First evidence towards chemical self-recognition in a gecko

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21 **Abstract**

22 Self-recognition is the ability to recognise stimuli originating from oneself. Humans and most

23 great apes show evidence of true self-recognition in the mirror test. They use their reflection

24 to remove a mark that is only visible in the mirror. Not all animals, however, rely primarily on

25 vision. In lizards, chemical cues are important in social interactions. A number of lizard species

26 show chemical self-recognition but it has never been investigated in a gecko species. Here,

27 we test the tokay gecko (*Gekko gecko*) a territorial species with parental care on their ability

28 to discriminate their own skin and faecal chemicals from those of same-sex, unfamiliar

29 conspecifics. Geckos show a higher response rate towards chemicals from unfamiliar

30 individuals compared to self-produced chemicals and a water control. Lizards showed self-

31 directed behaviour, responded stronger to skin chemicals and females responded more than

32 males. Our study provides first evidence towards self-recognition and for a social function of

33 chemical present on faeces in tokay geckos but further tests are needed to confirm true self-

34 recognition. Tokay geckos are an excellent model species to investigate individual recognition

35 to demonstrate more sophisticated social cognitive abilities than have previously been

36 attributed to reptiles.

37

38 *Keywords:* chemical discrimination, cognition, communication, *Gekko gecko*, reptile,

39

40

Introduction

41

42   Self-recognition is the ability to recognise cues that represent/ originate from oneself (visual

43   images, olfactory cues, acoustic stimuli) (Gallup, 1970; Gallup et al., 2011; Platek et al., 2004).

44   Research into self-recognition aims to uncover self-awareness (the ability to become the

45   object of one's own attention) and its' emergence across humans and non-human animals

46   (Gallup, 1970; Gallup et al., 2011). The method of choice is the mirror self-recognition (MSR)

47   test. In this test, a subject is confronted with a mirror and provided with a mark that can only

48   be seen using the reflection in the mirror. Confirmation of MSR occurs when the subject

49   inspects the mark and attempts to remove it using their reflection (Gallup, 1970). Two

50   important control conditions need to be implemented. First, an invisible mark has to be used

51   to exclude that physical irritation caused by the mark itself or the process of marking is

52   triggering the behaviour (Gallup, 1970). Second, a mark has to be applied in a spot that can

53   be seen without the use of the mirror to confirm the subjects' motivation to remove marks in

54   general (Gallup and Anderson, 2018). Humans and most great apes show MSR (Gallup, 1970;

55   Gallup et al., 2011) while evidence in other species has led to controversial discussion (Gallup

56   and Anderson, 2018; 2020).

57   Not all species primarily depend on their visual sense. This has led to the development

58   of the sniff-test for self-recognition used in dogs whose primary sense is smell (Cazzolla Gatti,

59   2016). These studies have demonstrated that dogs discriminate between their own odour and

60   that of conspecifics. They sniff the urine of unfamiliar dogs for longer than their own urine

61   (Cazzolla Gatti, 2016; Horowitz, 2017). Furthermore, they sniff their own odour longer when it

62   is modified than the modifier by itself (Horowitz, 2017). Nonetheless, some researchers have

63   criticised these studies as not being a true equivalent to the MSR test because dogs do not

64   show self-directed behaviour in the sniff-test which is an important criterion in the MSR test

65   (Gallup and Anderson, 2018). Interestingly, chemical self-recognition tests are a fairly

66   common test in lizards (Aguilar et al., 2009; Alberts, 1992; Aragón et al., 2001; Bull et al.,

67   2000; Cooper, 1999; Graves and Halpern, 1991; López et al., 1997). Particularly one study in

68   male desert iguanas (*Dipsosaurus dorsalis*) showed that these animals perform self-directed

69   behaviour after detection of their own femoral gland sections but did not show this behaviour

70   towards the secretions of unfamiliar males (Alberts, 1992). Following the critique voiced

71   regarding the results in dogs, this study demonstrates more conclusive evidence for self-

72   recognition using chemicals in lizards.

73   Generally, reptiles rely strongly on chemicals (i.e. pheromones) when it comes to

74   individual recognition, territoriality, social interactions and mate choice (Norris and Lopez,

75   2001). In lizards, pheromones might originate from the skin or specialised glands such as

76   femoral glands which are most pronounced in males (Norris and Lopez, 2001). Many species

77   also possess cloacal glands that deposit pheromones onto the faeces (Norris and Lopez,

78   2001). This is especially important in scat piling lizards which defecate repeatedly in the same

79   location (Bull et al., 1999a). Similar to latrines in mammals (e.g. Green et al., 2015; King et

80   al., 2017), these scat piles can have a social function by communicating, for example, territory

81   ownership (Bull et al., 1999a; 1999b) and group identity (Bull et al., 2000; but see Shah et al.,

82   2006). Lizards detect pheromones using tongue-flicks (TF), protrusions of the tongue forward

83   towards a stimulus (e.g. on the ground or on a swab) to collect chemicals (Cooper, 1994), and

84   generally show increased TF rates towards stimuli from unfamiliar conspecifics (e.g. Alberts,

85   1992; Aragón et al., 2001; Cooper et al., 1999; Graves and Halpern, 1991).

