

1 **Olfactory recognition of snake cues by fawn-footed mosaic-tailed rats *Melomys cervinipes***

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3 Olfactory recognition in *Melomys cervinipes*

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17

18 **Summary**

19 Rodents rely on their sensitive olfactory systems to detect and respond to predators. We

20 investigated the ability of a native Australian rodent, the fawn-footed mosaic-tailed rat *Melomys*

21 *cervinipes*, to detect, recognise, and discriminate between two species of native snakes. We used

22 snake sheds from a sympatric venomous red-bellied black snake *Pseudechis porphyriacus* and a

23 non-sympatric non-venomous Stimson's python *Antaresia stimsoni*. 20 mosaic-tailed rats each

24 experienced three olfactory tests using a Y-maze. Rats were first exposed to one snake shed
25 against a paper control, and then exposed to the other snake shed against a paper control. Which
26 rat experienced which shed first was allocated randomly. Mosaic-tailed rats were then exposed to
27 both sheds simultaneously. Rats could detect the snake sheds, spending longer investigating, and
28 making more visits to, the sheds than the paper control. They also recognised the sheds as
29 potentially dangerous, reducing their total investigation over time, but increasing their frequency
30 of visits. However, rats did not discriminate between sheds, suggesting a general strategy for
31 assessing the identity of reptilian predators.

32

33 **Key Words:** olfaction, olfactory, recognition, reptile, rodent, tropical

34

35 **Introduction**

36

37 The evolutionary arms race, constituted by ever-changing morphologies and behaviours of
38 conflicting species, has shaped the interactions of most predator-prey relationships (Dawkins &
39 Krebs, 1979). Few animals are considered to be apex predators (Polis & Strong, 1996), while the
40 majority are threatened by some level of predation. Consequently, prey must be equipped with
41 physical ornaments (e.g. stick insects *Carausius morosus*, Graham, 1972), armaments (e.g.
42 armoured crickets *Acanthopplus speiseri*, Mbata, 1985), or behavioural strategies (e.g. cryptic
43 behaviour in willow ptarmigan *Lagopus*, Steen et al., 1992), to reduce or avoid the risks
44 (Dawkins & Krebs, 1979).

45 Different species use different responses to avoid or reduce risks. For example, stoneflies
46 *Paragnetina media* use crypsis to hide (Feltmate & Williams, 1989), while swallowtail

47 butterflies *Papilio machaon* use noxious, distasteful chemicals and aposematic colouration to
48 deter predators (Pasteels et al., 1983). However, some prey species must first detect a predator
49 using one or more sensory cues before responding to minimise the risk of predation. These cues
50 can be visual, such as shadows cast from flying predatory birds (Sordahl, 2004), olfactory, such
51 as urine odours of roving carnivorous mammals (Hayes et al., 2006), auditory, such as the noise
52 of rustling in leaves by predatory reptiles (Kindermann et al., 2009), or a combination of these.
53 Following detection of the cue, prey should assess the threat, and respond to minimise the risk of
54 predation (Kindermann et al., 2009; Hodges et al., 2014). For example, pallid gerbils *Gerbillus*
55 *perpallidus* respond to auditory and visual cues of avian predators by avoiding the time of day
56 and locations where these birds occur (Kindermann et al., 2009).

57 Rodents play ecologically essential roles as prey items in numerous environments
58 (Wywiałowski, 1986; Cramer & Willig, 2002). Most use olfaction extensively to detect predators
59 (Apfelbach et al., 2005). Rodents possess complex vomeronasal (VNS) and olfactory systems
60 (ORS) that are sensitive to different types of chemical odourants, including allelochemicals and
61 pheromones (Ache & Young, 2005; Bind et al., 2013). The detection of these cues triggers
62 different neural pathways to corresponding regions of the brain, specifically the amygdala and
63 olfactory cortex (Bind et al., 2013), which elicit appropriate behavioural responses, such as
64 fleeing or freezing (Choi & Kim, 2010; Bind et al., 2013). For example, laboratory rats *Rattus*
65 *norvegicus* with inhibited amygdalae were less likely to retreat when presented with a predator
66 stimulus compared to rats with unmodified amygdalae (Choi & Kim, 2010).

