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Sex-specific discrimination of familiar and unfamiliar mates in the Tokay gecko

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Abstract

Social animals need to keep track of other individuals in their group to be able to adjust their behaviour accordingly and facilitate group cohesion. This recognition ability varies across species and is influenced by cognitive capacities such as learning and memory. In reptiles, particularly Squamates (lizards, snakes, and worm lizards), pheromonal communication is pivotal for territoriality, reproduction, and other social interactions. However, the cognitive processes underlying these social interactions remain relatively understudied. In our study, we examined the ability of male and female Tokay geckos (*Gekko gecko*) to chemically differentiate familiar and unfamiliar mating partners. Our findings suggest that both sexes can make this distinction, with males responding stronger to the odour of a familiar mate, and females responding more to unfamiliar mates. The lizards maintained their discriminatory abilities for two to three weeks but not up to six weeks after separation, indicating a potential involvement of memory. This research highlights the efficacy of using pheromones as social stimuli for investigating social cognition in lizards, a promising avenue to better understand social cognition in these animals.

Key words: mate choice, pheromones, recognition, Squamata, tongue flick, vomerolfaction

Introduction

The ability to recognise individuals is most important for social animals in order to adjust their behaviour appropriately in recurring encounters depending on the identity of the conspecific (e.g. kin, group or not a member, familiar/potential new mating partner or competitor; Yorzinski 2017) and the context (e.g. parental care, courtship or territory defence; Edward 2015; Yorzinski 2017). Individuals of highly social species that live in complex group structures might be able to recognize and differentiate single individuals (e.g. Bull et al. 2000; Sayigh et al. 1999), which is expected to facilitate group cohesion (Rios and Kraenkel 2017). In turn, individuals from other, less social species, might only be able to discriminate categories of individuals such as familiar versus unfamiliar (Yorzinski 2017). Variation in the ability to recognise individuals across taxa is caused by variation in the underlying cognitive capacity, such as learning or memory (Yorzinski 2017). These capacities have been largely tested and confirmed in mammals (e.g. Gilfillan et al. 2016; Proops et al. 2009), but information in other taxa are more scarce (Houck 2009).

Depending on the species, the cues used to discriminate between individuals can involve visual signals (e.g. Parr et al. 2000), acoustic signals (e.g. Miller 1979; Nichols and Yorzinski 2015; Warrington et al. 2015), olfactory signals such as pheromones (e.g. Kaur et al. 2014; Keller et al. 2009; Péron et al. 2014), or combinations of different sensory modalities (e.g. Gilfillan et al. 2016; Proops et al. 2009). Across taxa, pheromones are used for social communication (Keller et al. 2009). For example, in the crayfish *Orconectes limosus*, juveniles chemically discriminate their mothers from unfamiliar females, preferentially associate with them and females are less likely to prey on their own offspring (Mathews 2011). Furthermore, zebra finch chicks (*Taeniopygia guttata*) also chemically discriminate their parents from unfamiliar adults and beg more towards chemicals originating from their parents (Caspers et al. 2017). Moreover, male but not female *Trogonophis wiegmanni* discriminate sex based on chemical cues, and both sexes discriminate a familiar from an unfamiliar mating partner (Martin et al. 2020). Research in several mammalian species demonstrated that the main olfactory system is involved in the recognition of mates (Bakker 2003; Keller et al. 2009), and the vomeronasal system is the main pathway for chemical recognition in reptiles (Martín and López 2011; Mason 1992). Therefore, the olfactory system is the prominent pathway involved in chemical communication across taxa.

Reptiles strongly depend on their chemosensory perception, and therefore, have a highly developed vomeronasal system (Martín and López 2011; Mason 1992; Scott et al. 2015). Chemoreception, comprising pheromones, is deeply involved in reptile territoriality, reproduction, recognition of individuals, choice of partner, and social communication (Cooper 1994; Martín and López 2011; Mason 1992). Pheromones are produced by the femoral and precloacal glands (Cooper and Burghardt 1990; Houck 2009; Martín and López 2011; Mason 1992; Weldon et al. 2008). To perceive these pheromones, reptiles, specifically Squamates (lizards, snakes and worm lizards), are known to perform tongue flicks (Cooper 1994). A tongue flick is a protrusion of the tongue out of the mouth to act as a sampling instrument for the collection of volatile and non-volatile compounds (Cooper 1994). Using the tongue flick quantification method, studies have shown that some lizards species can discriminate their own odour from that of their conspecifics (e.g. Alberts 1992; Aragón et al. 2001; Cooper et al. 1999; Mangiacotti et al. 2020; Moreira et al. 2006; Szabo and Ringler 2023), familiar from unfamiliar conspecifics (e.g. Aragón et al. 2001; Font and Desfilis 2002), kin from non-kin (e.g. Bull et al. 1994, 2000; Main and Bull 1996) and can even discern dominance status (e.g. Moreira et al. 2006). Even though these studies have already demonstrated the capacities of different lizard species to use chemicals for social communication, so far, we know little about the levels of discrimination that different species are exhibiting (e.g. own group versus foreign group, kin versus non-kin, parent-offspring, familiar-unfamiliar). Furthermore, different levels of discrimination ability as well as memory might be adaptive depending on the level of sociality (e.g. short term versus long term groups) or mating system (e.g. monogamy versus polygamy) across species.

