CHAPTER 5

Chemosensory communication
ABSTRACT

Lizards in the *Egernia* genus are predominantly social. Communication is an important means by which social interactions can be facilitated. Chemosensory communication is one method by which lizards may transfer information. Scat piling has the potential to be an efficient source of such chemosensory information. The cooperatively scat piling *Egernia whitii* (White’s skink) has not previously been studied for chemosensory communication. To discover if *E. whitii* can transfer information about each other via scats, individual skinks were presented with three scats simultaneously while in their home enclosures, and filmed to discover responses to scent stimuli in the scats. The scat sources were their own, a conspecific, and a congeneric skink (*E. multiscutata*).

Data gathered from video taped experiments showed that *E. whitii* use tongue flicks, olfaction and scent marking to differentiate between scent stimuli. They scent mark locations bearing scats from within-species sources significantly more often than congeneric locations. The results imply that resident skinks are able to distinguish between their own scent and the scent of other skinks, both within their species, and within their genus, and that scent marking has a communicative role within the *E. whitii* species. The chemical signatures of other skinks have different connotations for males and females, and responses are size dependent. Information derived from scats would be advantageous for group living, territorial maintenance, agonism avoidance, dispersal, immigration and refuge sharing with ‘compatible species’.

INTRODUCTION

The *Egernia* genus is primarily distinguished by one morphological character, in which the palatines curl partially around the nasal passage anteriorly above the secondary palate and the vomers send back long posterior processes along the medial edge of the open palatine scroll (Hutchinson 1983 *op cit*. Greer 1989). Greer (1989) notes an additional behavioural characteristic for the genus in the strong attachment to a permanent retreat or shelter site around which all activities are centred, and that this is unique amongst Australian skinks.
Social behaviour

Species in the *Egernia* genus are unusual among lizard species in forming stable social aggregations (Greer 1989, Hutchinson 1993, Gardener et al. 2001, Fuller et al. 2005), with reports of social complexity in 23 of the 30 described species (Chapple 2003). Widespread social complexity within the genus has prompted a description of *Egernia* as a lineage that represents an independent origin of social organisation which is in some ways comparable to birds, mammals and social insects (Chapple 2003). Chapple (2006) describes *E. whitii* social groups as stable.

Social behaviour in a broad sense can be defined as behaviour displayed by a group of individuals that interact with each other (Martin & Hine 2000). Crozier (1999) narrows the description by specifying social behaviour as activity directed differentially amongst members of the same species, and including sexual behaviour. He identifies a society as a group of cooperating individuals of the same species involved in reciprocal communication, which goes beyond sexual behaviour.

Moore (1999) defines communication as ‘the transfer of information between animals using visual, audible or chemical means’. Social organisation then, according to these definitions, necessarily involves associations of interacting individual conspecifics, whose behavioural repertoires include cooperation and reciprocal communication.

Pamilo (1999) summarises the mutual attraction of conspecific individuals by suggesting that many species live either permanently or temporarily in social associations despite the likelihood of conflicting interests leading to territoriality and agonism by competing individuals. He ascribes this behaviour to regulation by the costs and benefits of communal interactions, and by reproductive competition among individuals.

Noë (2006) however, ameliorates the competitive endorsement of many animal behaviourists by suggesting that although the agreement of territorial neighbours on the location of a mutually respected border is usually seen as ‘conflict resolution’, it actually fulfils the criterion of cooperation as much as it does that of competition. He suggests that cooperation and conflict are two sides of the same coin with ‘taste’ prescribing which side receives more attention.
Communication is sometimes described as a signal from another individual only if it initiates a reaction, or has a modifying effect on the behaviour of the reactor (Krebs & Davies 1996). However, the ‘reactive’ behaviours may be secretive, cryptic and very difficult to demonstrate in early research. Therefore in this thesis, the inclusive definitions of Crozier (1999) for social behaviour, Moore (1999) for communication, and Pamilo (1999), with amendments from Noë (2006) where applicable, for cooperative behaviour will be accepted throughout the text as authoritative, and they will set the criteria for the test species.

**Communication systems**

Across lizard species behavioural (Alberts 1994), visual (Martins 1994), tactile (Jennings & Thompson 1999, Stamps & Krishnan 1998, Ferguson 1977), auditory (Greer 1989) and chemo-sensory (Simon et al. 1981, Cooper & Vitt 1984) processes have been identified in communication systems. Different families of lizards exhibit discrete assertive, fighting, territorial defence, non-dominant and contact behaviours which have conventionally been described as ‘stereotypical’ (Carpenter & Ferguson 1977).

Visually oriented lizard species such as iguanids and agamids tend to use overt signalling mechanisms in their interactions, and incorporate spontaneous displays and brilliant colours with more subtle movements like rapid head bobs and arm waves (Stamps 1977). However secretive and nocturnal lizards like the scincids and geckonids are more reliant on chemosensory and acoustic stimuli (Evans 1961 *op. cit.* Glinski & Krekorian 1985).

Although scincid lizards have not developed the varied and extensive range of postural behaviours for signalling that are present in some other families, they do have well developed olfactory and vomerolfactory structures. Snakes are acknowledged as vomeronasal specialists, but the vomeronasal sensory capacity in scincid lizards is essentially unknown (Rehorek et al. 2000).

Schwenk (1993) and Dial & Schwenk (1996) have put forward a case that gekkotan lizards may, in contrast, be olfactory specialists, although more information is required to confirm this, and there is evidence that gekkotan vomeronasal organs are similar to scincid lizards (Schwenk 1993, Gabe & Saint Girons 1976 *op cit.* Rehorek et al. 2000). The long posterior processes that are part of the vomers found in the
Egernia genus have not attracted research attention at this stage and whether they have a purpose other than to separate respiration from ingestion is unknown.