67 Recognition and discrimination may then follow detection of an odour cue (Ache & Young,
68 2005; Bind et al., 2013). It is important to distinguish between these concepts because detection
69 of a cue does not necessarily result in immediate recognition or discrimination of that cue, and

70 recognition does not always lead to discrimination. Recognition is defined as a response to a
71 stimulus that is either repeatable, if previously encountered, or predictable, if novel, but of a
72 similar nature to another experienced stimulus (Mendelson, 2015). Recognition may be innate.
73 For example, house mice *Mus musculus* bred and raised in captivity hide and freeze in response
74 to odours of cats, rats and snakes, even without prior exposure, suggesting that these olfactory
75 cues activate innate behavioural responses (Papes et al., 2010). However, recognition may rely
76 on learning the properties of a cue and the associated risk, such as the observed lack of
77 behavioural response of predator-naïve house mice to different predator and non-predator avian
78 auditory cues (Kindermann et al., 2009). In contrast to recognition, discrimination is the ability
79 to differentiate or distinguish between two or more cues because the animal has a specific
80 memory associated with each stimulus (Akkerman et al., 2012). For example, vervet monkeys
81 *Chlorocebus pygerythrus* respond to avian predators by looking upwards, whereas they respond
82 to land-based mammalian predators by climbing trees (Seyfarth et al., 1980). While recognition
83 without discrimination can occur (Overman et al., 1992), discrimination between different odour
84 cues cannot occur in the absence of recognition of those cues.

85 The ability to recognise and discriminate between odours could be critical for prey species
86 living in complex environments, such as tropical rainforests, as increased habitat complexity is
87 associated with increased predator abundance and diversity (Langellotto & Denno, 2004).
88 However, the ability to recognise and discriminate predator odour cues by species living in
89 tropical rainforests is poorly studied. Therefore, we investigated whether a native Australian
90 tropical rainforest rodent could recognise and discriminate between different reptilian predator
91 odours.

92 The fawn-footed mosaic-tailed rat *Melomys cervinipes* is a medium-sized (37-120g;
93 Callaway et al., 2018) nocturnal, endemic rodent found in the forests of coastal and subcoastal
94 Queensland and New South Wales, Australia (Moore & Burnett, 2008). It is semi-arboreal, using
95 both the canopy and forest floor for foraging and nesting (Wood, 1971). It is the primary prey of
96 numerous predators in the upland Wet Tropics of Queensland, including spotted tail *Dasyurus*
97 *maculatus* and northern *D. hallucatus* quolls (Hayes et al., 2006; Moore & Burnett, 2008),
98 dingoes *Canis lupus dingo* and feral cats *Felis catus* (Hayes et al., 2006), sooty *Tyto tenebricosa*
99 and lesser sooty *T. multipunctata* owls (Moore & Burnett, 2008; McDonald et al., 2013), and
100 red-bellied black snakes *Pseudechis porphyriacus* (Hayes et al., 2006).

101 Mosaic-tailed rats can detect the presence of mammalian predators and carpet pythons
102 *Morelia spilota variegata* in their natural environment via olfactory cues in predator faecal
103 matter (Hayes et al., 2006). While they avoided the mammal faeces, they did not avoid the
104 python faeces, which Hayes et al., (2006) attributed to the low frequency of python defecation,
105 making their faeces unreliable cues. However, two alternative explanations are possible: 1)
106 Mosaic-tailed rats may not have recognised the cues, and therefore treated them as they would a
107 non-predatory cue. Hayes et al. (2006) showed no significant difference in standardized
108 visitation rates of mosaic-tailed rats between a blank control and the carpet python odour,
109 suggesting that they could detect it, but did not recognise it. Because the blank used in Hayes et
110 al.'s (2006) study was an attractant (linseed oil), no difference in visitation rate between odour
111 stations and the blank does not give an indication of disinterest, and could actually reflect some
112 level of interest in the cue presented. Consequently, mosaic-tailed rats may not have avoided the
113 faeces because they were novel cues, and were curious about them (neophilia; Shapira et al.,
114 2013). Increased investigation of novel odours results from a need to gain more information

