Chemical mediation of reciprocal mother–offspring recognition in the Southern Water Skink
(*Eulamprus heatwolei*)

MEGAN L. HEAD,† PAUL DOUGHTY, SIMON P. BLOMBERG AND J. SCOTT KEOGH

1School of Botany and Zoology, The Australian National University Canberra, ACT 0200, Australia (Email: scott.keogh@anu.edu.au), 2Department of Terrestrial Vertebrates, Western Australian Museum, Welshpool, Western Australia, and 3Centre for Resource and Environmental Study, The Australian National University, Canberra, ACT, Australia

Abstract Kin recognition has been demonstrated to play an important role in the social structure of a wide range of animals. Most studies to date have examined parent–offspring recognition only in species that provide offspring with direct parental care, however, there are several advantages to parent–offspring recognition even in the absence of direct parental care. In this study we investigated reciprocal mother–offspring recognition in the Australian scincid lizard *Eulamprus heatwolei*, a species that does not show direct parental care. We examined whether neonates could discriminate between their mothers and unrelated females, and whether females could discriminate between their offspring and unrelated neonates, via chemical cues, using retreat site selection experiments. We conducted trials when neonates were 1 and 4 weeks old to investigate whether responses are maintained as neonates age. We found that both neonates and mothers could discriminate between related and unrelated individuals when neonates were 1 week old. Mothers were more likely to take refuge under tiles treated with the odours of their own offspring, while neonates spent less time in areas treated with the odours of unrelated females. At 4 weeks of age, mothers no longer exhibited discriminatory behaviour between their offspring and unrelated neonates, while neonates were more likely to associate with the odour of any female over the odourless control. We hypothesize that reciprocal mother–offspring recognition in *E. heatwolei* reduces interference competition between mothers and their offspring and also may be important in habitat selection and territory establishment.

Key words: Bradley–Terry model, chemical ecology, kin recognition, ontogenetic shift in behaviour, retreat site.

INTRODUCTION

Kin recognition is thought to play a significant role in the evolution of social behaviour (Hamilton 1964a). As kin share a large proportion of genes, individuals may be able to increase their own genetic fitness by biasing cooperative behaviour towards relatives or agonistic behaviour towards unrelated individuals. Both behavioural strategies may increase the likelihood of survival and reproductive success of related individuals (Hamilton 1964b).

One form of kin recognition that has received a great deal of attention among behavioural ecologists is mother–offspring recognition (Beecher et al. 1981; Barnett 1982; Porter 1986; Gibbons et al. 2003; Hayes et al. 2004). Most studies of mother–offspring recognition have focused only on species with direct parental care whereby mothers actively provide offspring with resources such as food or shelter. In such species, offspring and mothers are often in close association with each other for some time before it is necessary to discriminate between kin and non-kin. In at least some of these cases mother–offspring recognition is due to familiarity rather than relatedness (e.g. Noakes & Barlow 1973).

In species with direct parental care, mother–offspring recognition has obvious advantages. Mothers need to be able to distinguish offspring in order to direct parental resources to them and juveniles need to recognize mothers to avoid aggressive rejection by unrelated adults. However, there may be several advantages to mother–offspring recognition even in the absence of direct parental care. These benefits include avoiding kin competition, aiding juveniles in the selection of habitat, reduction of aggression towards offspring, or optimizing inbreeding and
outbreeding (Fletcher & Michener 1987). These benefits may constitute indirect parental care.

Chemical cues have been implicated in the mediation of kin recognition in a variety of taxa including fish (e.g. Brown & Smith 1994), amphibians (e.g. Blaustein & Waldman 1992), mammals (e.g. Hepper 1983; Hayes et al. 2004) and reptiles (e.g. Main & Bull 1996; Bull et al. 2001; O’Connor & Shine 2006). There are several reasons why chemicals may be useful cues for detecting kin. In particular, chemicals may be useful in habitats that are complex or have low visibility. Also, it is known that odours may be sufficiently distinct and diverse that they can be used by animals to distinguish between genetically different individuals and different classes of individuals (Brown & Eklund 1994).