**Chemosensory communication**

Body odours provide an innate and effortless means of signalling, even though they are assumed to be ultimately costly. Communication on a cellular level is defined by Fox (2004) as chemical signalling sensing, and response, and described as the essential and universal framework of all living organisms that unites all ecosystems. She goes on to say that chemical signals used either within an organism or between organisms rely on efficient and accurate interpretation by receptors. Chemicals carried in urine, faeces or external glands can be a rich source of information about the identity, sex, social status, aggressive motivation, and other attributes of an animal, combining effortlessly with other communication systems.

The release of urine, faeces or pheromones as complex signals regulating animal behaviour has been demonstrated in a range of taxa. Research by Breithaupt & Atema (2000) has documented the release of urine during dominance fights by male lobsters as related to offensive behaviour, which increases as the intensity of agonistic behaviour increases. The timing and frequency of urine releases appears to improve both the reliability and efficacy of the chemical threat signal.

Mice can recognise one another by individually characteristic phenotypic body odours that reflect their genetic constitution at the highly polymorphic major histocompatibility complex (MHC) of genes on chromosome 17 (Beauchamp et al. 2000). MHC-determined odours are produced by foetuses, and males associate differentially between genetically identical pregnant females carrying 8-18-day-old foetuses of differing MHC type. Previous housing and/or mating experience modulates male choice in this context.

Bees distribute pheromones during their dance sequences that appear to regulate recruitment for forager activity (Dornhaus & Chittka 2001), they deposit substances on particularly rewarding flowers resulting in more frequent visits (Cameron 1981) and repellent signals on unrewarding flowers (Giurfa 1993). Bee pheromones exert physiological effects on individuals and communicate group social structure (Pankiw et al. 2004).
Recent advances in avian research have detected drawn attention to the functional olfactory systems and the production of odours in birds which have previously been omitted in research (Hagelin et al. 2003). Male bower birds ‘paint’ the inside wall of the bower which they form with sticks, by wiping the surface with masticated plant material. During courtship visits to the bowers females nibble this plant-saliva mixture which is thought to function as a chemical sexual signal (Bravery et al. 2006). Crested auklets show an attraction to conspecific feather odour, showing preference for two chemical components of feather scent which are seasonally elevated, and they differentiate between odours from different sources (Hagelin et al. 2003).

Carnivores (Gorman & Trowbridge 1989 op. cit. Chame 2003), badgers (Stewart et al. 2001, Begg et al. 2002) and rabbits (Sneddon 1991) have all demonstrated scat piling or latrine behaviours and research suggests that their scats are deposited primarily as social signals. The complicated nature of the behaviours exhibited spans territorial marking, dominance, group membership, sexual assignment, reproductive readiness, and social status.


Individual odours can provide cues to guide behaviour concerning familiar and unfamiliar individuals (Cooper 1996, Bull et al. 2000), relatedness (Bull et al. 2001), intruders (Bull et al. 2001), seasonal pair bonds (Olsson & Shine 1998), and female receptivity (Head et al. 2005). In contrast, conspecific and congeneric and interspecific competition appear to be primarily size related (Bruyn 1994, Langkilde & Shine 2004, Bellamy 2006), and therefore visually assessed by the participants.

Scat piling is strongly linked with social function and communication. Because these elements play such an important role in social behaviour, understanding the role of scats in the transferral of information is essential if scat piling behaviour in lizards is to be understood. I conducted a further investigation to discover what basic
information could be obtained by allowing *E. whitii* access simultaneously to chemosensory scent contained in a scat of their own, a conspecific scat and a scat of a sympatric species of the same genus, *E. multiscutata*.

**MATERIALS AND METHODS**

**Test specimens and experimental arenas**

The study site was Wedge Island in the Spencer Gulf of South Australia (35°09'S, 136°27'E). Twenty four adult and subadult individuals were captured in March 2001, and 23 gravid females were captured during early December in 2002 and 2004. Captures were from haphazard locations across the island and the lizards were retained for laboratory experiments and observations. Each skink was maintained in an individual glass tank 600 mm long x 305 mm wide x 300 mm deep, lined with 20-30 mm of sand.

Each aquarium was provided with a 190 mm x 190 mm x 30 mm basking paver at one end supported 30 mm above the aquarium surface by two lengths of angle plastic. A flat plastic water dish of 85 mm diameter was positioned on top of the refuge. At the opposite end of the tank were several rocks for basking. The refuge and rocks were positioned on the tank floor before sand was introduced to prevent damage to the animals by subsidence.

All tanks were covered with cardboard sheets along each side to block out the visual reminder of neighbours that may have been familiar prior to capture. A heat lamp warmed the basking area at 32°C for eight hours a day from 8.30am to 4.30 pm, and photoperiods were 12 hours light and 12 hours dark during each 24 hour period. Experimental areas were kept at 25°C constant temperatures. Water was available continuously and skinks were fed three times a week – one meal of crickets, one meal of meal worms and one meal of vitamised vegetables and fruit sprinkled with reptile supplement.

At intervals each tank and its equipment was washed thoroughly with hot soapy water and sprayed with 70% ethanol before fresh sand was laid down. Equipment was handled using Microflex ® powder-free disposable latex gloves. Each skink was tested in its own enclosure to eliminate tongue flick behaviour and activity patterns associated with a new arena.
Experimental procedure

New half-pavers (dimensions 95mm x 190mm x 30mm) were painted with two coats of silicon sealant to reduce porosity and prevent retention of scent during the experimental period. The pavers were well aired and then permanently marked into three 20mm circles, 50mm apart. A container of different sized scats was collected from *E. multiscutata* aquariums over a period of time and allowed to age for ≥3 weeks to reflect potential field conditions before experimental trials began. All scats were matched to size during recognition tests.

