

# Molecular determination of paternity in a natural population of the multiply mating polygynous lizard *Eulamprus heatwolei*

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## Abstract

We studied the mating system of the southern water skink, *Eulamprus heatwolei*, during spring and summer (encompassing the breeding season) in a population in southeastern Australia. We examined potential attributes that might influence the mating system and male reproductive success including home range size, physical proximity of adults and body size, and then genotyped all mothers, offspring and potential sires. Home range overlap of both sexes was extensive, with adult females sharing the greatest amount of space with each other and adult males the least amount of space with each other. However, not all adults hold home ranges. We classified approximately one quarter of adult males as home range holders and the rest as 'floaters'. Adult females occupy home ranges more than males, with approximately three-quarters classified as home range holders. Home range ownership is not correlated with body size for either sex, however, male body size is positively correlated with the number of adult female home ranges that his home range overlaps and adult male home ranges are larger than those of females. We used microsatellite genotyping to assign paternities to 55 offspring from 17 litters and then compared this data with our home range and behavioural observations. This species displays extreme levels of multiple paternity given the small mean clutch size of three. Multiple paternity was confirmed in 11 (64.7%) of 17 clutches but three other clutches (for a total of 82.4%) also may display multiple paternity. A total of 30 offspring from 12 litters were assigned to 10 of the 32 genotyped adult males from our study site. Of these 10 adult males, half were home range holders. Five complete clutches and a total of 25 out of the 55 offspring could not be positively assigned to any male surveyed as part of the study and were attributed to floater males or resident males adjacent to our study site that had not been genotyped. While sample sizes are small, neither male home range ownership nor body size is significantly correlated with the number of paternities a male obtained. Our study suggests a polygynous mating system for this species.

*Keywords:* mating system, microsatellite DNA, multiple paternity, territory

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## Introduction

While mating systems and population structure have been described in a wide variety of mammals, birds and insects (Emlen & Oring 1977; Reynolds 1996; Dunn & Cockburn 1999), few studies have focused on lizard populations, which were considered relatively simple until

recently (Olsson & Madsen 1998). Within every reptilian order there are cases of male-male contests for females (Carpenter & Ferguson 1977) and in lizards body size, contest attributes, alternative mating tactics, female choice, and territory/home range quality may all play a role in determining a male's mating success (Olsson & Madsen 1998). While reproductive success in female lizards can be measured easily based on direct counts of eggs or offspring, estimates of individual male lizard reproductive success generally have been based on a measure of home

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range overlap with females (M'Closkey *et al.* 1990; Cooper & Vitt 1993; Censky 1995), primarily due to difficulties in observing copulations (Baird *et al.* 1996; Olsson & Shine 1996). These studies and others also suggest that large male size is positively correlated with male mating success (e.g. *Lacerta agilis*) (Olsson 1992); *Ameiva plei* (Censky 1995); *Crotaphytus collaris* (Baird & Timanus 1998); *C. ornatus* (LeBas 2001). This is hardly surprising as larger males would be expected to win contests more regularly than smaller ones (Stamps & Krishnan 1994, 1998). However, when there is a wide range of adult body sizes, younger, smaller males may opt for alternative mating strategies. For example, they may avoid combat with larger territorial males and instead adopt sneaker strategies in which they gain opportunistic matings (Wikelski *et al.* 1996; Berge 1998). A growing number of studies utilising molecular techniques on lizard systems have demonstrated that there is often a contrast between behavioural and genetic evaluations of male reproductive success (Olsson *et al.* 1996; Abell 1997; Gullberg *et al.* 1997; Bull *et al.* 1998; Gardner *et al.* 1999; Lewis *et al.* 2000; LaBas 2001).