86   Discrimination of self-produced chemicals and chemicals produced by unfamiliar,

87   same-sex conspecifics has never been shown in a gecko species although leopard geckos

88   (*Eublepharis macularius*) discriminate sex based on pheromones (Cooper and Steele, 1997;

89   Mason and Gutzke, 1990) and thick-tailed geckos (*Nephrurus milii*) recognise their own scats

90   to add additional faecal matter (Carpenter and Duvall, 1995). Many gecko species scat pile

91   which suggests either a communicative function aimed at conspecifics, an anti-predatory

92   function to avoid detection of refuges or both (Bull et al., 1999a; Carpenter and Duvall, 1995).

93   Here, we test the tokay gecko (*Gekko gecko*), a large (up to 185 mm Snout Vent Length),

94   nocturnal, insectivorous, scat piling gecko species from tropical South-East Asia (Grossmann,

95   2006). The aims of this study were to

96   (1) investigate if tokay geckos can discriminate between self-produced chemicals and

97   chemicals produced by unfamiliar, same-sex conspecifics on cotton swabs

98   (Cooper, 1998).

99   (2) We were also interested in finding out if chemicals originating from scats were as

100   effective as chemicals originating from the skin as stimuli.

101   We predicted, that if geckos are able to recognize their own odour they would show

102   lower responses towards their own odour than the conspecific odour (e.g. Alberts, 1992;

103   Cooper et al., 1999; Graves and Halpern, 1991). If geckos are capable of self-recognition, we

104   expected to find both stimulus directed and ground directed TFs as a sign of comparison

105   between the two stimuli when confronted with their own and unfamiliar conspecific odour. We

106   predicted, however, less ground directed responses when confronted with their own odour as

107   it is familiar and can be recognised easier. We also predicted that faecal chemicals were as

108   effective as skin chemicals in eliciting a response if scats had a communicative function.

109

Methods

111   Study animals, housing and husbandry

112   We tested 22 captive bred, adult tokay geckos, 10 males (SVL range = 11.35-15.02 cm) and

113   12 females (SVL range = 11.29-13.72 cm). Animals were acquired from different breeders

114   across Europe and approximately 2-6 years old at the time of the study. Animals were naïve

115   to the experimental procedure used in this study.

116   At our facility, geckos are kept singly in plastic terraria (females – 45 L x 45 B x 70 H

117   cm; males – 90 L x 45 B x 100 H cm). Enclosures are equipped with a drainage layer of clay

118   pebbles and a layer of organic rainforest soil (Dragon BIO-Ground) on top separated by a

119   mosquito mesh to prevent mixing of the layers. On the soil surface we spread autoclaved red

120   oak leaves. Collembola, isopods and earth worms in the soil break down the faecal matter

121   produced by the geckos. Each enclosure also includes a compressed cork back wall, cork

122   branches, refuges made out of cork branches cut in half and hung on the back wall as well as

123   plants.

124   Enclosures are located in a fully controlled environment with a reversed photo period.

125   Because tokay geckos are nocturnal, the dark cycle (when geckos are active) lasts from 6am

126   to 6pm while the light period (when geckos are asleep) lasts from 6pm to 6am. Each enclosure

127   is equipped with an additional light to provide lizards with UVB (Exo Terra Reptile UVB 100,

128   25 W) during the light cycle. The system automatically simulates a sunrise and sunset.

129   Temperature is automatically control and reaches approximately 25 °C during the night cycle

130   and 31 °C during the day cycle. To allow animals to thermoregulate, a heat mat (TropicShop)

131   is fixed to the outside of each enclosure increasing the temperature by ~5 °C. To simulate the

132   tropical condition this species experiences in the wild, the room humidity is kept at 50% and

133   daily rainfall (osmotic water, 30s every 12h at 5pm and 4am) increases the humidity within

134   enclosures to 100%. Humidity decreases with time until the next rainfall event. All enclosures

135   are set up on shelfs with small enclosures on the top and large enclosures on the bottom.

136   Animals are spread evenly across two rooms.

137   Lizards are fed three times per week on Monday, Wednesday and Friday with adult

138   crickets (*Acheta domesticus*). Before feeding, crickets are gut loaded using cricket mix (reptile

139   planet LDT), Purina Beyond Nature's Protein™ Adult dry cat food and fresh carrots to ensure

140   that they provided optimal nutrition (Vitamin D and calcium). Each individual lizard receives 3-

141   5 crickets each feeding with tweezers to be able to monitor the food intake. A water bowl

142   provides water *ad libitum*. Once a month geckos are captured and weighed to ensure healthy

143   weight.

144

145   Experimental setup and stimuli

146   Lizards were tested in their home enclosures to reduce stress of handling (Langkilde and

147   Shine, 2006) between 10th of August to 30th September 2021. Testing was conducted under

148   red light (PHILIPS TL-D 36W/15 RED). The light we use has a red component at 718 nm

149   which is not detectable by the tokay geckos' photoreceptors (Loew 1994). Furthermore, a blue

150   UV-C component at 282 nm is also produced which is visible to the geckos (Loew 1994) and

151   promotes gecko activity (personal observation).