Microflex ® powder-free disposable latex gloves were used for handling all equipment and pavers to ensure minimal scent contamination during experiments. The home tank of each of the *E. whitii* adults was prepared by removing all trace of scats or urine 24 hours before the trial began. In each 24 hour period during the trial fresh *E. whitii* scats were collected using sterile forceps, and stored in separate sealed containers individually labelled for each test skink. Unused scats were discarded after two days.

The marked experimental pavers were prepared for scent recognition immediately before recognition experiments began by placing a different scat in the centre of each of the three circles. Each animal received a paver with one fresh scat of their own, one fresh scat of an unfamiliar conspecific, and an aged *E. multiscutata* scat, placed in random order (figure 1). Scats were matched for size, and the conspecific donor SVL was matched to within 1 mm to the SVL of the resident. The experimental paver was positioned on top of the resident’s refuge, against the aquarium wall so that it created a step (Figure 1).

All skinks were filmed during their normal daily activity times and the time of day was recorded.
Figure 1: Diagrammatic representation showing plan view of experimental paver with scats *in situ*, and side view of filming arena with camera field of view indicated.

Early trials indicated that the resident might remain inside the refuge for the entire length of the test period, and as a consequence the struts were removed from underneath the refuge immediately prior to the test so that the resident had nowhere to retreat and was exposed to the scent cues. A video camera was focussed onto the refuge paver and ~20 mm of its surrounding edge. The camera was connected to a video recorder and each replicate was taped for three hours. Skinks that remained out of view were refilmed at a later time. Trials were commenced by starting the video recorder and placing the experimental paver in position. I then left the room to minimise observer influence.

At the completion of each experimental replicate the paver and the scent cues were removed, and the aquarium was restored to its original form. The paver was washed thoroughly with warm soapy water and dried with fresh paper towel to remove all traces of previous scent before being reused.

Twenty-four tests took place in June, July and August 2001, and a further 16 took place in January and February 2005 (N = 40). Twelve tests used female residents and female donor lizard scats, 12 tests used male residents and male donor lizard scats, and in 16 tests the sex of resident or donor skink was either unknown or the
sexes were mixed.

**Data collection**

There were 40 replicates in the trial using both male and female skinks. Experimental data were only collected when the skink’s head was in full view of the camera lens. Each video tape was viewed initially to discover common responses amongst test skinks and to interpret responses in the context of functional social behaviour. I wanted to isolate the recognition responses from the communication responses, and to establish a consistent method of interpretation for future chemosensory trials.

To this end I created a general template for use while viewing and interpreting films, recording the identification of the resident skink and the donor skink, the date and time of the experiment, the duration of the trial, location of each scent cue, SVL of resident skink, and each behavioural change (Table 1: Ethogram). Time discrimination was in seconds. The data gained were copied into files in the statistical analysis program SPSS ® version 11.5 for Windows for analysis.

I followed the recommendation of Pallant (2002) and used one-way repeated measures ANOVA, as subjects were exposed to two or more different conditions. Total durational values for overhead TF’s, snout in contact and scent marking were analysed and compared across the group for the three different scent cues using one-way repeated measures ANOVA. According to Stevens (1996) this analysis is powerful because by elimination of systematic bias and reduction of error by blocking on each subject, variability among the subjects due to individual differences is completely removed from the error term. When a significant result was obtained, paired t-tests were used to discover which responses differed from one another.

**Ethogram**

The design of recognition experiments vary among researchers according to the characteristics and responses of their subjects. Field captured *E. whittii* proved to be timid and reticent in my presence. An ethogram of recognition behaviours was developed following initial viewing of the tapes to investigate *E. whittii* idiosyncratic behaviour in response to chemosensory stimuli (Table 1). This is not a comprehensive repertoire but represents behaviour characteristic of the majority of
residents used in the trial. Behaviours are recorded as either durational data recorded in seconds or spike-event data. Data types are indicated on the ethogram.

Table 1: Ethogram based on observations of resident skink behaviour during chemosensory trial to detect differentiation between their own, conspecific and E. multiscutata scat scent cues. Asterisk marks comparative data.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Data type:</th>
<th>Description of behaviour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distant tongue flicks (TF’s)</td>
<td>S</td>
<td>Number of tongue extrusions into air or onto refuge surface before direct investigation of the scent cues</td>
</tr>
<tr>
<td>Order of interest</td>
<td>S</td>
<td>Order of approach to different scat cues</td>
</tr>
<tr>
<td>Period on refuge</td>
<td>D</td>
<td>Period of time spent on the refuge before approaching the experimental paver</td>
</tr>
<tr>
<td>Period on experimental paver</td>
<td>D</td>
<td>Period of time spent on the experimental paver</td>
</tr>
<tr>
<td>Number of passes</td>
<td>S</td>
<td>Number of times the resident skink stood, either fully or with front legs and majority of body, on the experimental paver and then left it again</td>
</tr>
<tr>
<td>Track</td>
<td>S</td>
<td>Attempts to follow a scent trail and/or dig under pavers</td>
</tr>
<tr>
<td>Rub</td>
<td>S</td>
<td>Rubbing chin or jaw on experimental paver</td>
</tr>
<tr>
<td>Touches</td>
<td>S</td>
<td>Number of times resident skink touched each scat (including taking scat in mouth or tossing it)</td>
</tr>
<tr>
<td>Overhead TF’s</td>
<td>S</td>
<td>Number of TF’s directed towards the scat at close range (\leq 20 mm)</td>
</tr>
<tr>
<td>Snout in contact</td>
<td>D</td>
<td>The period of time spent with snout in contact (SIC) or within \leq 20 mm of scat for both first 3 passes, and the total filming time</td>
</tr>
<tr>
<td>Scent marking</td>
<td>D</td>
<td>Period of time spent with cloacal area resting, rubbing or dragging over the scent area (paver partitioned into thirds for this assessment) for both first 3 passes, and the total filming time</td>
</tr>
<tr>
<td>Total contact time</td>
<td>D</td>
<td>Collective period of time spent on overhead TF’s, snout in contact and scent marking for first 3 passes, and the total filming time</td>
</tr>
</tbody>
</table>