We examined male reproductive success and female mate choice in the southern Australian water skink, *Eulamprus heatwolei*, one of seven closely related species in the *E. quoyi* group (Greer 1989; Cogger 2000). While there have been few studies of *E. heatwolei*, its sister species *E. tympanum* (from which *E. heatwolei* has only recently been taxonomically distinguished) has been the subject of considerable ecological research as the species is easy to study and manipulate (e.g. Schwarzkopf & Shine 1991; Schwarzkopf 1993, 1996; Blomberg 1994; Doughty & Shine 1997, 1998; Rohr 1997; Wilson & Booth 1998). Both *E. tympanum* and *E. heatwolei* are large (to 100 mm snout-vent length), diurnal, easily caught, easily maintained in captivity, and highly abundant and obvious members of riparian habitat (Greer 1989). Both species display sexual size dimorphism, occupy home ranges and display frequent aggression to other members of their species (Saylor Done & Heatwole 1977; Greer 1989). *E. tympanum* also has a small mean clutch size of three relatively large offspring, and females incur a large cost of reproduction (Doughty & Shine 1997, 1998). However, we know little about the degree of territoriality in these species or the movements of individuals and nothing is known of the mating systems of these lizards.

Based on what is known of *E. tympanum*, we predicted that *E. heatwolei* would display a polygynous mating system. We quantified body size and home range size in adults and observed their behaviour and spatial relationships in the field. We used six polymorphic microsatellite DNA loci to genotype offspring, their mothers and potential sires. Here we report on the occurrence, frequency and determinants of multiple paternity in a wild population of unmanipulated free-ranging *E. heatwolei*, and speculate on the attributes of sires vs. nonsires in this population.

## Methods

### Study Site

We conducted fieldwork on *Eulamprus heatwolei* over spring and summer from August 1999 to January 2000. The study site was located within the ~10 hectares Koala Enclosure of Tidbinbilla Nature Reserve, 25 km southwest of Canberra in the Australian Capital Territory. The enclosure is fenced but the fence does not restrict movement of lizards in and out of the enclosure. The study area extended approximately 150 m along the northwestern bank of Mountain Creek that formed one border, two sides of the site are bordered by wide gravel walking paths (2 m across) and the remaining boundary is approximately 100 m up the slope from the creek (Fig. 1). These features do not isolate the population. The site is characterized by a *Eucalyptus robertsonii*, *E. dives*, *E. viminalis* community with an understory of mixed shrubs, including *Bursaria spinosa*, *Leptospermum phyllicoides* and *Acacia dealbata*. *Poa* species and *Pteridium esculentum* dominate ground cover and there are numerous logs at various stages of decomposition (Ingwersen 1983; Ward & Ingwersen 1984). These logs provide refuges and basking sites for *E. heatwolei*, which is a log specialist.

### Sampling

We mapped the site by compass and tape measure with transects perpendicular and parallel to the creek. We numbered logs with paint and used these as reference points for lizard observations. In order to better understand the nature of home range size and quality, we recorded five habitat measures at each numbered log including: log width; length; state of decomposition on a scale of 0 (no decay) to 4 (heavily decayed); distance of log from the creek; and number of other logs within a 2-m radius. Every observed adult *E. heatwolei* in the study area was captured by noosing, and marked permanently and individually by toe clipping and semipermanently by numbered tape tags wrapped around the base of the tail for recognition without recapture. Natural toe loss was observed in the population and toe clipping has been shown to have no effect on lizard behaviour or fitness (Dodd 1993; Hudson 1996; Ott & Scott 1999). Clipped toes and tail tips were retained in 100% ethanol for genotyping. At first capture all lizards were measured for body variables [snout-vent length (SVL) and tail length to the nearest mm; head length; head width; head height; tail basal width; body width; body height and hind limb length to the nearest 0.5 mm; weight to the nearest 0.1 g] and their sex determined by checking for hemipenes.

Scan sampling consisted of walking through the area and recording locations and interactions of marked lizards.



**Fig. 1** Top – Map of the study site in Tidbinbilla Nature Reserve, Canberra, Australian Capital Territory. The study site is approximately 150 by 100 m, and all logs on the site are drawn. Bottom – A graphical representation of the site showing the density of lizard sightings, with the darker ellipses representing the largest number of sightings. Lizards preferred large logs near the creek.