In this study, our aim was to investigate whether Tokay geckos (*Gekko gecko*), a social lizard species, can discriminate the odour of a familiar from an unfamiliar mate. Furthermore, we wanted to know for how long individuals are able to discriminate, and if there are differences between males and females. To this end, we presented lizards with five stimuli: a blank control (untreated filter paper), pungency control (peppermint essential oil), their own odour, the odour of a familiar mate, and the odour of an unfamiliar, potential new mate. To assess how discrimination ability changes over time, we presented lizard with the stimuli four times across four months.

Male Tokay geckos are territorial and produce calls to defend their territory, but also to attract females (Grossmann 2007). Males and females stay together for at least one breeding season which lasts about four months (Nijman and Shepherd 2015). We, therefore, expected that geckos could discriminate between familiar and unfamiliar mates using pheromones. However, we expected a difference between the sexes that could be due to the fact that males are more territorial, and females can store sperm from different males up to three to four months (personal observation). Furthermore, we expected that geckos would show more tongue-flicks towards stimuli from unfamiliar mates, because mating with a new partner would increase their fitness; for example, if there are benefits of polygamy (i.e. having many mating partners). We also expected that individuals would produce more tongue flicks toward social stimuli than controls, and that they would show lower responses towards their own odour, because it is most familiar. Finally, we expected that the geckos' ability to discriminate would decrease over time: (1) If the mechanism underlying recognition is habituation (short-term change that at least partially reverts back to its original state after a certain period of time with no stimulation; Thorpe 1963; Rankin et al. 2009), we expected a fast decrease within the first two weeks (e.g. Glaudas 2004; Herzog et al. 1989). We expected to see a very low level of responses already in the first session (after 2 weeks of separation), because habituation would decline rapidly when no longer exposed to the odour of a familiar mate. (2) If the mechanism is learning and memory, we expected a slow decrease with individuals potentially still able to discriminate after four months.

Methods

Animals, captive conditions and husbandry

14 adult, naïve, captive bred Tokay geckos were tested in this study: 7 males (Snout vent length range across the testing period = 14.62-16.01 cm), and 7 females (Snout vent length range across the testing period = 12.64-13.88 cm). All individuals were purchased from different breeders and were between 3 and 8 years old. Sexes were determined by looking at the presence (for males) or absence (for females) of femoral glands (Grossmann 2007). Lizards were kept in pairs from January 2022 to January 2023, and were separated two weeks prior to the start of the experiment.

Animals are housed singly in terraria with a bioactive set-up: females tanks measure 45 L × 45 B × 70 H cm and males tanks measure 90 L × 45 B × 100 H cm. Terraria are made of rigid foam plates with a net top and glass front doors. They are fitted with a compressed cork wall fixed to the back, cork branches cut in half hooked on the back (functioning as shelters), cork branches allowing lizards to climb, and life plants as enrichment. Each terrarium has a drainage layer of expanded clay, separated by a mosquito mesh from the soil placed on top (organic tropical forest soil; Dragon BIO-Ground). We spread sphagnum moss and autoclaved red oak leaves on the soil as shelter and food for the isopods and earth worms that decompose the faecal material of the lizards. Animals are kept across two rooms. All enclosures are organized on shelves with small enclosures on the top and large enclosures on the bottom. To simulate natural environmental conditions, the room environment is controlled by an automatic system. Animals are exposed to a reversed 12h:12h photo period (i.e. light from 6 pm to 6 am, dark from 6 am to 6 pm). The system imitates sunrise and sunset, which are accompanied by changes in temperature reaching approximately 25 °C during night and 31°C during day. In addition, an UVB light (Exo Terra Reptile UVB 100, 25 W) is provided on top of the terraria during the day. A red light (PHILIPS TL-D 36W/15 RED) invisible to geckos (Loew 1994) is kept on for 24h so as to enable experimenters to work with the lizards. Furthermore, lizards can thermoregulate to their optimal body temperature at any time due to a heat mat (TropicShop) attached to the right outer wall of each enclosure, which locally increases the temperature by 4-5 °C. Humidity is kept at 50 %, but every 12 hours, at 5pm and 4am, 30 seconds of rainfall (with reverse osmotic water) briefly increases humidity to 100%.

Animals are fed three times per week (Monday, Wednesday, Friday) with between three and five mealworms (*Tenebrio molitor*), cockroaches (*Nauphoeta cinerea*), or adult house crickets (*Acheta domesticus*). In order to provide optimal nutrition to our animals (vitamin D and calcium), the insects are fed with high protein dry cat food (various brands), cricket mix (reptile planet LDT), and fresh carrots. Each gecko is fed with 25 cm long forceps in order to control food intake. Fresh water is supplied ad libitum in a water bowl. Moreover, the geckos are weighted every month and measured (snout vent length) approximately every three months, to track their body condition.

Set-up

The experiment was conducted from 31st of January to 30th of May 2023. Lizards were tested in a testing tank (45 L x 45 B x 60 H cm; Exo Terra). As our animals are kept in two different rooms, we placed one testing tank in each room. These testing tanks were made of glass (with a mesh top), and covered with a black plastic film on three sides (leaving the front transparent for video recording). Tanks were placed in the middle of the rooms, on a table of 77 cm height, with the transparent front facing a wall at a distance of 100 cm. Two dim white lights (LED, SPYLUX® LEDVANCE 3000K, 0.3 W, 17 lm) were placed, one on top in the back right corner and one in the middle front, to allow video recording of lizard behaviour in high quality. A GoPro camera (Hero 8; wide mode, 4k resolution, 24 FPS) mounted on a tripod (95 cm height, 55 cm distance from the testing tank) was placed in front of the transparent side. The order in which individuals were tested within a day was randomly assigned, as well as the order of the stimuli they were tested with (but counterbalanced to ensure that the order was different each session but even across individuals each session). Each animal was tested once a day, on non-feeding days (Tuesday and Thursday), for five trials across 2.5 weeks (i.e. session, together 3 Tuesdays and 2 Thursdays), a total of four sessions with an inter-session interval of 19 days.