Direct parental care after parturition is uncommon in reptiles (Shine 1988). Thus, reptiles offer an opportunity to gain a better understanding of the adaptive significance of parent–offspring recognition. To date, mother–offspring recognition has been found in a number of viviparous lizards (Tiliqua rugosa and Egernia stokesii – Main & Bull 1996; Lacerta vivipara – Lena & de Fraipont 1998; Egernia saxatilis – O’Connor & Shine 2006), but not in two oviparous lizards (Eumeces laticeps and Eumeces fasciatus – Vitt & Cooper 1989). These results may indicate the importance of mother–offspring recognition in those lizards where young are likely to encounter related females. Oviparous lizard species have little or no association with the eggs once laid and incubation can take several months, whereas the offspring of viviparous species often are associated with adults for some time before dispersal.

An ontogenetic shift in kin discrimination may be expected in some species. The ability to recognize kin is expected to vary through ontogeny because the context in which conspecifics encounter each other presumably changes as the animal matures. This has never been reported in lizards, although laboratory trials on T. rugosa demonstrate kin discrimination to last for up to two months (Main & Bull 1996). Most previous studies on ontogenetic changes in the ability to discriminate kin from non-kin, however, have concentrated on testing amphibians before and after metamorphosis. This is probably due to the obvious changes in ecology and behaviour that these amphibians undergo during this period. Some amphibian species retain sibling recognition abilities after metamorphosis (Blaustein et al. 1984; Walls 1991), while others do not (Blaustein & O’Hara 1986; Cornell et al. 1989; Gramapurohit et al. 2006). These patterns indicate that sibling recognition is important in species that aggregate during the larval stage and is retained in species that show low rates of dispersal after metamorphosis.

In this study we investigated the use of chemical cues in reciprocal mother–offspring recognition in the viviparous skink Eulamprus heatwolei, a species known to be able to chemically detect predators (Head et al. 2002) and to use chemical cues in the detection of female sexual receptivity (Head et al. 2005). It has been suggested that reciprocal mother–offspring recognition is likely to occur in lizard species with relatively long lives, delayed maturity and overlap of home ranges between adults and juveniles (Bull 1994; Main & Bull 1996), perhaps because it infers some fitness advantage. Eulamprus heatwolei fits all of these criteria (Schwarzkopf 1991; Morrison et al. 2002; Stapley & Keogh 2004; 2005). We predicted that offspring would discriminate between their mothers and unrelated females (mother recognition) and that mothers would discriminate between their offspring and unrelated neonates (offspring recognition) but we also were interested in changes to responses over time as neonate E. heatwolei do disperse over the first few weeks of life. We tested the response of mothers to their own offspring and to unrelated neonates as well as the response of neonates to their own mothers and to unrelated females and we conducted retreat site selection experiments at two neonate ages to test for any ontogenetic shift in kin recognition.

MATERIALS AND METHODS

Study species

The southern water skink, E. heatwolei, is a medium-sized diurnal skink of less than 110 mm snout–vent length that inhabits the forest floor of sclerophyll riparian zones in south-east Australia. Females are viviparous and give birth to an average of three offspring (range 1–6). Compared with other lizards of a similar size, Eulamprus spp. are relatively long-lived, slow growing and late maturing. They have an average lifespan of 8 years, with females maturing at 3–4 years of age and males maturing after 2–3 years (Schwarzkopf 1991). Eulamprus heatwolei is a semiterrestrial lizard with some males defending home-ranges and others roaming over the landscape (Morrison et al. 2002; Stapley & Keogh 2004; 2005).

Lizards were collected in late September and early October 2000 from the Tidbinbilla Nature Reserve (Canberra, ACT, Australia, 800 m elevation). This capture period occurred immediately after spring emergence and before mating began. The lizards were brought back to a laboratory at the Australian National University where they were measured, weighed and sexed via hemipene eversion.