RESULTS

General behaviour

Spike events and general durational behaviour not directed differentially towards individual scats are presented in Tables 2 & 3. Some animals remained out of camera view for several hours before investigating. Although the time of day was
recorded this was found to have no statistically significant effects on parameters recorded. There was no significant difference between mean time spent on the experimental paver and time spent on the refuge paver, although two resident skinks (5% of total sample) did not spend any time on the refuge paver, instead scaling the stacked paver sides to land directly on the experimental paver, and leaving the same way.

The pattern of skink activity normally includes time under the refuge paver, or time on the rocks under the heat lamp, and visits to the upper surface of the refuge paver to drink from the water dish (pers. obs.). Therefore these frequent visits suggest that they are attracted to scats bearing scents from other lizards.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distant tongue flicks</td>
<td>5</td>
<td>113</td>
<td>48.73</td>
<td>4.30</td>
</tr>
<tr>
<td>Period before approach</td>
<td>49</td>
<td>5013</td>
<td>838.58</td>
<td>148.47</td>
</tr>
<tr>
<td>Time on refuge paver</td>
<td>0</td>
<td>715</td>
<td>147.15</td>
<td>28.64</td>
</tr>
<tr>
<td>Time on experimental paver</td>
<td>21</td>
<td>656</td>
<td>160.17</td>
<td>22.26</td>
</tr>
<tr>
<td>Number of passes</td>
<td>1</td>
<td>70</td>
<td>11.15</td>
<td>2.17</td>
</tr>
</tbody>
</table>

Resident skinks showed no preference order in investigating experimental scent cues. While more test skinks touched the conspecific scat during their investigation of the scent cues than either their own or the *E. multiscutata* scat, the results of a Chi² test showed that the difference was not significant ($X^2 = 2.637$, d.f. = 2, $P = 0.2675$).

Figure 2 shows the proportion of trials in which resident skink came in contact with each scat scent cue used in the experiment.
Figure 2: Proportion of trials in which resident skink came in contact with each scat scent cue used in the experiment.

Table 3: Order of investigation for scent cues, and number of skinks with snout or tongue contacting the scat cue during the trial.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Own scat</th>
<th>E. multiscutata</th>
<th>Conspecific</th>
</tr>
</thead>
<tbody>
<tr>
<td>Investigated first</td>
<td>15</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>Investigated second</td>
<td>10</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>Investigated third</td>
<td>11</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Contacts</td>
<td>24</td>
<td>24</td>
<td>30</td>
</tr>
</tbody>
</table>

All resident skinks (100%) engaged in ‘chemosensory tracking/searching behaviour’. This consisted of one or more of three actions: 1) Tongue flicking or contacting the paver surfaces in directions radiating away from the scats, 2) Contacting the paver surfaces prior to directly approaching the scats, or, 3) Vigorous digging or scratching at the interfaces between the experimental and refuge pavers, or between the experimental paver and tank wall.

Twenty-seven resident skinks (67.5%) rubbed their chin/jaw area on the upper paver. This was a sideways twisting motion achieved by arching the neck, twisting the head, and applying downward pressure to the paver surface. It is possible that this is some form of scent marking but scent glands have not been investigated in this species. *E. whitii* lack femoral pores and must rely on other sources of scent to signal (pers.
A striking feature of the majority of resident reactions was the large number of tongue flicks directed towards the scent stimuli from a distance. Only five resident skinks (12.5%) directed < 20 tongue flicks towards the experimental paver from a distance, with a mean rate of > 48 tongue flicks before closer inspection (Table 2). The number of distant tongue flicks was also related to SVL. Smaller skinks directed a greater number of tongue flicks towards the experimental paver from a distance (Figure 3). I conducted a regression analysis and this showed that the effect was significant ($t = 3.779, \beta = -0.523, P = 0.001$).

![Figure 3: The number of distant tongue flicks as a function of body length (SVL). A regression line has been fitted.](image)

**Recognition and communication behaviour**

Data were collected over the length of three-hour filmed responses, irrespective of the time lapse before initial response to the scent cues. There was extreme variability in individual responses and between one and 70 passes per individual were made to investigate the scent cues. Some individuals repeatedly returned to the scent cues, moving their heads from side to side in rapid visual scanning movements, showing
jerky searching movements.

In the majority of tests one or more of the scats used as scent cues were dislodged from their position on the experimental paver by the third pass. Data were collected from all responses, but only those observations obtained during the period the scats remained inside the marked circles on the experimental paver were used for statistical analyses.

The majority of investigatory passes occurred in the early phase of investigation as shown in Figure 4. For this reason I analysed the data over two different time periods: 1) Immediate response - the first three passes, or less if a scat or scats were dislodged, and, 2) Response over time - the full period of response time. The data collected for the immediate response has been analysed to investigate recognition and communication responses. Data collected for the response over time are used in the section below on male and female behavioural differences.

![Figure 4: The number of times individual skinks passes across the experimental paver as a function of the period time spent investigating. A regression line has been fitted.](image-url)
**Overhead tongue flicks**

A one-way repeated measures ANOVA was conducted to compare the number of overhead TF’s directed towards the individual’s own scat, an *E. multiscutata* scat and a conspecific scat. The means and standard errors are presented in Table 4. There was a significant effect for TF’s (Wilks’ Lambda = 0.806, $F_{2, 38} = 4.578$, $P = 0.017$, multivariate partial eta squared = 0.194, indicating a large effect as per Cohen 1988).

