency to classify certain cognitive processes as complex on only introspective evidence. Instead, research continues to suggest that varieties of behaviors that require “complex” cognitive abilities (empathy; Pérez-Manrique & Sureda, 2018; tool use; Dinets et al., 2015; friendship; Skinner & Miller, 2020, Gerber et al., 2021) simply evolve under specific environmental pressures that may be more widespread across taxa than once thought.

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AI Statement: The authors declare that they have not used any AI technology in the preparation of the paper.

Ethics: All experimental procedures were approved by the Wilfrid Laurier University Animal Care Committee (AUP R17004) and complied with all Canadian Council on Animal Care guidelines.

Data availability: All the data analyzed in the current paper are available as an excel spreadsheet on our OSF repository, along with JASP files for replicating the analyses and sample experimental videos: https://osf.io/wd56g/?view_only=7b43fcb29ac4413bab7bc56228f763d9.

References

Anderson, J. R., & Gallup, G. G. (2015). Mirror self-recognition: a review and critique of attempts to promote and engineer self-recognition in primates. Primates, 56(4), 317–326. https://doi.org/10.1007/s10329-015-0488-9

Baragli, P., Scopa, C., Maglieri, V., Palagi, E. (2021). If horses had toes: demonstrating mirror self recognition at group level in *Equus caballus*. *Animal Cognition, 24*, 1099-1108.

Barth, J., Povinelli, D. J., Cant, J. H. (1994). Bodily origins of SELF. In D. R. Beike, J. M. Lampinen, & D. A. Behrend (Eds.), *The self and memory* (pp. 11–43). Psychology Press.

Boillat, M., Challet, L., Rossier, D., Kan, C., Carleton, A., Rodriguez, I. (2015). The vomeronasal system mediates sick conspecific avoidance. *Current Biology, 25*, 251-255.

Botvinick, M., Cohen, J. (1998). Rubber hands ‘feel’ touch that eyes see. *Nature, 391*, 756.

Brandl, J. L. (2016). The puzzle of mirror self-recognition. *Phenomenology and the Cognitive Sciences, 17*, 279–304. https://doi.org/10.1007/s11097-016-9486-7

Broom, D. M., Sena, H., Moynihan, K. L. (2009). Pigs learn what a mirror image represents and use it to obtain information. *Animal Behaviour, 78*, 1037-1041.

Burghardt, G. M., Partin, A. M., Pepper, H. E., Steele, J. M., Liske, S., Stokes, A. E., Lathan, A. N., Springer, C. M., & Jenkins, M. S. (2021). Chemically mediated self-recognition in sibling juvenile common gartersnakes (*Thamnophis sirtalis*) reared on same or different diets: evidence for a chemical mirror? *Behaviour*, *158*(12–13), 1169–1191.

Cazzolla Gatti, R. C., Velichevskaya, A., Gottesman, B. L., & Davis, K. D. (2020). Grey wolf may show signs of self-awareness with the sniff test of self-recognition. *Ethology Ecology & Evolution, 33(4)*, 444–467. https://doi.org/10.1080/03949370.2020.1846628

Clary, D., Kelly, D. M. (2016). Graded mirror self-recognition by Clark’s nutcrackers. *Scientific Reports, 6*, 36459.

Clary, D., Stow, M. K., Vernouillet, A., Kelly, D. M. (2020). Mirror-mediated responses of California scrub jays (*Aphelocoma californica*) during a caching task and the mark test. *Ethology, 126*,140-152.

Costanzo, J. P. (1989). Conspecific scent trailing by garter snakes (*Thamnophis sirtalis*) during autumn Further evidence for use of pheromones in den location. *Journal of Chemical Ecology*, *15*(11), 2531–2538.

Cote, J., & Clobert, J. (2007). Social personalities influence natal dispersal in a lizard. *Proceedings of the Royal Society B: Biological Sciences, 274(1608),* 383–390.

Chiszar, D., Smith, H. M., Bogert, C. M., & Vidaurri, J. (1991). A chemical sense of self in timber and prairie rattlesnakes. *Bulletin of the Psychonomic Society*, *29(2)*, 153–154.

Daghfous, G., Smargiassi, M., Libourel, P., Wattiez, R., & Bels, V. (2012). The Function of Oscillatory Tongue-Flicks in Snakes: Insights from Kinematics of Tongue-Flicking in the Banded Water Snake (*Nerodia fasciata*). *Chemical Senses, 37(9),* 883–896.