After females were collected from the field they were kept in outdoor enclosures with males to allow mating. Prior to parturition, gravid females were brought into a controlled temperature room (25°C) with natural
light where they were housed in individual cages under standard conditions. The mothers spent days housed individually in plastic ‘home’ boxes (43 × 32 × 23 cm) with bark bedding. During the day mothers’ boxes were heated with a 40 W light bulb at one end of the cage. This allowed lizards to thermoregulate naturally. During the day, when the light bulb was on, temperatures ranged from 33°C under the light bulb to 23°C at the opposite end of the cage. Within 2 h of birth neonates were separated from each other and their mothers and housed in individual plastic ‘home’ cages (19 × 13 × 6 cm) with paper towels for bedding. Neonates in their home boxes were kept in a separate controlled temperature room held at 23°C and exposed to the same natural photoperiod as their mother. All lizards were provided with food (*Tenebrio* larvae and dog food) and water *ad libitum*. For experimental trials, clean boxes were used for both mothers and neonates. Because the response of mothers was recorded at night when their heat light was turned off and the response of neonates was recorded during the day with no heat light, all trials took place when the lizards were approximately 23°C.

**Retreat site selection**

Retreat site selection experiments were designed to test whether *E. heatwolei* can use chemical cues to distinguish related and unrelated individuals. We examined whether mothers could distinguish between the scent of their own offspring and that of unrelated neonates, as well as whether offspring could distinguish between the scent of their own mother and that of unrelated females. Both adults and offspring frequently use retreat sites during the day, as well as at night to sleep, so mothers were tested at night and offspring were tested during the day so that both types of experiment could be conducted at the same time. Tests were conducted at two ages to examine ontogenic variation in response. The first series of trials were conducted from 29 to 31 January, when neonates were less than 1 week old. The second series of trials were carried out from 20 to 22 February, when neonates were 4 weeks old.

**Preferences of mothers**

Each test box (30 × 15 × 7 cm) contained a bark substrate and two retreat sites. Retreat sites consisted of a ceramic tile (10 × 10 cm) placed at either end of the test box. Tiled were raised 1 cm from the bark with sticks so that mothers could crawl underneath without disturbing the retreat site. A piece of paper towel (10 × 10 cm) was placed under each retreat site. Each piece of paper towel was treated in one of three ways; (i) housed with one of the mother’s offspring for 3 days; (ii) housed with an unrelated neonate of the same age as the mother’s offspring for 3 days; or (iii) placed in an empty home cage (odourless control). Each piece of paper towel was only used once.

For both ages, sample sizes consisted of 20 mothers and 20 neonates. Two neonates were randomly chosen from each clutch and one was used at 1 week of age and the other at 4 weeks of age. Each night mothers were placed in individual test boxes at 17.00 hours (for both ages) with one of three treatment combinations: offspring versus unrelated neonate; offspring versus odourless control; and unrelated neonate versus odourless control. Mothers were exposed to each of the three treatment combinations once over three consecutive nights. Approximately one-third of the mothers (test groups of six or seven females) experienced each of the three possible orders in which treatment combinations could be presented, thus controlling for order effects. We recorded the tile under which the mother was found 4 h after she had been placed in the test box. Mothers were returned to their home cages in the morning. All tiles and boxes were washed with detergent and dried between trials.

**Preferences of offspring**

Each test box (30 × 15 × 7 cm) contained two retreat sites. Retreat sites consisted of piles of bark 1 cm deep and spanning the width of the box. There was a gap of 20 cm between the bark piles. Bark was treated in one of three ways: (i) housed with the neonate’s mother for 3 days; (ii) housed with an unrelated neonate of the same age as their mother for 3 days; or (iii) housed in an empty home cage (odourless control). Individual bark piles were only used once.