Transects were started at a different point for each session to minimize observer bias, and observation time was distributed evenly throughout the site. The lizards were relatively desensitised to human presence and allowed close (< 1 m) approach, however, a distance of > 2 meters generally was maintained. Observations were aided by the use of binoculars. We conducted a census of the area twice each day with a morning session (before 1230 hours) and an afternoon session (after 13.00 hours). Total observation

time was 182 h. We scored a maximum of two independent sightings for an individual per day, one each for morning and afternoon sessions. We did not use further sightings on the same day in analyses. Lizards were scored as 'basking partners' on a day if they were within 20 cm of each other. The identity of lizards within 5 m of another lizard also was recorded. As not all individuals assigned paternities had calculated home ranges, we calculated proximity as the smallest distance between lizard sightings. Because of the

large body size of this species, the open nature of the woodland habitat they live in, and that their preferred basking sites are large logs, this species of lizard is highly observable. Thus, our scan sampling technique was very likely to allow us to accurately document lizards that were residents of the study site.

### *Home Range Calculation*

We recorded each capture or observation of an individual as a data point relative to mapped features in the environment. These points were transformed to graph paper and minimum convex polygons (MCP) drawn and areas determined (Rose 1982). We realize the MCP technique has shortcomings (Samietz & Berger 1997), so to supplement the MCP method, the graphed data points were analysed in the GIS (Geographic Information System) program ARCVIEW 3.0 with the Spatial Analyst extension (ESRI). Home ranges were calculated for skins using the kernel method that operates by assigning high-density estimates for areas with many observations, and low-density estimates for areas with few observations (Seaman & Powell 1996). This produces a home range that is robust to changes in grid size and has the potential to estimate any configuration of densities, provided an appropriate level of smoothing is selected (Seaman & Powell 1996; Hansteen *et al.* 1997; Samietz & Berger 1997). This method is stable to changes in sample size, but has been shown to overestimate home range size and can only be used in comparative research if the smoothing parameters are identical. In our analyses the grid size was set at 50 cm and the smoothing factor 200. After comparing the two methods, we determined that the 75% kernel best described the lizard home ranges as it assigned similar although greater areas than those assigned by the MCP method.

The number of sightings can be an influencing factor in estimates of home range size (Rose 1982; Samietz & Berger 1997; Abell 1999), thus we used the method of Smith (1995) to determine the minimum number of sightings needed to gauge an accurate estimation. We performed a series of regressions with home range as the dependent variable. Starting with all individuals having at least three sightings (seven males and 23 females), the number of sightings necessary for inclusion was increased until there was no longer a statistically significant relationship between home range size and number of sightings. For both sexes, six or more sightings were needed (sightings  $\leq 6$ :  $r = 0.312$ ,  $N = 30$ ,  $P = 0.093$ ). For the purposes of this study, all lizard-use-areas will be referred to as home ranges, encompassing the territory of each individual. We used this minimum number of sightings as an objective criterion to categorized the lizards as either home range holders (residents) or 'floaters'. While the difference between six or more sightings (lizards classified as resident) and five or fewer sightings

(lizards classified as floaters) may seem arbitrary, for almost all individuals, those classified as residents were sighted many more than six times and those classified as floaters had few sightings.

The amount of overlap between home ranges was calculated with ARCVIEW (ESRI). For each individual the number of overlapping males and females was calculated as well as three measures of the extent of overlap between individuals of the same and opposite sex: (i) percentage overlap; (ii) overlap pressure; and (iii) encroachment. Percentage overlap is the proportion of the focal individuals' home range shared by individuals of a given sex. It is the measure most commonly used in lizard home range studies and is given a value from 0 to 1 (Ferner 1974; Abell 1999). Overlap pressure is calculated by adding all areas that other individuals share with the focal individual and dividing this sum by the focal individuals' home range. This results in a score from 0-n where n is the number of overlapping individuals. It gives additional weight to areas shared by multiple individuals. Encroachment provides a measure of how much space the focal individual overlapped individuals rather than how much its space was overlapped (Abell 1999). The score for encroachment ranged from 0 to 1. We compared overlap data with morphological variables and microsatellite paternity analysis in an attempt to identify the potential for male variables to predict actual male mating success. All statistical analyses were performed on log transformed data. The analyses used are noted in the results.