152   Because animals were spread across two rooms, each room was tested on a different,

153   non-feeding day (either Tuesday or Thursday) once a week. Each individual was tested in a

154   random order each day and the stimuli (control, own, same sex unfamiliar) and treatment

155   (skin, faeces – i.e. scat) were also randomised across trials. As a positive control we used the

156   odour of tap water on a paper towel. To create the control stimulus, one side of a cotton swab

157   was taped 10 times on a moistened paper towel. As the familiar odour we used the individuals

158   own odour either from their skin collected by gently rubbing one side of a cotton swab over its'

159   back and/or sides 10 times or from a fresh (no older than 2 days) scat. The cotton swab was

160   rubbed on the scat until a stain was visible. To create the same-sex unfamiliar stimulus we

161   took chemicals from the skin or scats of a same sex individual from the second room. Although

162   animals never had direct contact with each other within a room we were unsure if the smell of

163   individuals could spread within a room. To ensure true unfamiliarity, we used the individuals

164   from the second room located across a small hallway. The same methods as for collecting

165   individuals own odour was used. Each individual was tested on their reaction towards the

166   odour of three different same-sex conspecifics. From each conspecific both chemicals from

167   skin and faeces were used to be able to compare the reaction across treatments while

168   controlling for identity. All cotton swabs were marked at the back to indicate on which side the

169   stimulus was applied. This was done so the experimenter could present each cotton swab

170   with the stimulus facing downwards to exclude the use of visual information originating from

171   faeces or UV-reflecting chemicals (Mason 1992).

172

Figure 1. Setup of cotton swabs in clay bowls. For each focal subject swabs were placed in a separate clay bowl. Swabs were placed in the testing order. The experimenter made sure that swab tips covered in chemical stimuli never touched each other. To prevent excessive degradation of stimuli, individuals within a room were divided into two groups and the second group set up after the first group finished testing.

182

183   Experimental procedure

184   At the start of a test day stimuli were set up as follows: First, all swabs for the control were

185   prepared. Next, all swabs with lizards own odour were prepared and lastly, all swabs with the

186   unfamiliar odour were prepared. This was done to leave enough time (20-30 minutes) between

187   stimulus collection and test of focal individuals to recover from stimulus collection (skin

188   treatment). All swabs were placed in clay bowls in the order of presentation (Figure 1). We

189   first set up half of the individuals, tested their reaction and then set up and tested the second

190   half. This was done to prevent excessive degradation of chemical stimuli. After all individuals

191   finished testing, cotton swabs were discarded and clay bowls thoroughly cleaned with hot

192   water and a sponge. Then they were dried upside down until the next test day. The

193   experimenter ensured that the inside of the bowls was never touched. Furthermore, they

194   ensured that the cotton swabs within a bowl never touched.

195   After set up, we first tested all individuals with the first cotton swab, then all with the

196   second and finally with the third. This was done to leave about 10-15 minutes between

197   stimulus presentations and reduce carry-over effects between stimuli. Both males and females

198   were tested using the same procedure.

199   In a given trial, we first placed a dim white light (LED, SPYLUX® LEDVANCE 3000K,

200   0.3 W, 17 lm) on top of the enclosure. Lizards were used to this light as it was used during

201   feeding and generally during testing. Next, we located an individual in its' enclosure. If the

202   individual was hiding we gently removed the refuge from the back to expose it. Next, a cotton

203   swab was presented holding it in a pair of 20 cm long metal tweezers. This ensured that the

204   hand of the experimenter was far enough away to prevent the experimenters' odour interfering

205   with the experiment. The experimenter was visible during trials similar as during regular

206   feeding. Trials from the first two test days were recorded with a GoPro (Hero 5). However,

207   videos were too dark and we had issues scoring the lizards behaviour. For all other trials we

208   switched to recording with a Samsung S20 smartphone (108 Megapixel, 8K-FUHD) which

209   produced far better quality videos under the light conditions. Furthermore, the ability to switch

210   between front and back camera enabled us to take videos even when lizards were sitting

211   above the tank entrance. By the second week of testing we detected a large decrease in bites

212   likely caused by lizards learning that the cotton swab was not edible. We, therefore, decided

213   to repeat the first trial at the end of the testing period to ensure that our measurements were

214   not confounded by changes in behaviour.

215

216   Data collection

217   Videos were scored from the start of a trial, when the cotton swab was first presented within

218   1 cm of the lizards snout. Trials lasted a maximum of 120 seconds (2 minutes). If a tongue

219   flick (TF) or bite occurred, the behaviour was video recorded for 60s after the initial event

220   following the procedure used in previous studies with squamates (e.g. Aragón et al., 2001;

221   López et al., 1997; Martin et al. 2020). If the lizard showed a turn (whole body movement away

222   from the swab, Table 1) and walked away from the swab the trial was terminated.

223   Videos were analysed blind as to which stimulus was presented but not to treatment

224   which was visible in some videos. We used VLC media player (Version 3.0.7.1, Vetinari, Intel

225   64 bit) to score behaviour (Table 1) shown during trials. We scored bites, TF, breaths if the

226   ventral side of the individual was visible (i.e. gular pumping, Norris and Lopez, 2011), deep

227   breaths, and turns. TFs were divided into flicks directed at the stimulus (tongue tip aimed at

228   the swab) and flicks directed towards the ground (tip aimed at the ground). We also measured

229   the trial time in seconds starting from the time the stimulus was presented within 1 cm of the

230   lizards' snout until either 120 seconds without a bite or TF elapsed, the lizard performed a turn

231   or 60 seconds after the first bite or TF. Enclosure temperature was recoded automatically to

232   an accuracy of 0.1 °C by the system responsible for regulating the environment within rooms.