**Table 4: Descriptive statistics for the number and direction of overhead tongue flicks directed towards three different scent cues by 40 individuals.**

<table>
<thead>
<tr>
<th>Scent cue origin</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual’s own scat</td>
<td>0</td>
<td>5</td>
<td>1.3</td>
<td>0.20</td>
</tr>
<tr>
<td><em>E. multiscutata</em> scat</td>
<td>0</td>
<td>11</td>
<td>2.35</td>
<td>0.45</td>
</tr>
<tr>
<td>Conspecific scat</td>
<td>0</td>
<td>16</td>
<td>2.58</td>
<td>0.53</td>
</tr>
</tbody>
</table>

Figure 5 shows the mean number of overhead tongue flicks directed towards the test skink’s own scat, an *E. multiscutata* scat and a conspecific scat. A paired samples t-test was conducted to determine the difference between the TF responses. There was a significant difference (2-tailed) between the number of TF’s directed towards their own scat and the *E. multiscutata* scat (Mean difference ($X$) = -1.05, SE = 0.43, $t_{39} = -2.44$, $P = 0.02$), and their own scat and a conspecific scat (Mean difference ($X$) = 1.28, SE = 0.58, $t_{39} = -2.21$, $P = 0.033$). This indicated that residents were able to distinguish between their own scent and the scent of other skinks, both within their species, and within their genus.
Figure 5: Error bar graph showing mean overhead tongue flick rate directed towards the test skinks’ own scat, an *E. multiscutata* scat, and a conspecific scat.

**Time in contact**

A one-way repeated measures ANOVA was conducted to compare the period of time spent (in seconds) with snout in contact (≤ 20 mm) with individual’s own scat, an *E. multiscutata* scat and a conspecific scat. The means and standard errors are presented in Table 5. There was a significant effect for contact time (Wilks’ Lambda = 0.791, $F_{2, 38} = 5.012$, $P = 0.012$, multivariate partial eta squared = 0.21, indicating a large effect as per Cohen 1988).

<table>
<thead>
<tr>
<th>Scent cue origin</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual’s own scat</td>
<td>0</td>
<td>32</td>
<td>3.70</td>
<td>0.86</td>
</tr>
<tr>
<td><em>E. multiscutata</em> scat</td>
<td>0</td>
<td>102</td>
<td>12.22</td>
<td>3.69</td>
</tr>
<tr>
<td>Conspecific scat</td>
<td>0</td>
<td>314</td>
<td>15.68</td>
<td>7.84</td>
</tr>
</tbody>
</table>
Figure 5 shows the mean time in contact directed towards the resident skink’s own scat, an *E. multiscutata* scat and a conspecific scat. A paired sample t-test was conducted to determine the difference between the contact time responses. There was a significant (2-tailed) difference between time in contact with their own scat and the *E. multiscutata* scat (Mean difference ($\bar{X}$) = 8.52, SE = 3.18, $t_{39} = 2.69$, $P = 0.011$). However there were no significant differences in the time spent in contact with their own scat and the conspecific scat ($\bar{X} = 11.98$, SE = 7.94, $t_{39} = 1.51$, $P = 0.14$), or the *E. multiscutata* scat and the conspecific scat ($\bar{X} = 0.48$, SE = 0.67, $t_{39} = 0.71$, $P = 0.48$).

The relationship of the error bars for *E. multiscutata* contact time and conspecific contact time in Figure 6, and the mean time spent in contact with the scent cues (Table 5), suggest that the possibility of a difference in response to *E. multiscutata* scats and conspecific scats should not be ruled out, and the high variability in responses to the conspecific scats should be noted (i.e. high standard error). A larger sample group may show a difference between these two scent cues. The results show that resident skinks allocate significantly less time to sampling the odour of their own scat than they do to sampling the odour of a congeneric species’ scat. This differentiation indicates that resident skinks are able to recognise their own scat as different from the scat of another species.
Chemosensory communication

Chemosensory cues

Figure 6: Error bar graph showing mean time in contact (in seconds) with resident skinks’ own scat, an *E. multiscutata* scat, and a conspecific scat.

**Scent marking**

A one-way repeated measures ANOVA was conducted to compare the period of time (in seconds) spent with the cloacal region resting, rubbing or dragging over the experimental area. The paver was divided into thirds during viewing of films for the collection of this data. Each third was centred by one of the scat cues: the individual’s own scat, an *E. multiscutata* scat and a conspecific scat. The means and standard errors are presented in Table 6. There was a significant effect for contact time (Wilks’ Lambda = 0.849, $F_{2, 38} = 3.37$, $P = 0.045$, multivariate partial eta squared = 0.151, indicating a large effect as per Cohen 1988).

Table 6: Descriptive statistics for the period of time spent (in seconds) by 40 individuals scent marking the experimental paver nearest to the scat of their own, an *E. multiscutata* scat, and a conspecific scat.