115 about the odour source (Hurst et al., 1997). If recognition of python cues is not innate, and
116 individuals had not encountered carpet pythons or their faecal matter before, they may merely
117 have been attempting to gain information about the novel odour in their environment. 2) Mosaic-
118 tailed rats may show a different behavioural response to snake faeces than the mammalian faeces
119 because they can discriminate between predator types. Investigation of the cue could thus
120 represent a different strategy for assessing the relative predation risk of a reptilian predator than a
121 mammalian predator.

122 We investigated whether mosaic-tailed rats could detect, recognise and discriminate predator
123 odours, using the sheds of venomous and non-venomous native snakes under controlled
124 environmental conditions in the laboratory. Sheds were used for two reasons: 1) Faeces may be
125 unreliable cues (Hayes et al., 2006), and skin and fur-derived predator odours may have longer-
126 lasting effects and greater potency compared to faeces (Apfelbach et al., 2005; but see Stabler,
127 1939 and Lillywhite et al., 2002). 2) While using live snakes would likely elicit a greater
128 response from the rats (aside from the ethical considerations), the likelihood of encountering
129 cues of snake presence (e.g. sheds and faeces) under natural settings would likely be higher than
130 encountering the actual snake itself, as snakes will move from one location to another. Mosaic-
131 tailed rats were presented with sheds from a venomous red-bellied black snake and a non-
132 venomous Stimson's python *Antaresia stimsoni* as well as a paper control. The two snake species
133 were chosen because they allowed us to control for potential behavioural responses due to
134 novelty of the odour cue because red-bellied black snakes occur sympatrically with mosaic-tailed
135 rats, whereas Stimson's python do not overlap in range.

136 We tested three hypotheses. First, we hypothesised that mosaic-tailed rats would be able
137 detect the odour cues, as snakes have particular skin-derived chemicals that play a role in species

138 recognition and social behaviour (Mason & Parker, 2010), as well as predatory behaviour
139 (Weldon & Schell, 1984). We predicted that, if mosaic-tailed rats could detect the cues, then the
140 amount of time the mosaic-tailed rats spent investigating the sheds, the number of visits to each
141 shed, and the duration of the first investigatory event would differ to the paper control, regardless
142 of the species of snake. Second, we hypothesised that mosaic-tailed rats would be able to
143 recognise a known reptile predator from the shed odour cues. We predicted that mosaic-tailed
144 rats would recognise, and show aversion (reduced time spent investigating, fewer visits, shorter
145 initial investigation event) to the snake odours in general. Third, we hypothesised that mosaic-
146 tailed rats would discriminate between different snake predator odours due to the presence of
147 species-specific skin-derived chemicals. While it was difficult to predict *a priori* the direction of
148 discrimination, we expected that mosaic-tailed rats would show an aversion (reduced time spent
149 investigating, fewer visits, shorter initial investigation event) to red-bellied black snake odours ,
150 as red-bellied black snakes occur sympatrically with, and are known natural predators of,
151 mosaic-tailed rats (Hayes et al., 2006). However, we also predicted that mosaic-tailed rats would
152 not show aversion to the Stimson's python shed because Stimson's pythons do not overlap in
153 range with mosaic-tailed rats.