For trials conducted when neonates were 1 week old, 16 mothers were used that had given birth to 44 neonates. For trials at age 4 weeks four additional mothers who had given birth to eight additional neonates were used, giving a total of 20 mothers (including the original 16 mothers) and 52 neonates. A single neonate from each clutch was randomly chosen for use in the experiment and the same neonates were used for the two age classes. Each day offspring were placed in test boxes at 11.00 hours (for both ages) with one of three treatment combinations: mother versus unrelated female; mother versus odourless control; and unrelated female versus odourless control. The unrelated female odour was different for each age group. Neonates were exposed to each of three treatment combinations once over three consecutive days. The sequence in which the neonates experienced the treatments was balanced to control for order effects.

We recorded the position (on either pile of the treated bark or in the space between these piles) of the
Data analysis

As there was the possibility of variation in preferences among individual lizards, we modelled individual female preferences by including lizard ID as a random factor in the analysis. Because the experiment was performed at two different ages, we included age as a fixed factor. Each treatment was also a fixed factor. The measured response variable was simply whether a treatment was chosen (1) or not chosen (0). Such a binomial response is most easily analysed as a logistic regression, and as we had both fixed and random factors, we used a generalized linear mixed model approach. Analyses were performed using the glmmPQL function in the MASS package for R 1.7.1 (Ihaka & Gentleman 1996; Venables & Ripley 2002).

First we looked to see whether there were differences between the data from the two age groups. Using likelihood ratio tests we compared the fit of a model allowing different preferences for both ages and a model allowing the same preferences for both ages. If the model of different preferences fitted the data better than an equal preference model, then it showed that there were differences of equal preference for both treatments in one paired comparison against a general alternative, the Bradley–Terry method tests the hypothesis of equal preference within the context of all three preference tests.

Likehood ratio tests were used to determine whether the data conformed to the Bradley–Terry model and whether the Bradley–Terry model fitted better than an equal preference model. If the Bradley–Terry model fitted the data significantly better than the equal preference model, then it showed that there were preferences among the treatments (De’ath & Moran 1998). Alternatively, if there was no significant difference between the models, then the simpler (no preference) model was retained. Relative preference ratings also were calculated using the Bradley–Terry model and graphed to show the order in which the three treatments were preferred.

RESULTS

Preferences of mothers

There were no cases in which the mothers were found outside retreat sites, so there were no cases of ties in our data. The most parsimonious model contained an interaction between age group and preference for the odour of a related neonate. This model explained significantly more of the variation in our data than the simpler model which did not include the interaction term ($\chi^2 = 10.951, \text{ d.f.} = 1, P < 0.0001$). It was chosen over a more complex model that included two interactions between the treatment conditions and age group because the addition of these interactions

offspring at five different times before neonates were returned to their home boxes for the night. Neonates were placed in the treatment box at 11.00 hours and the first recording was taken 1 h later and subsequent recordings were taken at 1 h intervals. All boxes were washed and dried between trials and bark was discarded.
did not significantly improve the fit of our model ($\chi^2 = 0.291$, d.f. = 1, $P = 0.590$). The dispersion parameter for the most parsimonious model was 0.9997, which is very close to the theoretical value of unity, suggesting that there was little extra-binomial variation that was unexplained by the model. The variance due to different preferences among mothers was very small (0.0010), suggesting that mothers usually made the same choices. We concluded that preferences differed for the two age groups. Therefore, we discuss the results from experiments with neonates at different ages separately.

Preferences at 1 week of age

At age 1 week, the equal preference model did not fit the data as well as the Bradley–Terry model ($\chi^2 = 24.28$, d.f. = 1, $P < 0.0001$). This indicated that mothers show a well-ordered hierarchy of preferences. Mothers preferred tiles treated with the odour of their own offspring over tiles treated with odour from unrelated offspring or the odourless control (Fig. 1a). The estimated probability of a mother choosing a retreat site treated with the odour of a related neonate over an odourless control was 0.85 (95% confidence interval: 0.69–0.93), while the probability of her choosing the odour of a related neonate over an unrelated neonate was 0.72 (0.41–0.86) and the odour of an unrelated neonate over an odourless control was 0.68 (0.40–0.86).