<table>
<thead>
<tr>
<th>Scent cue origin</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual’s own scat</td>
<td>0</td>
<td>269</td>
<td>41.30</td>
<td>9.06</td>
</tr>
<tr>
<td><em>E. multiscutata</em> scat</td>
<td>0</td>
<td>235</td>
<td>25.62</td>
<td>7.07</td>
</tr>
<tr>
<td>Conspecific scat</td>
<td>0</td>
<td>515</td>
<td>51.70</td>
<td>14.68</td>
</tr>
</tbody>
</table>

Figure 7 shows the mean scent marking time directed towards the resident skink’s
own scat, an *E. multiscutata* scat and a conspecific scat. Paired samples t-tests were conducted to examine potential difference between the scent marking responses. There was a significant (2-tailed) difference between scent marking time with their own scat and the *E. multiscutata* scat ($\bar{X} = 15.67$, SE = 6.16, $t_{39} = 2.55$, $P = 0.015$). However there were no significant differences in the time spent scent marking their own scat and the conspecific scat ($\bar{X} = 10.40$, SE = 14.48, $t_{39} = 0.72$, $P = 0.477$), or the *E. multiscutata* scat and the conspecific scat ($\bar{X} = 26.08$, SE = 15.85, $t_{39} = 1.65$, $P = 0.108$).

The relationship of the error bars for *E. multiscutata* scent cues and conspecific scent cues in Figure 7, and the mean time spent scent marking (Table 6), indicate that a difference in time spent scent marking may have been found if the variability in response to the conspecific stimulus had been smaller. A larger sample group may to show a difference between these two scent cues. The results show that resident skinks are leaving chemosensory information of their own in close proximity to scats from unknown conspecífics via scent marking, but not near the scats from another species. This indicates that they recognise their own species as different from another species in the genus, and attempt to communicate via chemosensory means with conspecifics.

![Figure 7: Error bar graph showing mean scent marking time (in seconds) with resident skinks' own scat, an *E. multiscutata* scat, and a conspecific scat.](image)
**Male and female behavioural differences**

Two resident skinks could not be reliably identified for sex. Analyses in which the effect of sex is shown therefore use N = 38.

The immediate response, as indicated by the first three passes for both male and female skinks, was similar (results of independent t-tests $P = > 0.05$), but when data for responses recorded over a longer period of time were analysed with independent t-tests, differences between the sexes emerged. Males tongue flicked the *E. multiscutata* scat more often than females ($X = 1.17, SE = 0.36$, males $X = 2.75, SE = 0.65$, $t_{36} = 2.07, P = 0.046$) (Figure 8). Females spent more time scent marking close to their own scats than males ($females \ X = 49.28, SE = 12.47, males \ X = 20.10, SE = 6.20, t_{36} = 2.16, P = 0.046$) (Figure 9). The total time spent in tongue flicking, snout in contact, and scent marking their own individuals scat was also significantly greater for females than males ($females \ X = 55.00, SE = 13.17, males \ X = 20.45, SE = 6.25, t_{36} = 2.45, P = 0.026$) (Figure 10).

![Figure 8: Mean number of tongue flicks to *E. multiscutata* scat of male and female residents (females N = 18, male N = 20).](image-url)
Figure 9: Mean time in seconds resident male and female skinks spent scent marking their own individual scat (female $N = 18$, male $N = 20$).

Figure 10: Mean total time in seconds resident male and female skinks spent with their own individual scat (female $N = 18$, male $N = 20$).
Independent t-tests showed a difference between the number of male and female distant TF’s (females $\bar{X} = 38.33$, SE = 6.69, males $\bar{X} = 58.65$, SE = 5.26, $t_{36} = -2.17$, $P = 0.036$) (Figure 11). Males tongue flicked towards the experimental paver from a distance more often than females (Figure 11), but spent less time investigating the scats in close proximity (Figure 12). There was a trend towards greater female investigation in close proximity (females $\bar{X} = 15.78$, SE = 4.22, males $\bar{X} = 6.65$, SE = 1.66, $t_{36} = 2.09$, $P = 0.057$) (Figure 12).

![Figure 11: Mean number of distant tongue flicks directed towards the experimental paver times by male and females resident skinks (female N = 18, male N = 20).](image)
Figure 12: Mean number of times resident skinks returned to the experimental paver to investigate three different scats in close proximity (female N = 18, male N = 20).

Figure 13: Male and female distant tongue flicks as a function of body length (SVL). Regression lines for each sex have been added.
A negative relationship between the number of distant tongue flicks and body length was apparent for both sexes, but the male regression slope appeared to be steeper than the female regression slope (Figure 13). I carried out a t-test comparison of the regression slopes to investigate this (Zar 1999) but this showed that the slopes were not significantly different ($t_{32} = 0.11858, P = >0.05$).

**DISCUSSION**

**Chemosensory cues**


addition to their role in communal and competitive interactions, chemosensory cues are used by lizards to adjust activity patterns and location to limit and avoid risks associated with known predators (Stapley 2003, Downes 2002, Amo et al. 2004).

Multiple complementary roles involving olfactory and vomerolfactory mechanisms appears to be the most likely explanation for the derivation of chemosensory cues from such a variety of different sources in lizards. However, there may be duplication within and between species and it is possible that scent cues derived from different sources provide similar information. In addition to these considerations different species may have developed different methods of communicating depending on their degree of social activity. The use of scat piling in *E. whitii* does not negate the employment of skin secretions or glandular activity as alternative or complementary chemosensory cues.

**Chemosensory cues in scats**

Male *Lacerta monticola* (Iberian rock lizards) discriminate between familiar and unfamiliar conspecifics through faecal pellet odours (Aragón et al. 2000) and their response is size dependent. The effect of faecal pellets on lizard behaviour suggests that they are signals in the intraspecific communication of these lizards (López et al. 1998) and that they may be used to scent mark home ranges and thus contribute to lowering the costs of aggressive interaction (Aragón et al. 2000).

Male and female Chilean lizards, *Liolaemus tenuis*, are able to discriminate between their own and conspecific odours from scats, but males are unable to discriminate between precloacal secretions (Labra et al. 2002). These findings suggest that signals arising from different areas of the body may have different uses, and that sex may influence the outcome. Investigation of faecal boli as visual signals in other species has shown that the presence of scats attracts conspecifics to the site of defaecation, and chemical investigation follows (Duvall et al. 1987, Lopez et al. 1998). In this way scats may act as composite signals.