### *Genotyping and Paternity Assignment*

Two full months after the mating season ended we began to capture all potentially gravid females from the study site. Starting on 10 January 2000, we continued to capture females on our study site until we observed no adult females for five consecutive days. Females were relocated to a temperature controlled room (18 °C) where they were housed individually in ventilated snap-lock containers (30 L  $\times$  21 W  $\times$  9 H cm) with a natural light cycle. Fine woodchip was used as bedding and a small piece of cardboard provided as a retreat site. Cat food, mealworms and water were provided *ad libitum*, and all lizards ate daily and appeared healthy. Basking opportunities were provided for six hours a day (between 10am and 4pm) by heat tape placed under one end of the container. Females were checked daily until parturition (early February). Offspring were toe clipped, weighed, sexed and measured (snout-vent length, hind limb length, tail length) within 24 hours of birth and each neonate was placed in a separate container. Mothers and offspring were released at the site of the mothers' last capture within 14 days of birth.

All mothers, their offspring and all sampled potential sires were genotyped for six polymorphic microsatellite

	n	Coefficient	Std. Error	t	P
Intercept	134	2.467	0.855	2.884	0.0046
# logs < 2 m	134	0.176	0.059	2.975	0.0035
Length cm (ln)	134	-0.079	0.104	-0.755	0.4516
Width cm (ln)	134	0.379	0.136	2.778	0.0063
Distance from creek (ln)	126	-0.420	0.082	-5.110	< 0.0001

**Table 1** Multiple regression table of habitat log variables on number of skink sightings

DNA loci that we describe elsewhere (Scott *et al.* 2001). Two methods were used to assign paternity. Initially, multiple paternity was detected by the presence of three or more paternal alleles per locus. We also used a database of genotypes for all sampled adults in the population, and searched for adult males that possessed genotypes with all the paternal alleles found in the offspring. This allowed a second assessment of multiple paternity by comparisons between successfully assigned male genotypes and the remaining offspring in a litter. Paternity was assigned as the result of a 100% match between offspring and a potential sire's genotype at all six microsatellite loci (alleles matched within 2 bp). While we cannot discount the possibility that more than one potential sire in the population has the same genotype for these six loci, we had no cases where there was a 100% match between an offspring and more than one potential sire.

## Results

Noosing resulted in 83 *Eulamprus heatwolei* captured, sampled and marked, of which 66 were adults (34 females and 32 males). Sub-adults and juveniles were under-represented in captures due to our focus on adults and were not included in analyses.

### Home Range Ownership, Size, Overlap and Habitat Use

Of the 32 adult males caught on the study site, we calculated home ranges for seven individuals with six or more sightings (mean  $\pm$  SE,  $60.03 \pm 5.78$  m<sup>2</sup>). Of the 34 females captured on the study site, we calculated home ranges for 23 females with six or more sightings (mean  $\pm$  SE,  $41.1 \pm 3.61$  m<sup>2</sup>). So adult males occupy significantly larger home ranges than adult females (ANOVA,  $F_{1,30} = 7.09$ ,  $P < 0.01$ ). The remaining 25 adult males and 11 adult females were classified as 'floaters' due to the low frequency of sightings on the study site, but some of these adults may be residents with home ranges adjacent to our study site.

We used multiple regression to analyse the type of habitat preferred. Three of the five habitat variables we measured significantly influenced where lizards (including those of juveniles and subadults) were found: distance from the creek; number of logs within 2 m and log width

(Table 1). Thus, lizards prefer large logs, logs that are close to the creek and logs that have other logs nearby. Within the group of logs where skinks had been sighted, no factors were found to significantly increase sighting frequency. Data points for all lizard sightings were visualised using ARCVIEW, allowing centres of activity within the population to be identified that correspond the home ranges of the animals (Fig. 1).