Procedure

First, the camera was fixed to the tripod in the room in which the focal individual was housed. Then, the filter paper (either an unused piece for the controls or taken from the enclosure of an individual) was taped to the middle of the back wall of the testing tank (centre of the paper at 21.75 cm from the top and 29.25 cm from the side walls). To make sure the position of the filter paper was always the same, the back wall was marked with a piece of tape. Next, the focal individual was caught in a transparent plastic container (22.8 L x 10.6 B x 7.2 H cm), and placed within the container inside the testing tank (in the middle, directly in front of the back wall). The individual was left alone for 5 minutes of acclimation after capture. Thereafter, the camera was turned on (activation of the preview), the plastic container’s lid was removed to allow the focal individual to explore the testing tank, and the testing tank’s doors locked. The experimenter then left the room and observed the focal individual live on a smartphone, using the preview of the GoPro quick app (version 11.16). The video recording was started as soon as the focal gecko showed its’ first tongue flick (TF) and lasted for 10 minutes thereafter (Supplementary video M1). If a lizard did not exit the plastic container within 10 minutes, the trial was considered as NA and ended (N = 7, NA trials were not repeated). At the end of the trial, the individual was caught in the same plastic container and released back into its enclosure. Before the next trial, the testing tank and the plastic container were cleaned using an ample amount of 70% ethanol and whipped dry with paper towels. Everything was left to dry for a minimum of 10 minutes to allow the alcohol to evaporate. The experimenter washed their hands with water and soap at the end of each trial in order to not contaminate other filter papers with odour remaining from previous trials.

Stimuli

Each animal was tested with five stimuli: the odour of a familiar mate (kept together and mating for one year but separated 2 weeks before the experiment started), the odour of an unfamiliar mate (potential new mate they had not mated with previously; a different individual was used each session), their own odour, no odour (C1 - paper control), and peppermint essential oil (farfalla AromaCare) odour (C2 - peppermint control). We included the paper control to make sure the responses of the lizards were consistent over time. We included the peppermint oil control to make sure novelty was not the cause of an increased response rate. All social stimuli were collected using a filter paper (Laboratory filter paper, 12.5 cm diameter, Betzold) pinned to the back wall within enclosures in the sleeping spot 1-5 days before a trial. Due to an error, in eight trials the filter paper was left inside an enclosure for only one day while in seven trials it was left for five days. In all other trials it was left for three days. To collect their own odour, we placed a filter paper in the enclosure of the focal lizard. To collect the odour of the familiar mate, we placed a filter paper in the enclosure of the focal individuals' familiar mate. Finally, to collect the odour of the unfamiliar mate, we placed a filter paper in the enclosure of a lizard located in the second room with which the focal individual had had no previous contact with. To create the pungency control, we spread peppermint oil onto an unused filter paper using a roll-on in four spots (top, bottom, left and right).

Data collection

The videos were scored blind as to the presented stimuli (Supplementary video M1). We used the Behavioural Observation Research Interactive Software (BORIS, Version 7.13.9.; Friard & Gamba, 2016) to score behaviours performed during trials. We scored two types of TF: (1) TF towards the stimulus (on the filter paper or within an area of one lizard head length around the filter paper), and (2) TF performed at any other location within the testing tank. We also scored the length of time the snout of the lizard was not visible (i.e. out of sight). This was necessary due to the lizards' arboreal lifestyle. When an individual was moving away from the camera the snout was obstructed by the lizards body during which time TF were not visible.

To gain an accurate measure of trial time in seconds, we subtracted the duration out of sight from the total video length (= trial time). We then calculated the relative number of TF by dividing the number of TF by the trial time, to gain a comparable measurement across individuals and trials. We did this for the TF towards the stimulus, the TF away from stimulus, and the total amount of TF. Videos could not be scored blind as to the individual ID. Therefore, approximately 30% of trials were watched by a second observer (80 of 280 trials) to calculate inter-observer reliability. We found very high correlation in latency scores across observers: $r_{stimulus} = 0.991$ (Pearson correlation, $CI_{low} = 0.985$, $CI_{up} = 0.994$,$t = 63.654$, $df = 77$, $p < 0.001$), $r_{other} = 0. 977$ (Pearson correlation, $CI_{low} = 0.964$, $CI_{up} = 0. 985$,$t = 39.988$, $df = 77$, $p < 0.001$), $r_{total} = 0.975$ (Pearson correlation, $CI_{low} = 0. 961$, $CI_{up} = 0. 984$,$t = 38.413$, $df = 77$, $p < 0.001$). As lizard behaviour can be affected by temperature, we also recorded room temperature every 15 minutes, with an accuracy of 0.1°C.