Dinets, V., Brueggen, J., & Brueggen, J. (2015). Crocodilians use tools for hunting. *Ethology Ecology & Evolution, 27(1),* 74–78.

Doody, J. S., Dinets, V., & Burghardt, G. M. (2021). *The Secret Social Lives of Reptiles*. JHU Press.

Gallup, G. G. (1970). Chimpanzees: self-recognition. *Science, 167,* 86-87.

Gallup, G. G. (1977). Self recognition in primates: A comparative approach to the bidirectional properties of consciousness. *American Psychologist, 32,* 329-338.

Gallup, G. G. (1982). Self-awareness and the emergence of mind in primates. American Journal of Primatology, 2, 237–248.

Gallup, G. G. (1985). Do minds exist in species other than our own? *Neuroscience & Biobehavioral Reviews, 9,* 631-641.

Gallup, G. G. (1998). Self-awareness and the evolution of social intelligence. Behavioral Processes, 42, 239–247.

Gallup, G. G., Anderson, J. R. (2018). The “olfactory mirror” and other recent attempts to demonstrate self-recognition in non-primate species. *Behavioral Processes, 147,*16-19.

Gallup, G. G., Anderson, J. R. (2019). Self-recognition in animals: where do we stand 50 years later? Lessons from cleaner wrasse and other species. *Psychology of Consciousness: Theory, Research, and Practice, 7(1)*, 46–58.

Gardner, M. G., Pearson, S. K., Johnston, G. R., Schwartz, M. P. (2015). Group living in squamate reptiles: a review of evidence for stable aggregations. *Biological Reviews, 91(4),* 925-936.

Gerber, L., Wittwer, S., Allen, S., Holmes, K., King, S. L., Sherwin, W. B., Wild, S., Willems, E. P., Connor, R., & Krützen, M. (2021). Cooperative partner choice in multi-level male dolphin alliances. *Scientific Reports, 11(1),* 6901.

Gove, D., & Burghardt, G. M. (1983). Context-correlated parameter of snake and lizard tongue-flicking. *Animal Behaviour, 31,* 718-723.

Graves, B. M., & Halpern, M. (1988). Neonate plains garter snakes (*Thamnophis radix*) are attracted to conspecific skin extracts. *Journal of Comparative and Physiological Psychology, 102(3),* 251-253.

Halpern, M. (1987). The Organization and Function of the Vomeronasal System. *Annual Review of Neuroscience, 10(1),* 325–362.

Halpern, M. & Martinez-Marcos, A. (2003). Structure and function of the vomeronasal system: an update. *Progress in Neurobiology, 70(3),* 245–318.

Halpin, Z. T. (1990). Responses of Juvenile Eastern Garter Snakes (*Thamnophis sirtalis sirtalis*) to Own, Conspecific and Clean Odors. *Copeia, 1990(4)*, 1157. https://doi.org/10.2307/1446504

Heyes, C. (1994). Reflections on self-recognition in primates. *Animal Behaviour, 47(4)*, 909–919.

Hillemacher, S., Ocklenburg, S., Güntürkün, O., & Tiemann, I. (2023). Roosters do not warn the bird in the mirror: The cognitive ecology of mirror self-recognition. PLOS ONE, 18(10), e0291416. https://doi.org/10.1371/journal.pone.0291416

Howell, T. J., Toukhsati, S., Conduit, R., Bennett, P. (2013). Do dogs use a mirror to find hidden food? *Journal of Veterinary Behavior, 8*, 425-430.Jeffreys, H. (1961). *Theory of Probability*. 3rd Edition, Clarendon Press, Oxford.

Kass, R. E. and Raftery, A. E. (1995) Bayes factors. Journal of the American Statistical Association, 90, 773-795.

Kohda, M., Hotta, T., Takeyama, T., Awata, S., Tanaka, H., Asai, J., Jordan A.L. (2019). If a fish can pass the mark test, what are the implications for consciousness and self-awareness testing in animals? PLOS Biology, 17, e3000021.

