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

Olfactory recognition in *Melomys cervinipes*

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Summary

Rodents rely on their sensitive olfactory systems to detect and respond to predators. We investigated the ability of a native Australian rodent, the fawn-footed mosaic-tailed rat *Melomys cervinipes*, to detect, recognise, and discriminate between two species of native snakes. We used snake sheds from a sympatric venomous red-bellied black snake *Pseudechis porphyriacus* and a non-sympatric non-venomous Stimson's python *Antaresia stimsoni*. 20 mosaic-tailed rats each experienced three olfactory tests using a Y-maze. Rats were first exposed to one snake shed against a paper control, and then exposed to the other snake shed against a paper control. Which rat experienced which shed first was allocated randomly. Mosaic-tailed rats were then exposed to both sheds simultaneously. Rats could detect the snake sheds, spending longer investigating, and making more visits to, the sheds than the paper control. They also recognised the sheds as potentially dangerous, reducing their total investigation over time, but increasing their frequency of visits. However, rats did not discriminate between sheds, suggesting a general strategy for assessing the identity of reptilian predators.

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

Introduction

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

Different species use different responses to avoid or reduce risks. For example, stoneflies *Paragnetina media* use crypsis to hide (Feltmate & Williams, 1989), while swallowtail butterflies *Papilio machaon* use noxious, distasteful chemicals and aposematic colouration to deter predators (Pasteels et al., 1983). However, some prey species must first detect a predator using one or more sensory cues before responding to minimise the risk of predation. These cues can be visual, such as shadows cast from flying predatory birds (Sordahl, 2004), olfactory, such as urine odours of roving carnivorous mammals (Hayes et al., 2006), auditory, such as the noise of rustling in leaves by predatory reptiles (Kindermann et al., 2009), or a combination of these. Following detection of the cue, prey should assess the threat, and respond to minimise the risk of predation (Kindermann et al., 2009; Hodges et al., 2014). For example, pallid gerbils *Gerbillus perpallidus* respond to auditory and visual cues of avian predators by avoiding the time of day and locations where these birds occur (Kindermann et al., 2009).

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

Recognition and discrimination may then follow detection of an odour cue (Ache & Young, 2005; Bind et al., 2013). It is important to distinguish between these concepts because detection of a cue does not necessarily result in immediate recognition or discrimination of that cue, and recognition does not always lead to discrimination. Recognition is defined as a response to a stimulus that is either repeatable, if previously encountered, or predictable, if novel, but of a similar nature to another experienced stimulus (Mendelson, 2015). Recognition may be innate. For example, house mice *Mus musculus* bred and raised in captivity hide and freeze in response to odours of cats, rats and snakes, even without prior exposure, suggesting that these olfactory cues activate innate behavioural responses (Papes et al., 2010). However, recognition may rely on learning the properties of a cue and the associated risk, such as the observed lack of behavioural response of predator-naïve house mice to different predator and non-predator avian auditory cues (Kindermann et al., 2009). In contrast to recognition, discrimination is the ability to differentiate or distinguish between two or more cues because the animal has a specific memory associated with each stimulus (Akkerman et al., 2012). For example, vervet monkeys *Chlorocebus pygerythrus* respond to avian predators by looking upwards, whereas they respond to land-based mammalian predators by climbing trees (Seyfarth et al., 1980). While recognition without discrimination can occur (Overman et al., 1992), discrimination between different odour cues cannot occur in the absence of recognition of those cues.

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

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

Mosaic-tailed rats can detect the presence of mammalian predators and carpet pythons *Morelia spilota variegata* in their natural environment via olfactory cues in predator faecal matter (Hayes et al., 2006). While they avoided the mammal faeces, they did not avoid the python faeces, which Hayes et al., (2006) attributed to the low frequency of python defecation, making their faeces unreliable cues. However, two alternative explanations are possible: 1) Mosaic-tailed rats may not have recognised the cues, and therefore treated them as they would a non-predatory cue. Hayes et al. (2006) showed no significant difference in standardized visitation rates of mosaic-tailed rats between a blank control and the carpet python odour, suggesting that they could detect it, but did not recognise it. Because the blank used in Hayes et al.'s (2006) study was an attractant (linseed oil), no difference in visitation rate between odour stations and the blank does not give an indication of disinterest, and could actually reflect some level of interest in the cue presented. Consequently, mosaic-tailed rats may not have avoided the faeces because they were novel cues, and were curious about them (neophilia; Shapira et al., 2013). Increased investigation of novel odours results from a need to gain more information about the odour source (Hurst et al., 1997). If recognition of python cues is not innate, and individuals had not encountered carpet pythons or their faecal matter before, they may merely have been attempting to gain information about the novel odour in their environment. 2) Mosaic-tailed rats may show a different behavioural response to snake faeces than the mammalian faeces because they can discriminate between predator types. Investigation of the cue could thus represent a different strategy for assessing the relative predation risk of a reptilian predator than a mammalian predator.