233

234   **Table 1.** Ethogram of behaviours shown by tokay geckos in response to chemical stimuli.

Name of behaviour	Description
Breath	One up and down movement of the lizards' throat = 1 breath. Only visible from the ventral side. Recorded as counts.
Deep breath	One extension and retraction of the flanks behind the front legs. Visible from the dorsal and ventral side. Recorded as counts.
Tongue flick	Quick protrusion of the tongue forward away from the mouth. NOT licking of the lips which is also a protrusion of the tongue but along the skin of the mouth. Recorded as counts.
Bite	The tip of the swab is taken between the upper and lower jaw. May be accompanied by shaking of the head. Recorded as counts.
Turn	The lizards' moves away from the swab. The whole body moved either past the swab, backwards away or involved a turn away from the swab. Recoded as yes or no. A trial was terminated if this behaviour was shown.

235

236   Ethical note

237   The test reported in this study are strictly non-invasive observations of behaviour. Introducing

238   odour of conspecifics is a practice used during enrichment in reptiles. We followed the

239   guidelines provided by the Association for the Study of Animal Behaviour/ Animal Behaviour

240   Society for the treatment of animals in behavioural research and Teaching (2018). All testing

241   was approved by the Suisse Federal Food Safety and Veterinary Office (National No. 33232,

242   Cantonal No. BE144/2020). Captive conditions were approved by the Suisse Federal Food

243   Safety and Veterinary Office (Laboratory animal husbandry license: No. BE4/11).

244

245   Statistical analysis

246   *Power analysis*

247   Before data collection, we performed a power analysis using G*power (Faul et al., 2007;

248   2009). As our study was designed as a 2x2x3 factorial designed, we calculated power based

249   on a within factor repeated measures ANOVA. The literature on chemical discrimination in

250   other lizard and worm lizard species (Alberts,1992; Cooper et al., 1999; López et al., 1997)

251   generally suggested large effect sizes. We were, however, unsure what effect size to expect

252   from our geckos and therefore calculated the minimal effect size that could be reliably detected

253   at a power of 0.8. We specified an alpha level of 0.05, a power of 0.8, six groups with three

254   measurements, a correlation among repeated measures of 0.5 and a correction of 1. With

255   these settings and a sample size of 24 individuals we are able to detect an effect size of 0.3

256   at an actual power of 0.99. The sample size used in our study was 22 individuals. We

257   expected, however, only a slight reduction in the actual power to detect a small effect size.

258

259   *Data analysis*

260   General reaction

261   We were interested if the reaction towards the presented stimuli was affected by the treatment

262   (scat or skin), stimulus (water control, own or unfamiliar odour), sex (male or female), the order

263   in which stimuli were presented, trial and temperature. These were used as fixed effects in

264   three models looking at all tongue flicks produced in 60 seconds, deep breaths per second

265   and breaths per second. For TFs we used a generalised linear mixed zero-inflation Poisson

266   model (GLMM, package glmmTMB, Brooks et al., 2017) because our dataset included a large

267   amount of 0 TFs. The conditional model included the above mentioned fixed effects while the

268   zero-inflation model only included treatment, stimulus and sex as fixed effects. We did not

269   expect all fixed effects to cause zero-inflation. Both the models included a random effect of

270   animal identity to account for repeated measures. We were also interested if the difference in

271   size between the test subject and the unfamiliar individual (delta SVL) from which the odour

272   was taken affected TFs. To this end, we looked at TFs produced in the unfamiliar condition

273   only as the response variable in another zero-inflation Poisson model. Both the conditional

274   model and zero-inflation model included delta SVL and treatment as fixed effects and trial and

275   animal identity as random effects. In both analyses we specified session for the over-

276   dispersion component.

277   For the two measures of breathing, we first divided the number of breaths by the trial

278   time to get a comparable measure for the breaths (breaths and deep breaths per second). We

279   used breaths and deep breaths per second as the response variable in linear mixed effects

280   models (LME, package lmerTest, Kuznetsova et al., 2017) with Gaussian family including the

281   above mentioned fixed effects. Both models conformed to the assumption of residual normality

282   (visual inspection of qqplots). Both models included a random effect of animal identity and

283   session to account for repeated measures. We did not analyse bites because they were shown

284   too infrequent to be analysed.

285

286   Differences between swab and ground directed tongue flicks

287   Across all trials, males only tongue flicked three times while females together produced 202

288   TFs. We, therefore, based the following analysis on the data from females only. To identify if

289   lizards compared their own odour to that of an unfamiliar individual we recorded TFs directed

290   at the swab and those directed at the ground (on which their own odour was present). We

291   analysed swab and ground directed TFs separately. We used generalised linear mixed zero-

292   inflation Poisson models with ground or swab TFs as the response variable. The conditional

293   models included stimulus as the only fixed effect as well as treatment, trial and animal identity

294   as random effects. Treatment and trial were included as random effects because the general

295   analysis revealed significant effects on TFs. In the zero-inflation models we used treatment

296   and stimulus as fixed effects and animal identity as the random effect. We also specified

297   session for the over-dispersion component. Finally, we also compared the two TFs within

298   stimulus conditions using their average across trials and treatments (to avoid pseudo-

299   replication) with Wilcoxon signed rank tests for paired data.