Preferences at 4 weeks of age

The difference in fit between the Bradley–Terry model and the equal preference model was not significant ($\chi^2 = 1.460$, d.f. = 1, $P = 0.227$). When juveniles were 4 weeks old, mothers showed no preferences for odours of their own offspring versus odours of unrelated offspring versus an odourless control (Fig. 1a). The estimated probability of a mother choosing a retreat site treated with the odour of a related neonate over an odourless control was 0.42 (0.25–0.60), while the probability of her choosing the odour of a related neonate over an unrelated neonate was 0.59 (0.41–0.75) and the odour of an unrelated neonate over an odourless control was 0.38 (0.22–0.57).

Preferences of offspring

Again, there were no cases where offspring were found outside of a retreat site, so there were no cases of ties in our data. The most parsimonious model for neonates’ preference for the odours of mothers was a model that included an interaction between age group and the preference of neonates for unrelated mothers versus the control. This model was preferred over a more complex model with two interactions between treatments and age group ($\chi^2 = 0.330$, d.f. = 1, $P = 0.566$) and a simpler model with no interactions ($\chi^2 = 11.882$, d.f. = 1, $P < 0.0001$). The dispersion parameter for the most parsimonious model was 0.981, which is close to the theoretical value of unity, suggesting that there was little extra-binomial variation that was unexplained by the model. The variance due to differences in preferences among offspring was again small (0.064), although larger than that for preferences among mothers. We concluded that neonates of different ages showed a significant difference in preference for odours of mothers. Therefore, we
analysed the results from experiments with different aged neonates separately.

**Preferences at 1 week of age**

At 1 week, the equal preference model did not fit the data as well as the Bradley–Terry model ($\chi^2 = 6.498$, d.f. = 1, $P = 0.011$). This result indicated a well-ordered hierarchy of offspring preferences. Neonates avoided tiles treated with the odour of unrelated females (Fig. 1b). The estimated probability of neonates choosing a retreat site treated with the odour of their own mother over one treated with the odour of an unrelated female was 0.68 (0.56–0.78), while the probability of a neonate choosing the odour of their mother over an odourless control was 0.50 (0.38–0.63). The probability of choosing an unrelated female over an odourless control was 0.32 (0.22–0.47).

**Preferences at 4 weeks of age**

At 4 weeks the equal preference model did not fit the data as well as the Bradley–Terry model ($\chi^2 = 5.759$, d.f. = 1, $P = 0.016$). This indicates a well-ordered hierarchy of preferences. Offspring preferred retreat sites covered with the odour from unrelated females and related mothers over the odourless control (Fig. 1b). The estimated probability of a neonate choosing a retreat site treated with their mother’s odour over an odourless control was 0.60 (0.48–0.70) and the probability of a neonate choosing the odour of an unrelated female over an odourless control was similar at 0.63 (0.52–0.73). The probability of a neonate choosing their own mother’s odour over that of an unrelated female was 0.46 (0.35–0.57).

**DISCUSSION**

In species that provide offspring with direct parental care, studies have demonstrated that mothers and offspring are able to recognize each other. However, there have been few demonstrations of this ability in species where mothers do not provide direct benefits for their young (such as food or protection, but see Main & Bull 1996; Lena & de Fraipont 1998; O’Connor & Shine 2006). Our study on *E. heatwolei* shows that mothers prefer to be with females in general rather than with no female. These results not only demonstrate that *E. heatwolei* are capable of kin discrimination, but also show an ontogenetic shift in behaviour. It is important to note that our experimental design can only examine the relative preferences of lizards with respect to the three treatments. A study of the absolute preferences of lizards for kin odours is beyond the scope of the present study.