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

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

Materials and Methods

Subjects

Twenty-four mosaic-tailed rats were collected from forested areas on the James Cook University Cairns campus (16º49'S 145º41' E) in 2016 using Elliott traps. They were transferred to individual cages in the Animal Behaviour Laboratory on the campus and allowed to acclimate to captivity for at least four months before experiments began. Nine offspring were born in captivity in 2017. All rats were housed individually or in same-sex sibling pairs (until adult) in wire-frame cages with a rectangular plastic base (36 cm x 29 cm x 47 cm). Approximately 10 cm of wood shavings was provided for bedding, and a cylindrical plastic nest box (10 cm x 21 cm), hay and paper towel were provided for nesting material. Plastic wheels, a cardboard roll, wire climbing platforms, and sticks and branches were provided for enrichment. Each rat had access to water *ad libitum*, and received ± 5 g of mixed seeds and rodent chow, and ± 5 g of fruits or vegetables (e.g. apple, cucumber) daily.

Study design

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

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

In Test 1, individual mosaic-tailed rats were presented with a choice between a snake shed and a paper control. The choice of shed (python or red-bellied black snake) was randomly selected for each individual. Immediately prior to testing, the shed was removed from the freezer, and a 1 cm^2 piece was cut and placed in a 1.5 ml plastic Eppendorf tube with small holes punched through the lid. The shed thawed within 5 min. A 1 cm^2 piece of plain white paper was placed into a second plastic Eppendorf tube, also with holes in the lid. The two tubes were then placed in the maze, one in each test box, with side selected randomly. The test rat was then placed in the neutral box of the maze, and allowed to acclimate to the neutral box for 5 min. Thereafter, the plastic barrier was removed, and the rat was given 20 min to explore the maze. The whole apparatus was filmed from above, and each treatment box was also filmed, using Panasonic HD HC-V110 video cameras. No observers were present in the room during recording sessions. Using continuous sampling, we scored the total duration of time spent investigating (sniffing and/or chewing) each tube, the duration of the first investigatory event for each tube (shed vs. paper control) and the number of separate investigation events of each tube.

Test 2 occurred as for Test 1. However, individuals were tested with the odour cues of the other snake shed against the paper control (i.e. if a rat experienced python and paper in Test 1, it received red-bellied black snake and paper in Test 2; and *vice versa*). Test 3 occurred as for Tests 1 and 2. However, in Test 3, individuals were presented with both snake sheds, each randomly allocated to a side. All individual rats were exposed to both snake sheds against the paper control before they were exposed to the two-shed direct comparison to eliminate the possibility that the response to the sheds was due to their novelty, rather than their odour quality. The same behaviours were recorded for Tests 2 and 3 as for Test 1. The Y-maze and boxes were thoroughly washed after each test and wiped with ethanol to remove any residual odours that could cause bias in subsequent tests (Bind et al., 2013). Tests occurred 1-2 weeks apart, with rats remaining in their home cages during the intervening period.

Statistical analyses

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

To determine if mosaic-tailed rats could detect and recognise the odour cues, we first ran separate linear mixed effects models (LMER) or generalised linear mixed effects models (GLMER with negative binomial distribution; lme4 package) for each behaviour (total duration of time spent investigating the cues, duration of the first investigatory event, and number of separate investigation events) for Tests 1 and 2 combined. Origin (captive-born or wild-caught) Sex, Test (1 or 2), Group (1 = received python shed first; 2 = received red-bellied black snake shed first) and Cue ("snake" or paper) were fixed factors, individual identity (ID) was the random factor, and behaviour was a continuous predictor. We included all interactions between fixed factors, except for Origin, given its low sample size, although we did include the interaction between Origin*Cue to assess whether captivity influenced olfactory ability. We chose to include all other statistical interactions in our model because excluding some factors (e.g. Origin, Sex and Group) could cause masking of trends, leading to generalisations for the species that may not be correct, although we acknowledge that fewer interactions would be more appropriate for analyses with small sample sizes. We then ran likelihood ratio tests to determine the significance of fixed factors. Specific differences were identified using the differences of least squares means method (available in the lmerTest package).

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

Ethical note

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

Results

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

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

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

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

Discussion

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

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

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

Discrimination is the differentiation between two or more cues, resulting in specific directed behavioural responses to these cues (e.g. avoid or investigate; Akkerman et al., 2012). Because species-specific skin-derived chemicals in snakes have been identified (Weldon & Schell, 1984; Mason & Parker, 2010), and given the highly developed VNS of rodents, we expected that mosaic-tailed rats would discriminate between the different snake predator odours. Instead, we found that the mosaic-tailed rats did not discriminate between snake species based on the sheds. This contrasts other studies showing that some animals can discriminate between different types of predators. For example, dwarf chameleons *Bradypodion taeniabronchum* showed different colour responses to boomslang *Dispholidus typus* or fiscal shrike *Lanius collaris* models (Stuart-Fox et al., 2008), and blue tits *Cyanistes caeruleus*, great tits *Parus major* and willow tits *Poecile montanus* spent more time mobbing a more dangerous sparrowhawk *Accipiter nisus* than a less dangerous Siberian jay *Periosoreus infaustus* (Hogstad, 2017). Instead, our results suggest two possibilities: 1) Mosaic-tailed rats can discriminate between snakes, but choose not to act on the information. However, because we cannot assess a rat's reasoning for not acting, we suggest rather 2) that investigation of these cues represents a general strategy for assessing information associated with reptilian predators. Generalised responses to particular predator types, such as reptiles, birds, and mammals, are common in mammals, as generalised responses provide protection from unknown or novel predators that may be similar to known predators (Ferrari et al., 2008).

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

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

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

Supplementary material

Supplementary data are available at online.

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List of figures

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

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

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

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

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