Ethical note

The experimental procedure used in this study was strictly non-invasive and followed the guidelines provided by the Association for the Study of Animal Behaviour/ Animal Behaviour Society for the treatment of animals in behavioural research and Teaching (2023). Experiments were approved by the Suisse Federal Food Safety and Veterinary Office (National No. 33232, Cantonal No. BE144/2020). Captive conditions were approved by the Suisse Federal Food Safety and Veterinary Office (Laboratory animal husbandry license: No. BE4/11).

Statistical analyses

To understand if lizards could discriminate familiar from unfamiliar mates, if there was a sex difference in this ability, and how long this discrimination ability lasted we ran one model. We used the relative number of TF towards the stimulus as the response variable in a censored Bayesian generalised linear mixed model with Gaussian distribution (GLMM, package *brms*;

Bürkner 2017, 2018, 2021). We only analysed TF towards the stimulus as we were not sure if other TF and total TF were impacted by other behaviours such as exploration of novel space. Trials in which a lizard did not visit the back wall on which the stimulus was presented were coded as censored (= 1; N = 34 out of 280 trials) as we assumed that if trials were run longer than 10 minutes lizards would have eventually visited the filter paper. As fixed effects we included (1) stimulus in interaction with sex to understand if there was a stimulus-specific sex difference in TF, (2) stimulus in interaction with session to understand if there was a stimulus specific change in responses over time and (3) temperature to account for differences in responses due to the ectothermic nature of lizards. Additionally, we included animal identity as the random effect to account for repeated measures. We made sure that model Rhat was 1, that the ESS was above 2000 and checked the density plots and correlation plots to ensure that the models had sampled appropriately. We used a diffuse normal prior with a mean of 0 and a standard deviation of 1 and ran 4 chains per model of 5000 iterations each. To investigate the results of interactions we used *post hoc* least square means comparisons (EMM) from the package *emmeans* (Lenth 2023). Finally, we were also interested to see if individuals were consistent in their response to these different stimuli over time while showing distinct differences from each other. We calculated adjusted repeatability of the relative stimulus directed TF adjusting for stimulus using the package *rptR* (Stoffel et al. 2017). All statistical analyses were run in R version 4.2.2 (R Core Team, 2022). We assumed statistical significance if the 95% confidence interval did not cross 0.

Results

We found evidence that both males and females were able to discriminate familiar from unfamiliar mates; however, in a sex specific manner (Figure 1; electronic supplementary material Table S1). Females produced more TF towards the odour of an unfamiliar mate compared to all other stimuli presented (EMM, Figure 1; Table 1). We found no evidence of a difference between any other presented stimuli in females (Figure 1; Table 1).

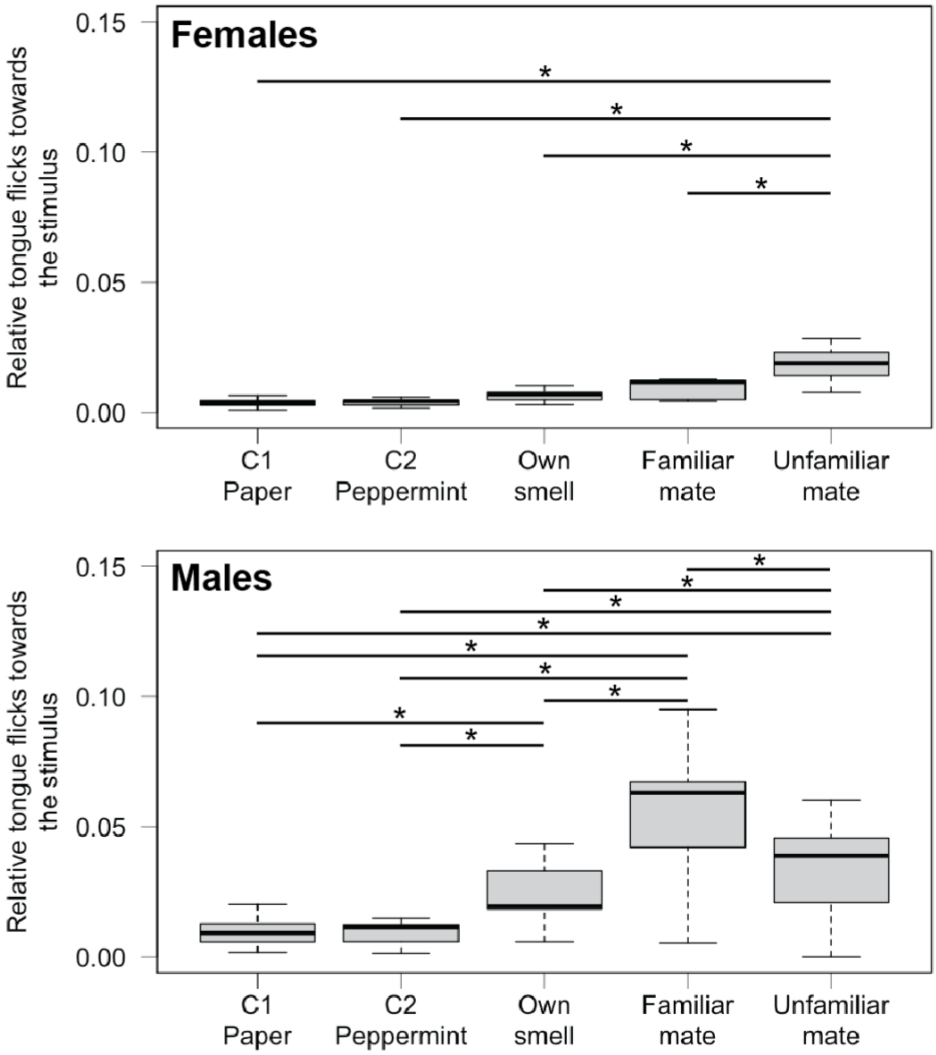

Fig. 1 Relative number of TF towards the different stimuli split across females (top) and males (bottom). The bold line within the boxes shows the median, the upper box edges show the upper quartile, the lower box edges the lower quartile, the top whisker ends show the maximum and the bottom whisker ends the minimum (outliers are not shown). * significant difference (confidence interval not crossing 0).