154

155 **Materials and Methods**

156

157 *Subjects*

158

159 Twenty-four mosaic-tailed rats were collected from forested areas on the James Cook University
160 Cairns campus (16°49'S 145°41' E) in 2016 using Elliott traps. They were transferred to

161 individual cages in the Animal Behaviour Laboratory on the campus and allowed to acclimate to
162 captivity for at least four months before experiments began. Nine offspring were born in
163 captivity in 2017. All rats were housed individually or in same-sex sibling pairs (until adult) in
164 wire-frame cages with a rectangular plastic base (36 cm x 29 cm x 47 cm). Approximately 10 cm
165 of wood shavings was provided for bedding, and a cylindrical plastic nest box (10 cm x 21 cm),
166 hay and paper towel were provided for nesting material. Plastic wheels, a cardboard roll, wire
167 climbing platforms, and sticks and branches were provided for enrichment. Each rat had access
168 to water *ad libitum*, and received ± 5 g of mixed seeds and rodent chow, and ± 5 g of fruits or
169 vegetables (e.g. apple, cucumber) daily.

170

171 *Study design*

172

173 20 individuals were chosen at random from the colony (males: $n = 12$; females: $n = 8$). Of these
174 20, six were captive-born (males: $n = 2$; females: $n = 4$). The number of captive born individuals
175 was low due to small numbers bred in captivity. Individuals were chosen at random from the
176 colony, and the person making the choice (KP) was blind to their origin to reduce bias. Each
177 individual was tested three times (see below) in random order in two-way choice tests using a
178 Perspex Y-shaped maze (34 cm x 28 cm x 5 cm) attached to three equal-sized rectangular boxes
179 (46 cm x 22 cm x 17 cm; see Rymer & Pillay, 2010). The rat could be confined to the neutral
180 box using a small plastic barrier prior to being released into the maze. All tests were conducted
181 between 18h00 and 21h00 during the peak period of mosaic-tailed rat activity (Wood, 1971)
182 under red light, which does not influence behaviour of other rodents (Castelhano-Carlos &
183 Baumans, 2009). Sheds were obtained from a local crocodile farm and a private owner. Sheds

184 were frozen at -20°C immediately following collection on site, then transported in an icebox to
185 the Animal Behaviour Laboratory, where they remained frozen at -20°C throughout the study.
186 Freezing does not affect chemical integrity (Pillay et al., 2006; Lenchova et al., 2008).

187 In Test 1, individual mosaic-tailed rats were presented with a choice between a snake shed
188 and a paper control. The choice of shed (python or red-bellied black snake) was randomly
189 selected for each individual. Immediately prior to testing, the shed was removed from the
190 freezer, and a 1 cm² piece was cut and placed in a 1.5 ml plastic Eppendorf tube with small holes
191 punched through the lid. The shed thawed within 5 min. A 1 cm² piece of plain white paper was
192 placed into a second plastic Eppendorf tube, also with holes in the lid. The two tubes were then
193 placed in the maze, one in each test box, with side selected randomly. The test rat was then
194 placed in the neutral box of the maze, and allowed to acclimate to the neutral box for 5 min.
195 Thereafter, the plastic barrier was removed, and the rat was given 20 min to explore the maze.
196 The whole apparatus was filmed from above, and each treatment box was also filmed, using
197 Panasonic HD HC-V110 video cameras. No observers were present in the room during recording
198 sessions. Using continuous sampling, we scored the total duration of time spent investigating
199 (sniffing and/or chewing) each tube, the duration of the first investigatory event for each tube
200 (shed vs. paper control) and the number of separate investigation events of each tube.

201 Test 2 occurred as for Test 1. However, individuals were tested with the odour cues of the
202 other snake shed against the paper control (i.e. if a rat experienced python and paper in Test 1, it
203 received red-bellied black snake and paper in Test 2; and *vice versa*). Test 3 occurred as for Tests
204 1 and 2. However, in Test 3, individuals were presented with both snake sheds, each randomly
205 allocated to a side. All individual rats were exposed to both snake sheds against the paper control
206 before they were exposed to the two-shed direct comparison to eliminate the possibility that the

207 response to the sheds was due to their novelty, rather than their odour quality. The same
208 behaviours were recorded for Tests 2 and 3 as for Test 1. The Y-maze and boxes were
209 thoroughly washed after each test and wiped with ethanol to remove any residual odours that
210 could cause bias in subsequent tests (Bind et al., 2013). Tests occurred 1-2 weeks apart, with rats
211 remaining in their home cages during the intervening period.