*Egernia inornata* has been anecdotally reported as scat piling underground (Hutchinson 1993), and as non-scat piling (Bull et al. 1999, Webber 1978). Bull et al. (1999) tested the responses of two *Egernia* species to skin secretions and scats, and found that *E. inornata* were able to discriminate between their own scent and unfamiliar conspecific scent using skin secretions but not using scats. *E. striolata*
however, which uses substrate surfaces for scat piling, were able to discriminate between their own scent and unfamiliar conspecific scent from both scats and skin secretions.

As a solitary species (Daniel 1998 op. cit. Chapple 2003) *E. inornata* has no need to use scat piling to gain information about group members. The production of social signals for individual recognition in skin secretions may render the use of scats containing identical information superfluous because in underground permanent retreats body odour arising from skin secretions is contained and concentrated. However underground scat piling may still be important in this species to advertise their presence in the area to congeneric species. A more detailed assessment would be required to comprehensively evaluate the use of scats in other aspects of their ecology.

**E. whitii responses to chemosensory cues**

The time taken to prepare for multiple replicates was usually \( \geq 90 \) minutes. During this time in the experimental room resident skinks were reluctant to leave their refuges. After the experiment began, some skinks stayed out of camera view for up to 84 minutes. This lack of activity may have been avoidance behaviour, but as the experimental focus was on unequivocal chemosensory responses, this aspect of behaviour was excluded from the analysis.

The discovery of scats from previously unknown lizards in their home territory clearly sends a signal that other individuals have recently been nearby, because every resident skink engaged in tracking or searching behaviour when investigating donor scats. This behaviour suggests that scat deposition signals the presence or possible appearance of another individual.

It became clear from the first tapes viewed that frequent tongue flicking from the sandy surface at the base of the refuge paver was the first active step in investigating unfamiliar scat cues. This unexpected behaviour was included as a variable. It yielded interesting results as analyses showed that the number of distant tongue flicks was related to the size of the resident skink. The smaller the skink the less likely it was to approach quickly without sampling the chemical cues from a distance.
Tests conducted by a colleague had previously established that *E. whitii* rarely tongue flick when left unattended in their familiar enclosures (S. Bellamy pers.com.). The usual pattern of skink activity entailed few visits to the upper surface of the refuge paver. However during the trials many skinks made repeated visits to the experimental paver to investigate the scent cues, which suggests that they are attracted to scats. The trial did not include masking the scent and distant tongue flicking suggested that they could discern scent from a distance. Large skinks approached more readily, and subjected the scent cues to less intensive chemosensory investigation from a distance than smaller skinks. Small skinks are more at risk from larger skinks as they are likely to lose in agonistic contests, therefore small skinks may be less willing to approach as a result.

Despite repeated visits to the experimental paver, the maximum time spent investigating the scats placed there was just under 11 minutes, and a period of only 21 seconds was recorded as the minimum time allocation. It is possible that closer investigation of the chemosensory cues was of relatively short duration because the majority of information had been gleaned earlier when tongue flicking was performed at a distance.

*E. whitii* are essentially social animals (Rawlinson 1974), with the majority living in small groups of two to six individuals and including closely related juveniles (Chapple & Keough 2006). Time was spent scent marking close to both their own and conspecific scats. While there are still unknown components in the process of information transfer, it is possible that the discovery of conspecific scent elicits reciprocal chemosensory marking. In this way individuals might identify themselves, sexual and other socially relevant information may be conveyed, and occupied territory may be advertised. The percentage of skinks (68%) that rubbed chins or jaws on the experimental paver indicates this anatomical area may contain scent glands, but microscopic examination would be required to confirm whether glands do exist and whether they are found in both sexes.

Tongue flicking at a distance relies on substantial considerable olfactory and/or vomerolfactory sensitivity, and is especially advantageous in a species known for its combination of sociality (Chapple & Keough 2006) and intraspecific aggression (Chapple 2003). This option allows choice in whether to approach directly or delay
or avoid an approach and is therefore discriminatory for the investigating resident skink. Any available information concerning size, sex, number or species of donor individuals via chemosensory cues has the potential to allow behaviour modification or adaptation by the receiver that might result in the reduction of costs or enhancement of benefits during encounters.

**Recognition and communication behaviour in *E. whitii***

*Overhead tongue flicks*

Resident skinks tongue flicked their own scats less often than they tongue flicked scats from a conspecific or congeneric lizard. However this did not translate to more time in contact with the conspecific scat than with their own scat, perhaps because of the high variability in responses to the conspecific scat. The variability may be related to varying levels of interest in the donor scent perhaps because of the degree of relatedness, the sex, or the size of the donor individual.

Chemosensory testing of skin secretions was carried out on *Lampropholis guichenoti* (unpublished data), a small Australian skink which does not maintain a home range or permanent social relationships (Bellamy 2000). Although these skinks are unable to differentiate between familiar and unfamiliar lizards after three days apart, both males and females tongue flicked female skin secretions at a higher rate than male skin secretions, indicating the importance of being able to differentiate sex from scent cues alone, even in a solitary skink. *E. whitii* have shown far greater complexity in their responses.

*Scent marking*

The discovery of cloacal scent marking activity is new to the *Egernia* genus. It is noteworthy that only conspecifics attract scent marking behaviour and this new finding implies that chemosensory information concerning the donor is purposefully transmitted via cloacal odours when a composite faecal signal is discovered nearby. The variability in response to the conspecific stimuli indicates that the behavioural repertoire of chemosensory communication in *E. whitii* is incomplete at this stage.