We found evidence that males produced more TF towards social odours (their own odour, the odour of a familiar and unfamiliar mate) compared to both controls (EMM, Figure 1; Table 1). Males also showed less TF towards their own odour compared to the odour of a familiar (EMM, Figure 1; Table 1) or unfamiliar mate (EMM, Figure 1; Table 1) and showed the most TF towards the odour of their familiar mate (EMM, Figure 1; Table 1). Males produced more TF in response to social stimuli but not in response to the controls than females (Figure 1; electronic supplementary material Table S2).

Table 1 Estimates and test statistics comparing the relative number of tongue flicks directed towards the different stimuli within males and females separately. Significant results (95% confidence interval – CI – not crossing 0) are highlighted in bold. HPD – Higher Posterior Density, C1 – blank paper control, C2 – peppermint control.

FEMALES			
Difference	Estimate	Lower HPD Interval	Upper HPD Interval
C1 – C2	-0.0006	-0.0107	0.0105
C1 – own odour	-0.0028	-0.0137	0.0079
C1 – familiar mate	-0.0050	-0.0160	0.0059
C1 – unfamiliar mate	**-0.0161**	**-0.0274**	**-0.0054**
C2 – own odour	-0.0022	-0.0125	0.0083
C2 – familiar mate	-0.0045	-0.0148	0.0063
C2 – unfamiliar mate	**-0.0156**	**-0.0261**	**-0.0050**
Own smell – familiar mate	-0.0021	-0.0125	0.0084
Own odour – unfamiliar mate	**-0.0133**	**-0.0238**	**-0.0029**
Familiar mate – unfamiliar mate	**-0.0111**	**-0.0223**	**-0.0007**
MALES			
C1 – C2	-0.0003	-0.0104	0.0105
C1 – own odour	**-0.0170**	**-0.0276**	**-0.0062**
C1 – familiar mate	**-0.0444**	**-0.0551**	**-0.0341**
C1 – unfamiliar mate	**-0.0289**	**-0.0398**	**-0.0182**
C2 – own odour	**-0.0166**	**-0.0270**	**-0.0058**
C2 – familiar mate	**-0.0440**	**-0.0547**	**-0.0340**
C2 – unfamiliar mate	**-0.0286**	**-0.0397**	**-0.0179**
Own odour – familiar mate	**-0.0274**	**-0.0379**	**-0.0170**
Own odour – unfamiliar mate	**-0.0120**	**-0.0234**	**-0.0010**
Familiar mate – unfamiliar mate	**0.0515**	**0.0047**	**0.0265**

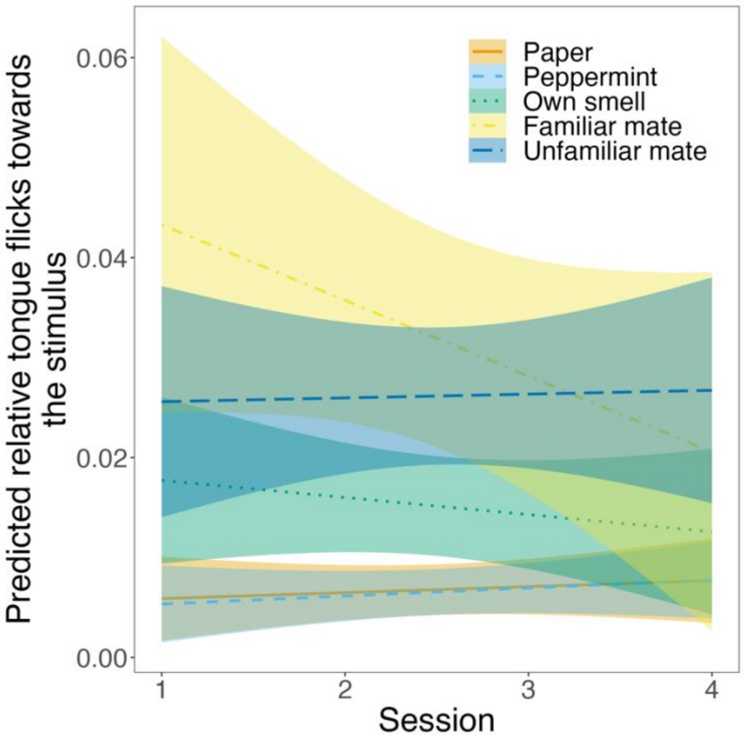

Fig. 2 Change in the relative number of TF towards the different stimuli across sessions (1-4). The results indicate a significant decrease in TF between trial 1 and 4 in the familiar mate condition only.