212

213 *Statistical analyses*

214

215 All analyses were performed using RStudio (version 1.0.153; <https://www.rproject.org>; R
216 version 3.5.0, <https://cran.rstudio.com>). The model-level significance was set at $\alpha = 0.05$. Prior to
217 analyses, all data were tested for normality (Shapiro-Wilk test) and homogeneity of variance
218 (Levene's test). We also examined QQ plots to assess the distribution. Data were transformed
219 where possible, and where not, appropriate non-parametric statistics were applied.

220 To determine if mosaic-tailed rats could detect and recognise the odour cues, we first ran
221 separate linear mixed effects models (LMER) or generalised linear mixed effects models
222 (GLMER with negative binomial distribution; lme4 package) for each behaviour (total duration
223 of time spent investigating the cues, duration of the first investigatory event, and number of
224 separate investigation events) for Tests 1 and 2 combined. Origin (captive-born or wild-caught)
225 Sex, Test (1 or 2), Group (1 = received python shed first; 2 = received red-bellied black snake
226 shed first) and Cue ("snake" or paper) were fixed factors, individual identity (ID) was the
227 random factor, and behaviour was a continuous predictor. We included all interactions between
228 fixed factors, except for Origin, given its low sample size, although we did include the
229 interaction between Origin*Cue to assess whether captivity influenced olfactory ability. We

230 chose to include all other statistical interactions in our model because excluding some factors
231 (e.g. Origin, Sex and Group) could cause masking of trends, leading to generalisations for the
232 species that may not be correct, although we acknowledge that fewer interactions would be more
233 appropriate for analyses with small sample sizes. We then ran likelihood ratio tests to determine
234 the significance of fixed factors. Specific differences were identified using the differences of
235 least squares means method (available in the lmerTest package).

236 To assess whether the rats could discriminate between the two snake odour cues (Test 3
237 only), we ran separate LMERS or GLMERS with Origin, Sex, Group and Cue as fixed factors, ID
238 as a random factor, and behaviour as a continuous predictor. We included all interactions
239 between fixed factors, except for Origin (we did include the interaction between Origin*Cue).
240 Again, we ran likelihood ratio tests to determine the significance of factors, and specific
241 differences were identified using the differences of least squares means method. Individual data,
242 and transformed means and standard errors (unless specified) are presented graphically.

243

244 *Ethical note*

245

246 Animals received environmental enrichment, and their welfare was monitored daily. The
247 experimental procedures did not have any negative effects on the welfare of the animals. At the
248 end of the study, all animals were returned to the colony. The research adhered to the
249 ABS/ASAB guidelines for the ethical treatment of animals (Vitale et al., 2018), as well as the
250 Australian Code for the Care and Use of Animals for Scientific Purposes (NHMRC, 2013). The
251 study was approved by the Animal Ethics Screening Committee of James Cook University
252 (clearance numbers: A2020 and A2246).

253

254 **Results**

255

256 We found a significant effect of the type of cue on total duration of investigation (LMER: $\chi^2_1 =$
257 36.33, $p < 0.001$; Figure 1), duration of the first investigatory event (GLMER: $\chi^2_1 = 11.42$, $p =$
258 0.001; Figure 2) and number of separate investigation events (LMER: $\chi^2_1 = 9.52$, $p = 0.002$;
259 Figure 3). Mosaic-tailed rats spent significantly longer investigating the snake shed, irrespective
260 of the species of snake, than the paper control (1.44x; Figure 1). Similarly, the duration of the
261 first investigatory event of the snake shed was 1.94x longer than that of the paper control (Figure
262 2), and the rats visited the snake shed 1.27x more often than the paper control (Figure 3).