300

301   Data analysis was done in the free, open source software R (Version 4.0.3; R Core Team,

302   2020). All data and code produced during this study are available on the Open Science

303   Framework (OSF; link for review purposes:

304   https://osf.io/jp7h8/?view_only=b4c0eac3792f4adaaef2ff6745aebf45)

305

306   **Results**

307   One female (G015) could not be tested as she was too anxious and was only used as a

308   stimulus individual. All other geckos habituated fast to being rubbed on their back with a swab

309   and did not flee during stimulus collection by the second week of testing (the first week of

310   testing was not used for analysis).

311

312   General reaction to the presented stimuli

313   Our analysis revealed that males tongue flicked much less than females (GLMM, estimate = -

314   4.249, z-value = -4.674, CI_{low} = -6.031, CI_{up} = -2.467, p-value < 0.001; Figure 2A) and that

315   lizards tongue flicked less towards odour originating from scats than from skin (GLMM,

316   estimate = -0.406, z-value = -2.413, CI_{low} = -0.736, CI_{up} = -0.076, p-value = 0.016, Figure 2B).

317   Compared to swabs containing the odour of unfamiliar individuals, lizards directed less TFs

318   towards tap water from a paper towel (GLMM, estimate = -0.556, z-value = -2.733, CI_{low} = -

319   0.954, CI_{up} = -0.157, p-value = 0.006; Figure 2A) and their own odour (GLMM, estimate = -

320   0.698, z-value = -3.562, CI_{low} = -1.083, CI_{up} = -0.314, p-value = 0.0004; Figure 2A). Overall,

321   lizards decreased TFs over the course of the experiment (GLMM, estimate = -0.196, z-value

322   = -2.099, CI_{low} = -0.378, CI_{up} = -0.013, p-value = 0.036). We detected no effect of stimulus

323   order (GLMM, estimate = 0.043, z-value = 0.481, CI_{low} = -0.133, CI_{up} = 0.219, p-value = 0.631)

324   and temperature (GLMM, estimate = 0.086, z-value = 0.277, CI_{low} = -0.524, CI_{up} = 0.697, p-

325   value = 0.782) and the size of the stimulus individual had no effect on the number of TFs

326   (GLMM, estimate = -0.123, z-value = -0.667, CI_{low} = -0.524, CI_{up} = 0.697, p-value = 0.505).

327   The zero-inflation models did not produce any significant results (GLMM, p-value > 0.05; Table

328   A1 and A2).

329   We found no significant effects of any of the fixed effects on deep breaths per second

330   (LME, *p*-value > 0.05, Table A3) but geckos showed a higher breathing rate in response to

331   stimuli originating from scats (when responses towards all stimuli were lumped together)

332   indicating a stronger involvement of olfaction (LME, estimate = 0.106, *t*-value = 3.132, CI_{low} =

333   0.039, CI_{up} = 0.170, *p*-value = 0.002, Table A4). None of the other fixed effects were significant

334   (LME, *p*-value > 0.05, Table A4).

335
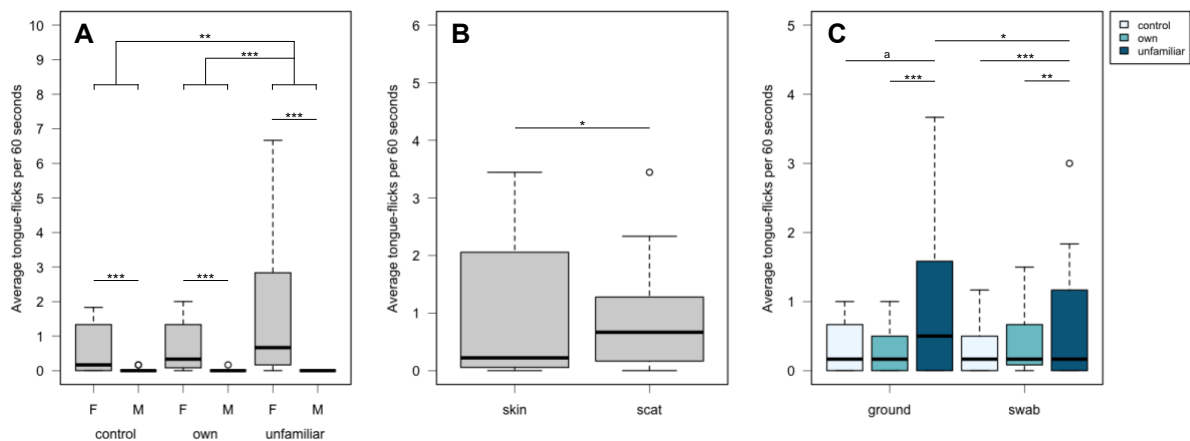
336

337   **Figure 2.** (A) Average tongue flicks produced within 60 seconds in the three stimulus

338   conditions separated between males and females. Overall, lizards tongue flicked the most in

339   the unfamiliar conditions and males tongue flicks less than females. (B) Average tongue flicks

340   produced within 60 seconds across the skin and scat treatment. Lizards tongue flicked less in

341   response to scats. (C) Average tongue flicks produced within 60 seconds directed towards the

342   ground and the swab separated into stimulus conditions. Only data from females are shown.