We found evidence that responses decreased across the four sessions only towards the odour of a familiar mate but not towards the other stimuli (Figure 2; electronic supplementary material Table S3). Lizards already decreased responses from session 1 to session 2 (EMM, estimate = 0.019, credible interval = 0.003 – 0.034) but not from session 2 to session 3 (EMM, estimate = -0.003, credible interval = -0.017 – 0.012) or from session 3 to session 4 (EMM, estimate = 0.011, credible interval = -0.003 – 0.026). We detected the biggest difference when comparing session 1 to session 4 (EMM, estimate = 0.027, credible interval = 0.011 – 0.042). Discriminability (the difference in responses towards the familiar versus the unfamiliar mates odour) also decreased over sessions. While lizards still discriminated in session 1 (EMM, estimate = 0.020, credible interval = 0.004 – 0.036; Figure 3; electronic supplementary material Table S4), this was not the case in session 2 (EMM, estimate = -0.006, credible interval = -0.021 – 0.009), session 3 (EMM, estimate = 0.006, credible interval

= -0.009 – 0.022) or session 4 (EMM, estimate = -0.011, credible interval = -0.026 – 0.003). Additionally, we found no effect of temperature on lizard behaviour (GLMM, estimate = -0.0008, credible interval = -0.005 – 0.003, electronic supplementary material Table S1). Finally, we found lizards relative stimulus directed TF to be repeatable at R = 0.258 (confidence interval = 0.087 – 0.412).

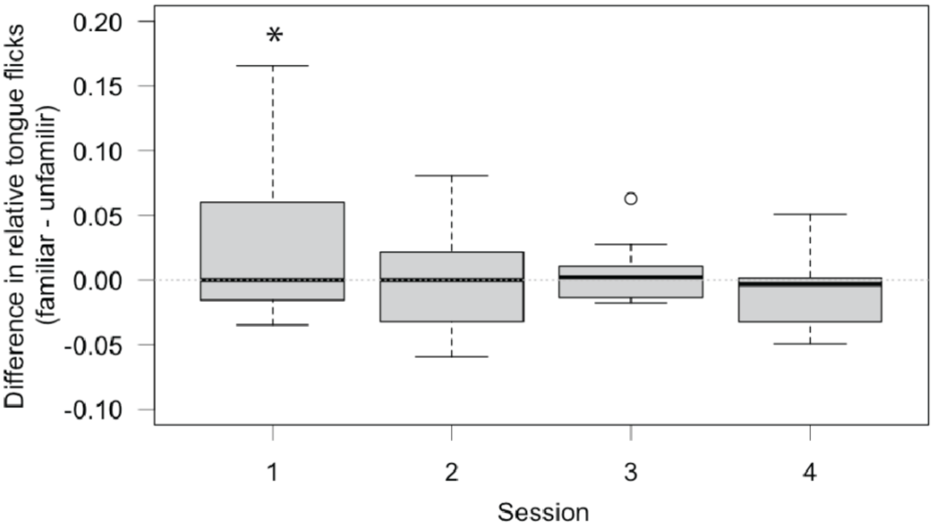

Fig. 3 Difference in the relative number of TF directed towards the odour of a familiar mate in comparison to the odour of an unfamiliar mate. Positive numbers indicate more TF directed towards the odour of a familiar mate. The bold line within the boxes shows the median, the upper box edges show the upper quartile, the lower box edges the lower quartile, the top whisker ends show the maximum and the bottom whisker ends the minimum (outliers are not shown). The dotted line indicates no difference. * significant difference (confidence interval not crossing 0).

Discussion

The results of our study show that both male and female Tokay geckos can discriminate between a familiar and unfamiliar mate. However, males directed more TF toward the odour of a familiar compared to an unfamiliar mate, while females directed more TF towards the unfamiliar mate. Females, overall, did respond less to all of the social stimuli than males.

However, both sexes directed more TF towards social stimuli (familiar, unfamiliar and their own odour) compared to the controls (paper only and peppermint oil). Furthermore, TF towards the familiar mate only, decreased across the four months testing period with significant discriminability between the familiar and unfamiliar mate vanishing approximately six weeks into the experiment. Finally, lizards TF behaviour was repeatable at R = 0.258.

We predicted that geckos could use pheromones to discriminate between familiar and unfamiliar mates and that they would show more TF towards stimuli from unfamiliar mates. Furthermore, we expected a difference between the sexes due to male territoriality and females' ability to store sperm. Our results support most of these predictions. Both males and females discriminated between the odour of a familiar and unfamiliar mate. Overall, males produced more TF towards social stimuli (their own odour, the odour of a familiar and unfamiliar mate) than females. Males directed more TF towards the odour of a familiar mate, while females directed more TF towards the odour of an unfamiliar, potential new mate. The reason for this sex difference could be due to sex specific mating strategies. Female's ability to store sperm points towards multiple mating each breeding season before or even after they pair up with a male. Males, on the other hand, are territorial and attract females through advertisement calls (Grossmann 2007). Mating, egg deposition as well as parental care are performed in the male's territory (Grossmann 2007). Males might, therefore, have a greater need to discriminate familiar from unfamiliar females. Recognition of the familiar mate might be highly beneficial, especially in the context of parental care (O'Connor and Shine 2004), as unrelated females are a threat to the eggs and offspring (Grossmann 2007) and should, therefore, be guarded against. A study on mate choice in female leopard geckos (*Eublepharis macularius*) did not find a clear preference by females for familiar or novel males (La Dage and Ferkin 2007). Currently, we lack information about Tokay geckos mating system, details about the benefits of parental care and we have no data on mate choice available. It is, therefore, not possible to interpret the difference in responses between the sexes as a preference for familiar/ unfamiliar mates. Hence, more research into these topics is needed. Additionally, the lower response rate of females might be related to issues with the method of collecting the pheromones. Males' enclosures were bigger and, although we placed the filter papers in their sleeping spot, males might have deposited less pheromones onto the papers compared to females in smaller enclosures. In the future, it would be better to place filter paper in a small box together with the individual for a set amount of time to control how much pheromone is deposited.