Kohda, M., Sogawa, S., Jordan, A.L., Kubo, N., Awata, S., Satoh, S., Kobayashi, T., Fujita, A., Bshary, R. (2022). Further evidence for the capacity of mirror self-recognition in. cleaner fish and the significance of ecologically relevant marks. PLOS Biology, 20, e3001529.

Krachun, C., Lurz, R., Mahovetz, L. M., Hopkins, W. D. (2019). Mirror self-recognition and its relationship to social cognition in chimpanzees. *Animal Cognition, 22*, 1171-1183.

LeMaster, M. P., & Mason, R. J. (2002). Variation in a Female Sexual Attractiveness Pheromone Controls Male Mate Choice in Garter Snakes. *Journal of Chemical Ecology, 28(6)*, 1269–1285.

Luiselli, L., & Akani, G. C. (2002). An investigation into the composition, complexity and functioning of snake communities in the mangroves of south-eastern Nigeria. *African Journal of Ecology, 40(3),* 220–227.

Luiselli, L., & Angelici, F. M. (1998). Sexual size dimorphism and natural history traits are correlated with intersexual dietary divergence in royal pythons (python regius)from the rainforests of southeastern Nigeria. *The Italian Journal of Zoology, 65(2),* 183–185.

Lyman-Henley, L. P., & Burghardt, G. M. (1994). Opposites attract: effects of social and dietary experience on snake aggregation behaviour. *Animal Behaviour, 47(4),* 980–982.

Mangiacotti, M., Martín, J., López, P., Reyes-Olivares, C., Rodríguez-Ruiz, G., Coladonato, A. J., Zuffi, M. a. L., Zuffi, M. a. L., & Sacchi, R. (2020). Proteins from femoral gland secretions of male rock lizards *Iberolacerta cyreni* allow self—but not individual—recognition of unfamiliar males. *Behavioral Ecology and Sociobiology*, *74(6)*, 68.

Mason, R.T., Fales, H.M., Jones, T.H., Pannell, L.K., Chinn, J.W., Crews, D. (1989). Sex pheromones in snakes. Science, 245, 290-293.

Mason, R. T., Parker, M. R. (2010). Social behavior and pheromonal communication in reptiles. *Journal of Comparative Physiology A*, *196*, 729–749.

Morey R, Rouder J (2021). BayesFactor: Computation of Bayes Factors for Common Designs. R package version 0.9.12-4.3, <https://CRAN.R-project.org/package=BayesFactor>.

Morrison, R., & Reiss, D. (2018). Precocious development of self-awareness in dolphins. PLoS One, 13(1), e0189813.

Pepperberg, I. M., Garcia, S. E., Jackson, E. C., Marconi, S. (1995). Mirror use by African grey parrots (*Psittacus erithacus*). *Journal of Comparative Psychology, 109(2)*, 182-195.

Pérez-Manrique, A., & Sureda, A. (2018). The comparative study of empathy: sympathetic concern and empathic perspective-taking in non-human animals. *Biological Reviews, 93(1)*, 248–269.

Platek, S.M., Thomson, J.W., Gallup, G.G. (2004). Cross-modal self-recognition: the role of visual, auditory, and olfactory primes. Consciousness and Cognition, 13, 197-210.

Povinelli, D. J. (1989). Failure to find self-recognition in Asian elephants (*Elephas maximus*) in contrast to their use of mirror cues to discover hidden food. *Journal of Comparative Psychology, 103(2)*, 122-131.

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rossman DA, Ford NB, Seigel RA. 1996. The garter snakes: Evolution and ecology. University of Oklahoma Press.

Schmid-Hempel, P. (2017). Parasites and Their Social Hosts. *Trends in Parasitology, 33(6)*, 453–462.

Shorland, G., Genty, E., Guéry, J-P., Zuberbühler, K. (2020). Investigating self-recognition in bonobos: mirror exposure reduces looking time to self but not unfamiliar conspecifics. *PeerJ, 8*, e9685.

Stockmaier, S., Stroeymeyt, N., Shattuck, E. C., Hawley, D. M., Meyers, L. A., & Bolnick, D. I. (2021). Infectious diseases and social distancing in nature. *Science, 371(6533)*, 1007.

Schwenkler, John L. (2008). Mental vs. Embodied Models of Mirrored Self-Recognition: Some Preliminary Considerations. In B. Hardy-Valeé & N. Payette (eds.), Beyond the Brain: Embodied, Situated, and Distributed Cognition. Cambridge Scholars Press.