**Possible advantages of kin recognition**

There are a number of possible advantages to reciprocal mother–offspring recognition in *E. heatwolei*, despite the absence of direct parental care. Lena and de Fraipont (2000) suggest that kin recognition in *L. vivipara* may have evolved to alleviate kin competition. If kin competition is important in *E. heatwolei* we would expect neonates to avoid mothers and mothers to avoid neonates. In contrast, we found neonates avoided unrelated females at 1 week of age and avoided the odourless control at 4 weeks of age. Also, mothers preferred related neonates at 1 week of age. These results suggest kin competition does not play an important role in kin discrimination in this species.

An alternative explanation for the presence of kin recognition in *E. heatwolei* is the avoidance of interference competition. This may occur in the form of mothers reducing aggression (or possibly cannibalism) towards related neonates, or aiding in territory establishment and selection of optimal habitat. Interference competition is likely to be most important in species where neonates are precocious and often encounter unrelated adults (Bull 1994). If interference competition is important in the evolution of kin recognition in *E. heatwolei*, we would expect neonates to avoid unrelated females in order to avoid aggressive encounters and when establishing territories. Mothers on the other hand would be more tolerant of offspring than unrelated neonates. Our results support these predictions for offspring at 1 week of age, but not at 4 weeks of age.

One important aspect of interference competition is the establishment of territories. For rainbow trout, it has been demonstrated that kin make better neighbours (Brown & Brown 1993). Fish with territories adjacent to kin possessed smaller territories yet had higher growth rates than fish neighbouring non-kin owing to decreased aggression between related
individuals. Advantages to establishing territories near kin also have been reported in mammals (e.g. Waser & Jones 1983; Caley & Boutin 1987), and may be relevant in the present system.

**Reasons for an ontogenetic loss of kin recognition**

There could be several reasons for loss of kin recognition by both mothers and offspring, when the latter are between 1 and 4 weeks of age. First, as mothers and offspring were separated at birth, mothers may have ‘forgotten’ offspring odours. Many animals however, have a long-term olfactory memory and do respond to the odours of kin months after separation (e.g. lizards – Main & Bull 1996; fish – Olsen & Winberg 1996). This indicates that mothers have the ability to recognize kin after long periods of separation if it is beneficial to do so. Second, mothers may use alternative senses (e.g. vision) for kin recognition as neonates age. In this experiment, the lizards did not view each other and this exposure may have been necessary to maintain kin recognition. Third, recognition of offspring may no longer be relevant after 4 weeks of age. This may lead to a loss of kin recognition or simply no display of kin discrimination. Mothers may still have the ability to recognize kin, but do not discriminate between related and unrelated neonates because it is not advantageous to do so (Waldman 1988). As neonates age they may be less vulnerable to aggression. Shortly after birth, neonates are unfamiliar with their surroundings and are more susceptible to attack from predators and conspecifics. Also, in a natural situation 4-week-old neonates may have dispersed away from their natal home-range and thus mothers and neonates may no longer benefit from the ability to discriminate between related and unrelated individuals. That kin discrimination ability is lost also may suggest that there is some disadvantage to discriminating between young once they are older.

Neonates at 4 weeks of age also seem to lose the ability to discriminate between related and unrelated females. This supports the idea that neonates no longer have the need to avoid aggressive encounters with unrelated females. Neonates prefer bark treated with any female odour over no odour. This may indicate that neonates prefer to associate with other lizards. Associating with adult lizards may aid in the avoidance of predators or in the selection of suitable habitat.

In conclusion, this study has demonstrated reciprocal mother–offspring recognition via chemical cues in *E. heatwolei*, a skink that does not provide neonates with direct parental care. Although, the adaptive significance of this recognition is necessarily speculative, we hypothesize that kin recognition plays a role in reducing interference competition between mothers and their offspring, and also perhaps in territory establishment and habitat selection. Similar conclusions have been suggested for *L. virens* (Lena & de Fraipont 1998). Further studies are needed to test if these explanations are true and to determine how widespread mother–offspring recognition is in species without direct parental care.

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CHEMICAL MEDIATION OF KIN RECOGNITION IN A LIZARD


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