263 Mosaic-tailed rats showed a significant increase in the number of separate investigation
264 events of the odour cues from Test 1 to Test 2 ($\chi^2_1 = 4.43$, $p = 0.035$; Figure 3). On average, the
265 number of separate investigation events increased by 1.17x in Test 2 (Figure 3). Test*Cue was
266 also a significant predictor of duration of investigation ($\chi^2_1 = 4.55$, $p = 0.033$; Figure 1). Mosaic-
267 tailed rats showed a significant decrease in the duration of time spent investigating the snake
268 shed from Test 1 to Test 2 (*post hoc* test: $p = 0.027$), but not the paper control (Figure 1).
269 Sex*Cue was also a significant predictor of number of separate investigation events ($\chi^2_1 = 7.15$,
270 $p = 0.007$), with males visiting the snake shed significantly more frequently than females (*post*
271 *hoc* test: $p = 0.038$; Figure 3). Males also visited the paper control significantly less frequently
272 than the snake shed (*post hoc* test: $p < 0.001$; Figure 3). There were no other significant effects or
273 interactions for any of the behaviours for Tests 1 and 2 (Supplementary Table S1).

274 In Test 3, no significant effects were found for any of the factors or behaviours
275 (Supplementary Table S2), except for ID ($\chi^2_1 = 6.01$, $p = 0.014$) and Origin ($\chi^2_1 = 4.75$, $p =$

276 0.029), which were both significant predictors of the number of separate investigation events in
277 Test 3 (Figure 4). Individuals HS36 and RF51 spent significantly more time investigating the
278 odour cues than individuals HP31 and HS23 (Figure 4). In addition, captive individuals visited
279 the odour cues 1.49x more often than wild-caught individuals (Figure 4).

280

281 **Discussion**

282

283 The ability to detect predators is critical for survival of prey species. We predicted that mosaic-
284 tailed rats would be able to detect the odour cues from the snake sheds due to the presence of
285 specific skin-derived chemicals in the sheds (Weldon & Schell, 1984; Mason & Parker, 2010).
286 Mosaic-tailed rats spent significantly more time investigating, and made more visits to, the snake
287 shed than the paper control, indicating that they could detect it, and that it invoked curiosity. The
288 ability to detect the cues was not unexpected, as rodents possess highly sensitive VNS and ORS
289 (Ache & Young, 2005; Bind et al., 2013) that were likely sensitive to the skin-derived chemicals
290 in the sheds.

291 We also expected that, if mosaic-tailed rats could recognise a known reptile predator from
292 the shed odour cues, then they should show an aversion to red-bellied snake odours, which are
293 naturally occurring, sympatric predators (Hayes et al., 2006), whereas the mosaic-tailed rats
294 should show increased interest in the Stimson's python shed because it was novel (i.e. does not
295 occur sympatrically with mosaic-tailed rats; Bevins & Besheer, 2006). While our results are
296 consistent with the finding in the Hayes et al. (2006) study that mosaic-tailed rats did not avoid
297 snake cues, we suggest that mosaic-tailed rats showed some, albeit limited, capacity for
298 recognition of the odour cues, as they responded to a cue that did not originate from the perceiver

309 (Akkerman et al., 2012). While the mosaic-tailed rats did not show an obvious aversion to red-
300 bellied black snakes, nor an increased interest in the Stimson's python shed, they appeared to
301 recognise a generic "snake" because, irrespective of the snake shed, mosaic-tailed rats showed an
302 increase in the frequency of investigation events, but a decrease in the total duration of
303 investigation, from Test 1 to Test 2. This indicates that they were likely not habituating to the
304 cues. In addition, while rats and mice, in general, have a tendency to approach, explore and
305 interact with novel objects to gain more information about the odour source (Hurst et al., 1997;
306 Bevins & Besheer, 2006), if they were merely responding to the sheds as something novel, we
307 would expect both duration and frequency to decrease. The decrease in the total duration of
308 investigation suggests either active avoidance of the snake sheds, or a possible increase in
309 anxiety in response to the sheds, where rats may have identified the sheds as a remnant of a
310 snake, but not the actual snake itself (Mitchell et al., 2015). The increase in the frequency of
311 investigation events could also suggest increased exploration in an attempt to gain information
312 from the sheds (Chiszar et al., 1976; Misslin & Ropartz, 1981), as seen in faecal matter age
313 assessment by skinks (*Egernia* sp., Bull et al., 1999).