Szabo, B., & Ringler, E. (2023). Geckos differentiate self from other using both skin and faecal chemicals: evidence towards self-recognition? *Animal Cognition*.

Vanhooland, L-C., Szabó, A., Bugnyar, T., Massen, J. J. M. (2023). A comparative study of mirror self-recognition in three corvid species. *Animal Cognition, 26*, 229-248.

Vonk, J. (2020). A fish eye view of the mirror test. Learning & Behavior, 48, 193–194. https://doi.org/10.3758/s13420-019-00385-6

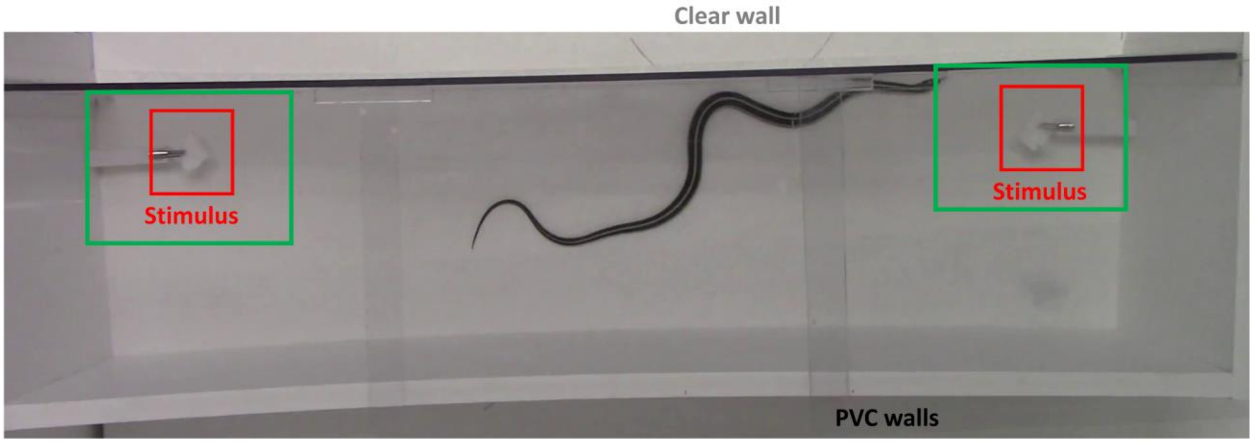
Warwick, C., Arena, P., Steedman, C. (2019). Spatial considerations for captive snakes. Journal of Veterinary Behavior, 30, 37-48.

Wilmes, A.J., Rheubert, J.L., Gruenewald, D.L., Kotaru, T., Aldridge, R.D. (2012). Conspecific pheromone trailing and pheromone trail longevity in the African Colubrid Boaedon fuliginosus. African Journal of Herpetology, 61, 159-168.

Yeager, C. P., & Burghardt, G. M. (1991). Effect of food competition on aggregation: Evidence for social recognition in the plains garter snake (*Thamnophis radix*). *Journal of Comparative and Physiological Psychology, 105(4)*, 380–386.

Supplementary Materials

Figure S1: photograph of the testing arena (still from a trial video) with measurements. The green line shows the extent of the zones within which snakes were considered to be inspecting each stimulus.



Arena size: 100.5 cm x 30.5 cm x 9.5 cm high
Stimulus clips extend 11 cm from each side wall; stimuli are 4 cm above the floor, and 78 cm apart

Figure S2: the proportion of short tongue-flicks (A) and time spent in each zone (B) for gartersnakes in each condition. The graph shows the proportion of tongue flicks towards or the time spent near the stimulus of interest. In each x-axis label, the stimulus of interest is listed first. Black dots show all the raw data; white lines inside the bars give means; the bars extend from the 25th to the 75th quantiles, and error bars show 95% confidence intervals. S = self, M = mark, SM = self + mark, F = familiar conspecific, FM = familiar conspecific + mark.