It is apparent that these skinks differentiate between their own species and another species in the genus. By leaving chemosensory cues they are leaving information about themselves – a process consistent with communication among conspecifics. Information transfer then relies on the return of the unknown individual that
deposited the scat, and in this context it is conceivable that *E. whitis* have selected for scent marking behaviour specifically for intraspecific communication.

**Male and female behavioural differences over time**

The immediate response of both male and female resident skinks was similar, but when data were analysed as response over time, some differences in strategy between the sexes become apparent. In contrast to an immediate response (first three passes or less), analyses over a longer period of time showed that males tongue flicked *E. multiscutata* scats more often than females. It could be inferred from this that males might have a greater role in territorial defence against congeners than females, and therefore a greater interest in sympatric competitors.

Females spent more time scent marking beside their own scats than males. This implies that it is important for females to reinforce their chemosensory ‘signature’, perhaps to emphasise their genetic or sexual attributes in the process of mate choice.

Male resident skinks recorded a higher TF rate before a direct approach was made than female residents. There are several possible reasons for this. It may indicate that because there is a greater likelihood of aggression if a male skink confronts an unknown individual, that a slower approach will allow the opportunity for the intruder to be visually assessed before a face to face agonistic encounter ensues. It may also indicate that female skinks have higher status, or that their approach is seen as less threatening by other males and females. Perhaps females are less reticent to approach because one or more of these factors operate and they have experienced favourable receptions in past experience.

As larger sized males and females were inclined to approach comparatively more quickly than smaller individuals and direct less tongue flicks at a distance, there may be a learned element in the responses. Ultimately the response differences between sexes highlights the complex nature of communication in reptiles, and the need to separate the sexes to obtain accurate chemosensory communication data.

**Social signalling within groups**

Cooperation in group scat piling allows individual members of the group to deposit chemical cues that have the potential to indicate relatedness, or social and sexual status within the group. By their practice of scat piling individuals belonging to
groups may reinforce their group membership while at the same time providing individual scent cues and ensuring their own safety within the group.

Dispersing transient and potential immigrant conspecifics, or even intruders and competitors, may readily gain information concerning the social environment before any physical encounter with the inhabitants via scat piling. This would allow advantageous settlement choices to be made while avoiding agonistic encounters. The occupant group may require recruits of one sex or the other, or the niche may be fully occupied. Information concerning the sexes of individuals within the group and the number of group members would allow the potential newcomer to make behavioural choices concerning settlement before any confrontation occurs, thus avoiding some potential conflicts.

**E. whitii refuge sharing**

Many skinks (Stamps 1977) show specific site defense rather than home range defense and *E. whitii* usually remain within a core area, close to burrow entrances (Bruyn 1994). *E. whitii* on Wedge Island inhabit relatively small home ranges and appear to have a small buffer zone separating them from conspecific groups (pers. obs.). They are both cryptic and secretive, spending most of their time concealed beneath rocks or partially concealed in foliage, even when foraging (unpublished data). Scat piling in this context could confer both cooperative and competitive benefits via chemosensory communication.

The aggression exhibited by *E. whitii* (Chapple 2003) may impose restrictions on other species, although interspecific dominance hierarchies based on size in the *Egernia* genus (Langkilde & Shine 2004) indicate that, as a generally heavier species, *E. multiscutata* take precedence (Bellamy 2006). However, mutual avoidance may still be an advantage in sympatric species where size differences are small. Territorial markers (scat piles) positioned within the core area of group territories would benefit conspecifics, and smaller reptile species, as the chemical signals provide information that could be the means of avoiding potentially damaging or unequal agonistic interactions.

The high incidence of *E. whitii* refuging with *Nephrurus milii* on Wedge Island (Chapter 4) provides an indicator of interspecific tolerance. A low recorded incidence of retreat sharing with other skinks and centipedes among *E. whitii* points
to the avoidance of other species, apart from the exceptional circumstance of fleeing from human predators. However there appears to be some plasticity when it comes to thick-tailed geckos (*N. milii*). *N. milii* individuals and pairs have been shown to scat pile (Shah et al. 2006). One mutual advantage of scat piling for both species might be that by utilising a mutual recognition system (scat piling) between the nocturnal species (*N. milii*) and the diurnal species (*E. whitii*), the skinks are able to safely use an increased number of retreats during the day while their gecko neighbours rest after their night-time activities, and vice versa when skinks sleep at night. Theoretically if no threat is perceived by either species concerning the other, greater access to refuges can be attained by both.

**Summary**

Scat deposition by *E. whitii* appears to serve a number of purposes. 1) It provides a composite visual and chemical signal, 2) it signals the presence of an unknown lizard, 3) it initiates different responses in males and females over a period of time, 4) it initiates a response in the form of scent marking when detected by a conspecific, 5) it may function to advertise the presence of *E. whitii* to other species, and, 6) in the form of scat piling it has the capacity to provide information about group members to other conspecifics. It is possible that *E. whitii* individuals reinforce their group membership by cooperating in scat piling, and that other information about sex, size, relatedness and familiarity become available to conspecifics through scat deposition.

Further research is required to assess these possibilities. Additional hypothesis testing based on these experimental results could include identifying the cause of the considerable variation in individual response, and determining other characteristics of chemosensory signals. An extensive series of tests in which both females and males were presented simultaneously with pairs of scats derived from: 1) male and female individuals, 2) mature and juvenile individuals, 3), familiar and unfamiliar individuals, and 4) related and unrelated individuals, conducted to control for the effects of sex and age, could yield intriguing results.

If a series of individual signals confirming sex, age, familiarity and relatedness were confirmed the combined chemosensory cues would have the capacity to provide complex information to elicit behavioural responses relevant to the formation of
adaptive social groupings, reproductive activity, juvenile protection until maturity and dispersal.
REFERENCES