343   Females tongue flicked more when stimuli originated from unfamiliar individuals (dark blue)

344   compared to the own (medium blue) and control condition (light blue). Females tongue flicked

345   the ground more than the swab in the unfamiliar condition only. a *p* < 0.06, * *p* < 0.05, ** *p* <

346   0.01, *** *p* < 0.001.

347

348   Differences between swab and ground directed tongue flicks

349   Females directed less ground TFs towards their own odour compared to odour from unfamiliar

350   individuals (GLMM, estimate = -1.133, z-value = -3.872, CI_{low} = -1.707, CI_{up} = -0.560, p-value

351   = 0.00011, Figure 2C). Females also directed less swab TFs towards their own odour (GLMM,

352   estimate = -0.927, z-value = -2.979, CI_{low} = -1.538, CI_{up} = -0.317, p-value = 0.003, Figure 2C)

353   and even less towards the water control (GLMM, estimate = -1.405, z-value = -4.614, CI_{low} =

354   -2.002, CI_{up} = -0.808, p-value < 0.001, Figure 2C). The zero-inflation models did not produce

355   any significant results (GLMM, p-value > 0.05, Table A5 and A6). Within conditions females

356   directed more TFs towards the ground in the unfamiliar condition (Wilcoxon signed rank tests,

357   V = 3, N = 11, p-value = 0.012, Figure 2C). We found no differences in the control (Wilcoxon

358   signed rank tests, V = 18.5, N = 11, p-value = 0.801, Figure 2C) and own odour condition

359   (Wilcoxon signed rank tests, V = 14.5, N = 11, p-value = 0.829, Figure 2C).

360

361   **Discussion**

362   Our experiment demonstrated that, at least female tokay geckos, discriminate between their

363   own odour and that of an unfamiliar female using chemicals originating from the skin and scats

364   but they show a weaker response to chemicals originating from scats. More TFs occurred

365   towards the odour of unfamiliar individuals and females produced more ground directed TFs

366   in response to the unfamiliar conspecific odour. In general, lizards sampled the stimulus most

367   (swab directed TFs) in the unfamiliar condition and we found no differences in TFs directed

368   towards their own odour and the water control.

369   Based on previous studies in other lizards (e.g. Alberts, 1992; Cooper et al., 1999;

370   Graves and Halpern, 1991), we predicted that tokay geckos would show more TFs towards

371   chemical stimuli originating from unfamiliar, same-sex conspecifics. Our results are in line with

372   these studies but only in females. Males only tongue flicked a total of three times during the

373   course of the experiment. Either, males do not rely as strongly on skin and scat chemicals for

374   individual recognition or they show a delayed response which we did not record using our

375   methodology. Indeed, we observed an increase in activity including TFs in some males after

376   trials had ended. Male tokay geckos are territorial (Grossmann, 2006) and their behaviour

377   might be interpreted as searching for the intruder. It is, however, necessary to run additional

378   test recording not just the immediate response of males within two minutes but record

379   behaviour for a longer time such as 10-15 minutes after stimulus presentation. Furthermore,

380   males might react stronger to femoral gland secretions similar to male amphisbaenians

381   (*Blanus cinereus*; Cooper et al., 1994) which should be tested in the future.

382   We also predicted that lizards would show chemical self-recognition by producing more

383   ground directed TFs in response to the unfamiliar odour compared to their own odour. Our

384   results confirm our prediction. Testing individuals inside their own enclosure posed an

385   experimental advantage. Their enclosures are saturated with their own odour which made it

386   possible to detect "self-directed" behaviour which would not have been possible in a neutral

387   environment. Although male desert iguanas showed self-directed TFs towards their femoral

388   glands (Alberts, 1992), we did not expect to find such behaviour in our geckos. Tokay geckos

389   are, however, territorial, show site fidelity and scat pile. We expected, therefore, that if any

390   comparison between the presented stimuli and self-produced odour did take place, this would

391   likely be shown by TFs towards the ground. These ground directed TFs were very pronounced

392   and easy to score because animals would always turn their heads away from the swab before

393   tongue flicking the ground. Our results point towards similar difficulty to recognise tap water

394   and their own scent because ground and swab directed TFs did not differ across these test

395   conditions. They did differ in the unfamiliar condition. We recorded higher rates of ground

396   directed TFs compared to swab directed TFs demonstrating a need for increased comparison

397   with their own odour. Interestingly, a study in male Iberian rock-lizards (*Lacerta monticola*)

398   showed no differences in non-swab directed TF between males own and unfamiliar males

399   femoral gland secretion (Aragón et al., 2001). This study tested wild caught males that were

400   kept together with a second individual on their reaction to femoral gland secretions. We used

401   chemical from skin and scats from captive bred individuals kept singly and mainly analysed

402   the reaction from females to these stimuli. It is possible that the scent of the second individual

403   present in the enclosure interfered with "self-directed" TFs in rock-lizards. A comparison to our

404   results is, however, difficult due to the many differences between studies.