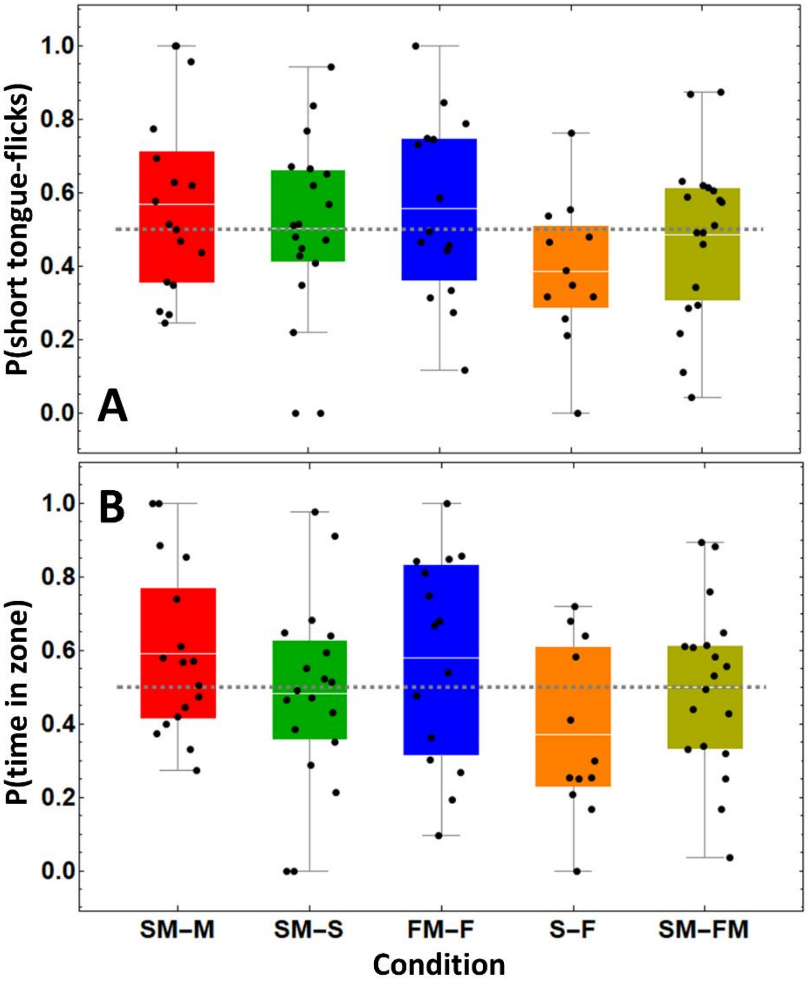


Figure S3: the proportion of short tongue-flicks (A) and time spent in each zone (B) for ball pythons in each condition. All other details are the same as in Figure S2.