314 Discrimination is the differentiation between two or more cues, resulting in specific directed
315 behavioural responses to these cues (e.g. avoid or investigate; Akkerman et al., 2012). Because
316 species-specific skin-derived chemicals in snakes have been identified (Weldon & Schell, 1984;
317 Mason & Parker, 2010), and given the highly developed VNS of rodents, we expected that
318 mosaic-tailed rats would discriminate between the different snake predator odours. Instead, we
319 found that the mosaic-tailed rats did not discriminate between snake species based on the sheds.
320 This contrasts other studies showing that some animals can discriminate between different types
321 of predators. For example, dwarf chameleons *Bradypodion taeniabronchum* showed different

322 colour responses to boomslang *Dispholidus typus* or fiscal shrike *Lanius collaris* models (Stuart-
323 Fox et al., 2008), and blue tits *Cyanistes caeruleus*, great tits *Parus major* and willow tits *Poecile*
324 *montanus* spent more time mobbing a more dangerous sparrowhawk *Accipiter nisus* than a less
325 dangerous Siberian jay *Perisoreus infaustus* (Hogstad, 2017). Instead, our results suggest two
326 possibilities: 1) Mosaic-tailed rats can discriminate between snakes, but choose not to act on the
327 information. However, because we cannot assess a rat's reasoning for not acting, we suggest
328 rather 2) that investigation of these cues represents a general strategy for assessing information
329 associated with reptilian predators. Generalised responses to particular predator types, such as
330 reptiles, birds, and mammals, are common in mammals, as generalised responses provide
331 protection from unknown or novel predators that may be similar to known predators (Ferrari et
332 al., 2008).

333 Several studies have suggested sex-specific differences in behaviour (e.g. activity and
334 aggression, Beatty, 1979; exploratory behaviour, King et al., 2013). We found that mosaic-tailed
335 rats displayed some sex-specific responses to the snake sheds, with males visiting the snake
336 sheds in Tests 1 and 2 significantly more frequently than females. This suggests some sexual
337 differentiation in decision-making, as seen in three-spine sticklebacks *Gasterosteus aculeatus*,
338 where males are bolder, and take more risks than females (King et al., 2013). This could be due
339 to differences in gonadal hormone expression (Beatty, 1979), which influence neural circuitry in
340 the decision-making centres of the brain, namely the amygdala and the hypothalamus (Beatty,
341 1979; Choi & Kim, 2010; Bind et al., 2013). In addition, in Test 3, captive-born individuals
342 investigated the sheds more frequently than wild-caught individuals, suggesting that the general
343 novelty of the cues may have impacted their assessment. However, no other patterns were
344 observed between captive-born and wild-caught individuals. As the sample size of captive-born

345 rats was small, these results should be treated with caution, and future studies should explore this
346 in more detail.

347 We also found that individual rats responded differently to the snake sheds, with some
348 showing significantly different behaviours compared to others. Investigation rates have been
349 shown to differ between individuals in other species (e.g. minnows *Phoxinus phoxinus*,
350 Magurran, 1986). Studying a group collectively can lead to masking of specific individual
351 responses and underlying variation (Vilhunen & Hirvonen, 2003), presenting an inaccurate view
352 of predation risk assessment at the individual level. In addition, it is important to focus on
353 individual differences, because it represents the phenotypic variation on which selection acts
354 (Pavlicev et al., 2010).