405   A next step towards more conclusive evidence for true self-recognition would be to test

406   geckos' reaction towards a change in their own odour similar to what was done with dogs

407   (Horowitz, 2017). Dogs are more interested in their own odour when it was marked but where

408   less interested in the mark alone. If geckos similarly increase ground directed TFs towards

409   their marked odour compared to the mark alone then this would further support our geckos'

410   ability to show true self-recognition.

411   Previous studies have considered that an increased rate of TFs towards the odour of

412   unfamiliar individuals could be caused by novelty of the stimulus (Bull et al., 1999a; 2000).

413   Bull and colleagues (1999a; 2000) used chemical stimuli from heterospecific individuals that

414   were unfamiliar to the test lizards as a control. If novelty was causing increased TF rates then

415   lizards would also show an increased response towards the heteropsecifics' odour which they

416   did not ruling out novelty as a cause for increased TF rates (Bull et al., 1999a; 2000). In our

417   study, we used tap water from a paper towel as a control instead of odourless deionised water

418   which elicited a lower rate of TFs compared to the unfamiliar odour. The fact that similar

419   numbers of TFs (ground and swab directed) were directed towards their own odour and the

420   odour of tap water and a paper towel shows that novelty was not solely responsible for our

421   lizards' reaction. As tap water and paper towels are not odourless, we would expect increased

422   TF rates to inspect the novel odour which we did not find. We acknowledge, however, that an

423   additional control similar to what was used in previous studies (Bull et al., 1999a; 2000) is

424   needed to completely rule our novelty as a cause for the strong effect we found. Furthermore,

425   we can also rule out that diet differences caused the difference in response towards own and

426   unfamiliar odour because all our lizards were fed the same diet.

427   Finally, our results also point towards a social function of scat piling. Although geckos

428   produced less TFs towards scats this difference was small. Additional research could

429   determine if geckos inspect scat piles of other individuals when available, if they are more

430   likely to defecate in locations with their own scat present (Carpenter and Duvall, 1995), and

431   could investigate if lipids are deposited on scats by glands (Bull et al., 1999b). Furthermore,

432   scat piling might have a possible function related to predator avoidance when predators use

433   the odour of scats to locate refuges (Bull et al., 1999a; Carpenter and Duvall, 1995; Norris and

434   Lopez, 2011). Studies on wild lizards should document the location of scat piles to determine

435   if scat piles have an anti-predator function as well. Scat piles in locations that are not frequently

436   visited by geckos would point towards an anti-predator function.

437   In summary, we provide fist evidence for chemical self-recognition in a gecko species

438   and a possible social function of scat piles. Further investigations are, however, needed to

439   confirm true self-recognition in tokay geckos and to better understand the communicative

440   function of scats. Future studies could also look at other forms of recognition such as

441   discrimination between familiar and unfamiliar individuals, mate recognition and kin

442   recognition of skin, femoral gland and scat odours. Tokay geckos are a good model species

443   to investigate recognition in general as they show biparental care and form temporary family

444   groups with their offspring (Grossmann, 2006; Somma, 2003). Such studies can potentially

445   demonstrate more sophisticated social cognitive abilities than have previously been attributed

446   to reptiles (Doody et al., 2013).

447

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452

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558

559   **Appendix**

560

561   Results tables

562

563   **Table A1.** Parameter estimates and test statistics for the generalised linear mixed zero-

564   inflation Poisson model looking at all tongue flicks produced by all tested individuals. The

565   models included a random effect of animal identity and an over-dispersion parameter of

566   session. Significant results are highlighted in bold. CI – confidence interval.

Conditional model					
Parameter	**Estimate**	*z*-value	**CI$_{low}$**	**CI$_{up}$**	*p*-value
Intercept	-1.924	-0.239	-17.681	13.833	0.811
Scat	**-0.406**	**-2.413**	**-0.736**	**-0.076**	**0.016**
Water control	**-0.556**	**-2.733**	**-0.954**	**-0.157**	**0.006**
Own odour	**-0.698**	**-3.562**	**-1.083**	**-0.314**	**0.0004**
Male	**-4.249**	**-4.674**	**-6.031**	**-2.467**	**2.95*10^{-6}**
Stimulus order	0.043	0.481	-0.133	0.219	0.631
Trial	**-0.196**	**-2.099**	**-0.378**	**-0.013**	**0.036**
Temperature	0.086	0.277	-0.524	0.697	0.782
Zero-inflation model					
Parameter	**Estimate**	*z*-value	**CI$_{low}$**	**CI$_{up}$**	*p*-value
Intercept	-1.462	-1.083	-4.107	1.184	0.279
Scat	-1.959	-1.820	-4.069	0.151	0.069
Water control	1.393	1.282	-0.737	3.523	0.200
Own odour	0.469	0.450	-1.574	2.511	0.653
Male	-17.667	-0.003	0.554	5.785	0.998

567

568   **Table A2.** Parameter estimates and test statistics for the generalised linear mixed zero-

569   inflation Poisson model looking at all tongue flicks produced in the unfamiliar condition by all

570   tested individuals. The models included trial and animal identity as random effects. Significant

571   results are highlighted in bold. CI – confidence interval.