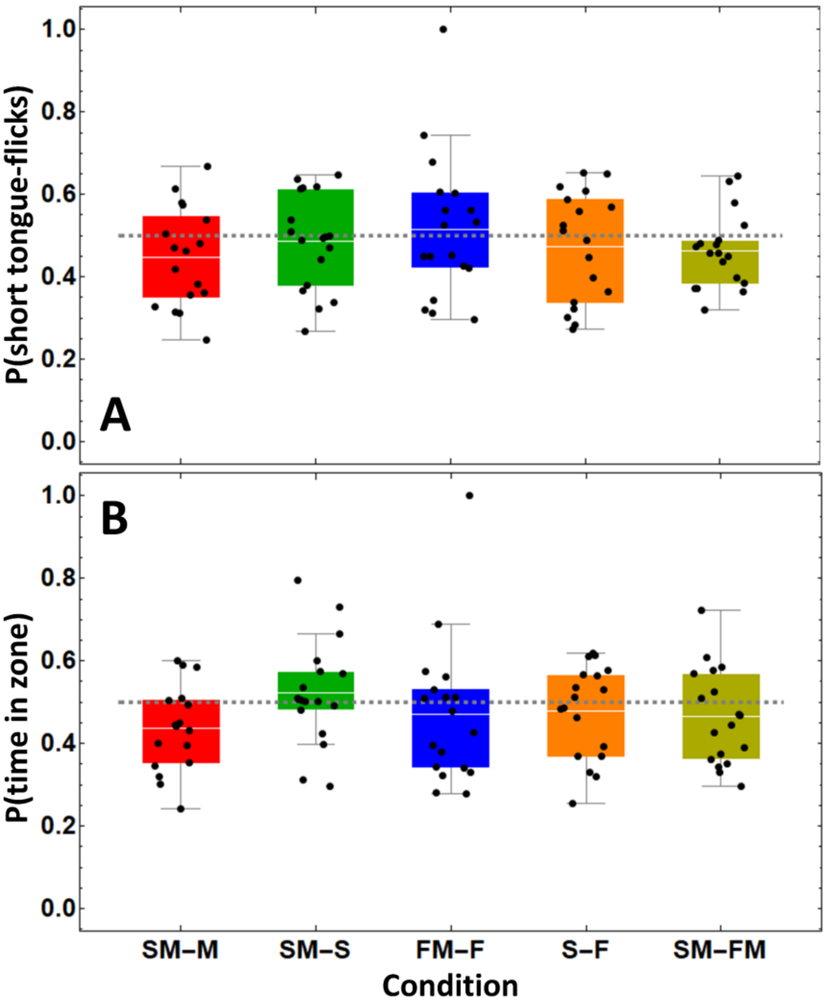


Table S1. Age, sources, sexes, and weights of the gartersnakes (A) and ball pythons (B) used in the experiment, along with the conditions that each snake completed. For familiar scents, snakes of the same sex but from a different source were used. Snakes identified with an asterisk were only used as stimulus snakes. Ages are based on approximate birth dates and testing windows. All weights are in grams. Trials each snake completed are listed using the same abbreviations as in Figure S2.

A. Gartersnakes					
ID	Age @ testing	Source	Sex	Weight (g)	Trials
A31	2-2.5 years	Lab born	F	77	SM-M, SM-S, FM-F
A32	2-2.5 years	Lab born	M	52	SM-M, SM-S, S-F
A33	2-2.5 years	Lab born	F	121	SM-M, SM-S, FM-F
B1	Unknown	Wild caught	M	34	SM-M, SM-S, FM-F
B10	Unknown	Wild caught	F	60	SM-M, SM-S, S-F
B11	Unknown	Wild caught	M	-	SM-M, SM-S, FM-F
B12	Unknown	Wild caught	M	53	SM-M, SM-S, S-F
B16	Unknown	Wild caught	M	47	SM-M, SM-S, FM-F
B17	Unknown	Wild caught	F	53	SM-M, SM-S, S-F
B18	Unknown	Wild caught	F	45	SM-M, SM-S, FM-F
B2	Unknown	Wild caught	F	17	SM-M, SM-S, FM-F
B20	Unknown	Wild caught	F	33	SM-M, SM-S, FM-F, S-F
B4	Unknown	Wild caught	M	22	SM-M, SM-S, FM-F
B5	Unknown	Wild caught	M	31	SM-M, SM-S
B6	Unknown	Wild caught	M	33	SM-M, SM-S
B7	Unknown	Wild caught	F	12	SM-S, S-F
B8	Unknown	Wild caught	F	75	SM-M, SM-S, FM-F
B9	Unknown	Wild caught	M	27	SM-M, SM-S, FM-F, S-F
9-2	6-9 months	Breeder 3	F	28	SM-FM
2-1	6-9 months	Breeder 3	F	11	SM-FM
4-2	6-9 months	Breeder 3	F	34	SM-FM
10-5	6-9 months	Breeder 3	M	16	SM-FM
2-4	6-9 months	Breeder 3	F	28	SM-FM
4-1	6-9 months	Breeder 3	F	13	SM-FM
5-2	6-9 months	Breeder 3	F	16	SM-FM
13-1	6-9 months	Breeder 3	F	16	SM-FM
8-3	6-9 months	Breeder 3	F	11	SM-FM
8-1	6-9 months	Breeder 3	F	16	SM-FM
8-4	6-9 months	Breeder 3	M	14	SM-FM
5-3	6-9 months	Breeder 3	F	26	SM-FM
12-3	6-9 months	Breeder 3	F	25	SM-FM
4-4	6-9 months	Breeder 3	F	12	SM-FM
3-4	6-9 months	Breeder 3	F	22	SM-FM
6-3	8-12 months	Breeder 4	F	19	SM-FM
7-4	8-12 months	Breeder 4	M	26	SM-FM