We also predicted that geckos would show greater responses toward social stimuli, rather than towards the controls, and among social stimuli, less responses towards their own odour, all of which is supported by our results, but most prominently in males. This is likely linked to the information content of the different stimuli, with controls providing the least information, their own odour being very familiar as part of their environment providing no new information (Szabo and Ringler 2023), and the social stimuli giving the most information about potential mating partners. The low response rate towards the peppermint oil control also rules out that novelty was a factor influencing response rates but rather social information encoded in the different social pheromone stimuli.

Our last prediction was that discrimination would decrease over time. We predicated a fast decrease if habituation was the main mechanism, while we predicted a slow decrease if learning and memory were involved. Our results show that the response rate towards the odour of the familiar mate decreased over the four month period with the difference in response towards the familiar and unfamiliar mate being significant only in the first test session conducted after two to three weeks of separation. This difference was not detectable in the second session another two to three weeks later. Previously, blackbelly garter snakes (*Thamnophis melanogaster*) and cottonmouths (*Agkistrodon piscivorus*) habituated to a threatening stimulus over five days, but showed a recovery of anti-predator behaviour after an average of 14 days with no stimulation (Glaudas 2004; Herzog et al. 1989). Based on these findings, our results point more towards learning and memory, not habituation, as the mechanism for discrimination of familiar versus unfamiliar mates, but habituation cannot fully be ruled out as of yet. Importantly, our results make ecological sense if we assume that Tokay geckos have a polygamous mating system, in which it is beneficial to mate with multiple mating partners. Depending on the exact point in the breeding cycle, both the ability to discriminate or not might be beneficial in maximising mating partners, especially for females (the moving sex). Being able to discriminate might be beneficial to avoid mating with the same individual multiple times if enough mating partners are available. However, if the pool becomes limited, then not differentiating between familiar and new partners could be more beneficial. However, further studies are needed, especially in the wild, to better understand Tokay gecko mating strategies and the associated cognitive abilities.

In summary, we found that Tokay geckos can discriminate familiar from unfamiliar mates but males direct more TF towards the odour of a familiar while females direct more TF towards the odour of an unfamiliar mate. Furthermore, only responses towards the odour of a familiar mate decreased across time. Further research should be done to understand if this discrimination ability is individual specific or categorial. Additionally, studies in the wild are needed to be able to fully interpret the results of our study. Our method is suitable to study chemical recognition in Tokay geckos, but some aspects of the methodology still need to be improved for future investigations into, for example, kin recognition. Interestingly, we find that geckos TF responses are repeatable, showing that studies looking at TF rate should use a within-individual design to account for differences in TF rate across individuals. Similar to previous work (Szabo and Ringler 2023), we showed that using pheromones as social stimuli is a good tool to investigate social cognition in this species. Overall, our results provide further evidence that lizards are suitable models to investigate chemical communication, especially in a social context.

Statements and Declarations

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Author contribution: BS - Conceptualization; BS - Data curation; BS - Formal analysis; BS - Funding acquisition; MOV, MD, BS - Investigation; MD, BS - Methodology; BS - Project administration; BS - Resources; BS - Validation; BS - Visualization; MOV, MD, ER, BS - Roles/Writing - original draft; MOV, MD, ER, BS - Writing - review & editing.

Data availability statement: Data generated during this study are available for download from the Open Science Framework (OSF, link for review purposes: https://osf.io/7ecxq/?view_only=f9bf5f69b55e4239b520c0d7449f504b).

Code availability statement: All code generated to analyse the collected data is available for download from the Open Science Framework (OSF, link for review purposes: https://osf.io/7ecxq/?view_only=f9bf5f69b55e4239b520c0d7449f504b).

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Supplementary tables

Table S1. Estimates and test statistics from the censored Bayesian generalised linear model looking at stimulus, sex, session and temperature effects on the relative number of tongue flicks towards the stimulus. Significant results (95% confidence interval – CI – not crossing 0) are highlighted in bold.

Parameter	Estimate	Estimated error	95% lower Credible interval	95% upper Credible interval
Intercept	0.02613	0.05491	-0.08190	0.13330
C2 – peppermint control	0.00002	0.00846	-0.01626	0.01649
Own odour	0.01020	0.00865	-0.00679	0.02736
Familiar mate	**0.02158**	**0.00881**	**0.00451**	**0.03891**
Unfamiliar mate	0.01509	0.00893	-0.00235	0.03221
Male	0.00561	0.00666	-0.00753	0.01863
Session 2	0.00638	0.00793	-0.00914	0.02191
Session 3	-0.00004	0.00793	-0.01556	0.01551
Session 4	0.00049	0.00797	-0.01524	0.01607
Temperature	-0.00081	0.00209	-0.00492	0.00331
Interactions				
C2 - male	-0.00005	0.00769	-0.01516	0.01513
Own odour - male	0.01438	0.00775	-0.00078	0.02963
Familiar mate - male	**0.02971**	**0.00764**	**0.02457**	**0.05458**
Unfamiliar mate - male	0.01300	0.00793	-0.00229	0.02855
C2 – session 2	-0.00771	0.01067	-0.02819	0.01345
Own odour – session 2	-0.00940	0.01117	-0.03141	0.01248
Familiar mate – session 2	**-0.02464**	**0.01091**	**-0.04604**	**-0.00350**
Unfamiliar mate – session 2	0.00151	0.01123	-0.02026	0.02393
C2 – session 3	0.00866	0.01100	-0.01290	0.03008
Own odour – session 3	-0.01306	0.01082	-0.03434	0.00811
Familiar mate – session 3	-0.02523	0.01115	-0.03753	0.00624
Unfamiliar mate – session 3	-0.00155	0.01158	-0.02418	0.02138
C2 – session 4	0.00099	0.01056	-0.01953	0.02152
Own odour – session 4	-0.00747	0.01074	-0.02840	0.01373
Familiar mate – session 4	**-0.02719**	**0.01073**	**-0.04800**	**-0.00611**
Unfamiliar mate – session 4	0.00384	0.01108	-0.01791	0.02576