Conditional model					
Parameter	**Estimate**	*z*-value	**CI$_{low}$**	**CI$_{up}$**	*p*-value
Intercept	**-2.662**	**-2.233**	**-4.998**	**-0.325**	**0.026**
Scat	-0.318	-1.405	-0.761	0.126	0.160
Delta SVL	-0.123	-0.667	-0.485	0.239	0.505
Zero-inflation model					
Parameter	**Estimate**	*z*-value	**CI$_{low}$**	**CI$_{up}$**	*p*-value
Intercept	-1.518	-1.817	-3.155	0.119	0.069
Scat	-0.158	-0.143	-2.314	1.998	0.886
Delta SVL	-0.662	-1.490	-1.533	0.209	0.136

572

573

574 **Table A3.** Parameter estimates and test statistics for the linear mixed model looking at deep

575 breaths per second. The model included session and animal identity as random effects. CI –

576 confidence interval.

Parameter	Estimate	*df*	*t*-value	CI$_{low}$	CI$_{up}$	*p*-value
Intercept	0.489	35.2	0.352	-2.142	3.112	0.727
Scat	0.015	315.8	0.559	-0.036	0.065	0.576
Water control	0.023	315.3	0.727	-0.038	0.085	0.468
Own odour	-0.005	315.5	-0.151	-0.066	0.057	0.880
Male	0.108	18.9	1.879	-0.004	0.219	0.076
Stimulus order	-0.019	315.4	-1.191	-0.050	0.012	0.235
Trial	-0.025	39.0	-1.117	-0.070	0.023	0.271
Temperature	-0.006	35.1	-0.118	-0.108	0.096	0.907

577

578

579 **Table A4.** Parameter estimates and test statistics for the linear mixed model looking at breaths

580 per second. The model included a random intercept of animal identity and a random slope of

581 session. Significant results are highlighted in bold. CI – confidence interval.

Parameter	Estimate	*df*	*t*-value	CI$_{low}$	CI$_{up}$	*p*-value
Intercept	0.124	111.9	0.099	-2.296	2.514	0.921
Scat	**0.106**	**154.7**	**2.132**	**0.039**	**0.170**	**0.002**
Water control	0.044	142.9	1.163	-0.030	0.116	0.247
Own odour	0.020	140.7	0.512	-0.055	0.096	0.610
Male	0.008	19.1	0.143	-0.094	0.113	0.888
Stimulus order	-0.013	137.8	-0.686	-0.050	0.024	0.494
Trial	-0.032	59.2	-1.418	-0.077	0.014	0.162
Temperature	0.033	112.2	0.684	-0.059	0.127	0.496

582

583

584   **Table A5.** Parameter estimates and test statistics for the generalised linear mixed zero-

585   inflation Poisson model looking at all ground tongue flicks across stimulus conditions in

586   females. The conditional model included treatment, trial and animal identity as random effects,

587   the zero-inflation model included animal identity as the random effect. Significant results are

588   highlighted in bold. CI – confidence interval.

Conditional model					
Parameter	**Estimate**	*z*-value	**CI$_{low}$**	**CI$_{up}$**	*p*-value
Intercept	-1.064	-1.239	-2.746	0.618	0.215
Water control	-0.637	-1.899	-1.294	0.021	0.058
Own odour	**-1.133**	**-3.872**	**-1.707**	**-0.560**	**0.0001**
Zero-inflation model					
Parameter	**Estimate**	*z*-value	**CI$_{low}$**	**CI$_{up}$**	*p*-value
Intercept	-1.463	-1.157	-3.942	1.016	0.247
Scat	-1.693	-1.621	-3.739	0.354	0.105
Water control	1.998	1.680	-0.333	4.329	0.093
Own odour	0.240	0.203	-2.069	2.548	0.839

589

590

591   **Table A6.** Parameter estimates and test statistics for the generalised linear mixed zero-

592   inflation Poisson model looking at all swab tongue flicks across stimulus conditions in females.

593   The conditional model included treatment, trial and animal identity as random effects, the zero-

594   inflation model included animal identity as the random effect. Significant results are highlighted

595   in bold. CI – confidence interval.

Conditional model					
Parameter	**Estimate**	*z*-value	**CI**$_{low}$	**CI**$_{up}$	*p*-value
Intercept	-0.483	-0.838	-1.613	0.647	0.402
Water control	**-1.405**	**-4.614**	**-2.002**	**-0.808**	**3.96*10^{-6}**
Own odour	**-0.927**	**-2.979**	**-1.538**	**-0.317**	**0.003**
Zero-inflation model					
Parameter	**Estimate**	*z*-value	**CI**$_{low}$	**CI**$_{up}$	*p*-value
Intercept	-1.018	-0.895	-3.249	1.213	0.371
Scat	1.912	1.597	-0.435	4.259	0.110
Water control	-21.217	-0.002	-16347.2	16304.9	0.998
Own odour	-1.809	-1.445	-4.262	0.645	0.148

596