355 Our study suggests that mosaic-tailed rats recognise snake predators using olfaction, but
356 they do not discriminate between different snakes, indicating that they may show a generalised
357 response to snake predator cues. Mosaic-tailed rats visited the perceived threat with increasing
358 frequency from Test 1 to Test 2, possibly to gain information from the sheds, rather than
359 showing innate recognition and avoidance, as seen in house mice (Papes et al., 2010).
360 Understanding the behavioural responses of small tropical rainforest mammals to the presence of
361 predators could give greater insight into their responses to other potential threats. As tropical
362 rainforests are highly dynamic and unpredictable, how animals respond to threats in these
363 landscapes will also give us a greater understanding of predator-prey dynamics in these complex
364 ecosystems.

365

366 *Supplementary material*

367 Supplementary data are available at online.

368

369 **Acknowledgements**

370

371 This work was supported by College of Science and Engineering support funds from James Cook
372 University (to KP and TLR). We thank Michael Grigg, Ryan Henson, and Angela Thurston for
373 generously volunteering their time to assist with data collection, Dr Tobin Northfield for
374 statistical assistance, and Dr Will Edwards for use of laboratory space. We thank Hartley's
375 Crocodile Farm and Vanessa Neale for their generous donation of snake sheds for this
376 experiment. We also thank two anonymous reviewers whose comments greatly improved the
377 manuscript.

378

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502 **List of figures**

503

504 **Figure 1.** Log total duration of investigation (s) in Tests 1 and 2 by individual fawn-footed
505 mosaic-tailed rats *Melomys cervinipes* of snake odour cues (Group 1: solid line received
506 Stimson's python shed *Antaresia stimsoni* in Test 1 and red-bellied black snake shed *Pseudechis*
507 *porphyriacus* in Test 2; Group 2: dotted line received the opposite shed in each test) and a paper
508 control presented over two tests in a Y-maze task. Inset figures show general statistical trends
509 (Mean \pm SE) for the factor Cue (top: Test 1; bottom: Test 2) and Cue*Test (bottom: Test 1), with
510 an asterisk indicating significant differences.

511 **Figure 2.** Raw duration of the first investigatory event (s) in Tests 1 and 2 by individual fawn-
512 footed mosaic-tailed rats *Melomys cervinipes* of snake odour cues (Group 1: solid line received
513 Stimson's python shed *Antaresia stimsoni* in Test 1 and red-bellied black snake shed *Pseudechis*
514 *porphyriacus* in Test 2; Group 2: dotted line received the opposite shed in each test) and a paper
515 control presented over two tests in a Y-maze task. Inset figures show general statistical trends
516 (Mean \pm SE) for the factor Cue (both tests), with an asterisk indicating significant differences.
517 Note: Individual HS23.1 was not included in the analysis for this behaviour only because of an
518 inability to accurately assess the first investigatory event in Test 1.

519 **Figure 3.** Log number of investigation events in Tests 1 and 2 by individual fawn-footed mosaic-
520 tailed rats *Melomys cervinipes* of snake odour cues (Group 1: solid line received Stimson's
521 python shed *Antaresia stimsoni* in Test 1 and red-bellied black snake shed *Pseudechis*
522 *porphyriacus* in Test 2; Group 2: dotted line received the opposite shed in each test) and a paper
523 control presented over two tests in a Y-maze task. Inset figures show general statistical trends

524 (Mean \pm SE) for the factor Cue (top in both tests), Sex*Cue (bottom: Test 1) and Test (bottom:
525 Test 2), with an asterisk indicating significant differences.

526 **Figure 4.** Raw number of investigation events in Test 3 by individual fawn-footed mosaic-tailed
527 rats *Melomys cervinipes* (Group 1: solid line received Stimson's python shed *Antaresia stimsoni*
528 in Test 1 and red-bellied black snake shed *Pseudechis porphyriacus* in Test 2; Group 2: dotted
529 line received the opposite shed in each test) presented with both snake odour cues (python or red-
530 bellied black snake) in a discrimination Y-maze task. Inset figures show (top) significant
531 differences for five individual fawn-footed mosaic-tailed rats (females = HS36, HP31 and HS23;
532 males = RF51 and HS38), and general statistical trends (Mean \pm SE) for the factor Origin, with
533 an asterisk indicating significant differences.