6-2	6-9 months	Breeder 3	M	27	SM-FM
11-3*	8-12 months	Breeder 4	M	20	[NONE; stimulus only]
11-1*	8-12 months	Breeder 4	F	45	[NONE; stimulus only]
B. Ball Pythons					
ID	Age @ testing	Source	Sex	Weight (g)	Trials
C2	2-2.5 years	Breeder 1	F	937	SM-M, SM-S, FM-F, S-F, SM-FM
C3	2-2.5 years	Breeder 1	F	984	SM-M, SM-S, FM-F, S-F, SM-FM
C4	2-2.5 years	Breeder 1	F	978	SM-M, SM-S, FM-F, S-F, SM-FM
C5	2-2.5 years	Breeder 1	F	1249	SM-S, FM-F, S-F, SM-FM
C6	2-2.5 years	Breeder 1	F	1107	SM-M, SM-S, SM-FM
D1	1-1.5 year	Breeder 2	F	729	SM-M, SM-S, FM-F, S-F
D2	1-1.5 year	Breeder 2	F	1148	SM-M, SM-S, FM-F, S-F, SM-FM
D3	1-1.5 year	Breeder 2	M	876	SM-M, SM-S, FM-F, S-F, SM-FM
D4	1-1.5 year	Breeder 2	M	1000	SM-M, SM-S, FM-F, S-F, SM-FM
D5	1-1.5 year	Breeder 2	M	931	SM-M, SM-S, FM-F, S-F, SM-FM
D6	1-1.5 year	Breeder 2	M	980	SM-M, FM-F, S-F, SM-FM
D7	1-1.5 year	Breeder 2	M	958	SM-M, SM-S, FM-F, S-F, SM-FM
E1	1-1.5 year	Breeder 2	M	849	SM-M, SM-S, FM-F, S-F, SM-FM
E2	1-1.5 year	Breeder 1	F	482	SM-M, SM-S, FM-F, S-F, SM-FM
E3	1-1.5 year	Breeder 1	F	965	SM-M, SM-S, FM-F, S-F, SM-FM
E4	1-1.5 year	Breeder 1	F	1039	SM-M, SM-S, FM-F, S-F, SM-FM
E5	1-1.5 year	Breeder 1	F	917	SM-M, SM-S, FM-F, S-F, SM-FM
E6	1-1.5 year	Breeder 1	F	1023	SM-S, FM-F, S-F, SM-FM
E7	1-1.5 year	Breeder 1	F	1030	SM-M, SM-S, FM-F, S-F, SM-FM

Table S2: Bayesian ANOVAs on total number of long (A) or short (B) tongue flicks, or total time spent near either stimulus (C) as a function of species and condition. The tables show, for each model considered, the Bayes Factor (BF) for that model compared to the null, and the Bayes Factor for inclusion (BF$_{incl}$) for each factor, similar to a main effect. The best model is highlighted.

A: long tongue flicks

Model	**BF**	**Main effect (BF$_{incl}$)**
Null (ID)	1	
Condition	0.35	0.236
Species	5.27	3.51
Species + Condition	1.72	
Species + Condition + Species*Condition	0.15	0.07

B: short tongue flicks

Model	**BF**	**Main effect (BF$_{incl}$)**
Null (ID)	1	
Condition	0.04	0.07
Species	0.54	0.424
Species + Condition	0.02	
Species + Condition + Species*Condition	0.1	0.25

C: time spent near stimulus

Model	**BF**	**Main effect (BF$_{incl}$)**
Null (ID)	1	
Condition	<0.001	<0.001
Species	0.011	0.008
Species + Condition	<0.001	
Species + Condition + Species*Condition	<0.001	<0.001

Table S3: effects of sex on long and short tongue flicks (TF) and time spent near the stimulus for gartersnakes (A) and ball pythons (B). For each measure in each condition, we conducted a Bayesian T-test comparing male to female snakes. We report the Bayes Factor (BF) for each comparison.

A: Gartersnakes

Condition	Long TF	Short TF	Time
SM vs. M	0.68	0.43	0.45
SM vs. S	0.41	0.41	0.41
SM vs. FM	0.51	0.46	0.47
FM vs. F	0.54	0.48	0.48
S vs. F	1.00	0.56	0.55

B: Ball pythons

Condition	Long TF	Short TF	Time
SM vs. M	0.45	0.59	0.49
SM vs. S	0.78	0.65	0.51
SM vs. FM	0.51	1.29	1.34
FM vs. F	0.50	0.53	0.48
S vs. F	0.64	0.46	0.51