Table S2. Estimates and test statistics comparing the relative number of tongue flicks directed towards the different stimuli between the sexes. Significant results (95% confidence interval – CI – not crossing 0) are highlighted in bold. HPD – Higher Posterior Density.

C1 – blank paper control			
Difference	Estimate	Lower HPD Interval	Upper HPD Interval
Female - male	-0.0056	-0.0189	0.0072
C2 – peppermint control			

Difference	Estimate	Lower HPD Interval	Upper HPD Interval
Female - male	-0.0056	-0.0187	0.0068
Own smell			
Difference	Estimate	Lower HPD Interval	Upper HPD Interval
Female - male	**-0.0201**	**-0.0329**	**-0.0070**
Familiar mate			
Difference	Estimate	Lower HPD Interval	Upper HPD Interval
Female - male	**-0.0454**	**-0.0583**	**-0.0322**
Unfamiliar mate			
Difference	Estimate	Lower HPD Interval	Upper HPD Interval
Female - male	**-0.0186**	**-0.0318**	**-0.0056**

Table S3. Estimates and test statistics comparing the relative number of tongue flicks directed towards the different stimuli across sessions. Significant results (95% confidence interval – CI – not crossing 0) are highlighted in bold. HPD – Higher Posterior Density.

C1 – blank paper control			
Difference	Estimate	Lower HPD Interval	Upper HPD Interval
Session 1 – 2	-0.0063	-0.0219	0.0095
Session 1 – 3	-0.0001	-0.0153	0.0156
Session 1 – 4	-0.0005	-0.0164	0.0150
Session 2 – 3	0.0063	-0.0095	0.0216
Session 2 – 4	0.0059	-0.0085	0.0212
Session 3 – 4	-0.0003	-0.0153	0.0159
C2 – peppermint control			
Difference	Estimate	Lower HPD Interval	Upper HPD Interval
Session 1 – 2	0.0015	-0.0134	0.0164
Session 1 – 3	-0.0085	-0.0235	0.0068
Session 1 – 4	-0.0015	-0.0171	0.0143
Session 2 – 3	-0.0099	-0.0242	0.0048
Session 2 – 4	-0.0029	-0.0170	0.0116
Session 3 – 4	0.0070	-0.0081	0.0224
Own smell			
Difference	Estimate	Lower HPD Interval	Upper HPD Interval
Session 1 – 2	0.0029	-0.0134	0.0187
Session 1 – 3	0.0130	-0.0012	0.0278
Session 1 – 4	0.0068	-0.0084	0.0225
Session 2 – 3	0.0101	-0.0043	0.0263
Session 2 – 4	0.0039	-0.0119	0.0193
Session 3 – 4	-0.0061	-0.0205	0.0088
Familiar mate			
Difference	Estimate	Lower HPD Interval	Upper HPD Interval
Session 1 – 2	**0.0186**	**0.0030**	**0.0341**
Session 1 – 3	0.0154	-0.0005	0.0306
Session 1 – 4	**0.0267**	**0.0108**	**0.0418**
Session 2 – 3	-0.0032	-0.0172	0.0115
Session 2 – 4	0.0082	-0.0060	0.0226
Session 3 – 4	0.0113	-0.0028	0.0259
Unfamiliar mate			
Difference	Estimate	Lower HPD Interval	Upper HPD Interval

Session 1 – 2	-0.0078	-0.0236	0.0085
Session 1 – 3	0.0015	-0.0146	0.0182
Session 1 – 4	-0.0042	-0.0213	0.0125
Session 2 – 3	0.0094	-0.0067	0.0243
Session 2 – 4	0.0035	-0.0117	0.0185
Session 3 – 4	-0.0058	-0.0206	0.0103

Table S4. Estimates and test statistics comparing the relative number of tongue flicks directed towards the odour of a familiar compared to an unfamiliar mate within sessions. Significant results (95% confidence interval – CI – not crossing 0) are highlighted in bold. HPD – Higher Posterior Density.

Difference	Estimate	Lower HPD Interval	Upper HPD Interval
Session 1: familiar - unfamiliar	**0.0198**	**0.0042**	**0.0357**
Session 2: familiar - unfamiliar	-0.0063	-0.0209	0.0088
Session 3: familiar - unfamiliar	0.0062	-0.0092	0.0219
Session 4: familiar - unfamiliar	-0.0111	-0.0261	0.0031