The SVL of male (ANOVA,  $F_{1,30} = 1.734$ ,  $P = 0.198$ ) and female (ANOVA,  $F_{1,32} = 1.86$ ,  $P = 0.18$ ) home range holders did not differ from floaters of the same sex, although there was a weak but nonsignificant trend for larger individuals to occupy home ranges in both sexes. So both small and large males may be home range holders or 'floaters'. Of those classified as home range holders, neither male SVL (Spearman's;  $r_s = 0.38$ ,  $N = 7$ ,  $P = 0.31$ ) or female SVL (Spearman's;  $r_s = 0.21$ ,  $N = 23$ ,  $P = 0.31$ ) or male head width (Spearman's;  $r_s = 0.595$ ,  $N = 7$ ,  $P = 0.115$ ) or female head width (Spearman's;  $r_s = 0.08$ ,  $N = 23$ ,  $P = 0.72$ ) was significantly correlated with home range area.

Both males and females overlapped multiple individuals. All three measures of overlap suggest extensive sharing of space between and within the sexes with males on males having the lowest percentage overlap, overlap pressure and encroachment measure (Table 2). A male overlapped 2.29 females on average and covered 57% of a female's home range, compared with a female who overlapped 1.5 males and 21% of male area. Female home ranges were overlapped an average of 59% by at least one other female with an average of 2.83 females found in a focal female's home range. There was an average of 1.04 males within each female's home range although 10 out of 23 females were not observed to be overlapped by the home range of any male. Within the home ranges of individual females were core areas that other adult females were not observed entering. Males also frequently displayed in the form of chases towards other males and subadults in close proximity. We have observed male-male combat bouts in the field and many adult males exhibit combat scars on the throat, head and neck.

There was a significant positive correlation between male SVL and female percentage overlap (Spearman's;  $r_s = 0.95$ ,  $N = 31$ ,  $P = 0.019$ ), overlap pressure (Spearman's;  $r_s = 0.92$ ,  $N = 31$ ,  $P = 0.022$ ) and encroachment (Spearman's;  $r_s = 0.81$ ,  $N = 31$ ,  $P = 0.047$ ). The relationship between male

**Table 2** Summary of home range overlap measures in *Eulamprus heatwolei* for adults with at least 6 sightings (male  $n = 7$ , female  $n = 23$ ). As an example, on the first line an average of 1.04 males are found in each female's home range, 32% of female space is shared with at least one male, the index of overlap pressure of males on females is 0.53, and males overlap an average of 10% of the home range of overlapping females

	Number overlapped Mean $\pm$ SE (n)	Percent overlap Mean $\pm$ SE (n)	Overlap pressure Mean $\pm$ SE (n)	Encroachment Mean $\pm$ SE (n)
M on F	1.04 $\pm$ 2.39 (23)	0.32 $\pm$ 0.08 (23)	0.53 $\pm$ 0.14 (23)	0.10 $\pm$ 0.03 (23)
M on M	1.50 $\pm$ 0.50 (7)	0.21 $\pm$ 0.11 (7)	0.30 $\pm$ 0.14 (7)	0.07 $\pm$ 0.03 (7)
F on M	2.29 $\pm$ 0.61 (7)	0.57 $\pm$ 0.15 (7)	1.01 $\pm$ 0.31 (7)	0.32 $\pm$ 0.09 (7)
F on F	2.83 $\pm$ 0.47 (23)	0.59 $\pm$ 0.08 (23)	1.66 $\pm$ 0.32 (23)	0.33 $\pm$ 0.05 (23)

**Table 3** The presence of multiple paternity inferred by number of paternal alleles and by the paternity assignment. The number of alleles at each locus are in parentheses (see Scott *et al.* 2001)

Litter	No. of paternal alleles at each locus						Multiple paternity		Offspring
	Ek8 ( $n = 5$ )	Ek33 ( $n = 4$ )	Ek37 ( $n = 17$ )	Ek39 ( $n = 4$ )	Ek100 ( $n = 19$ )	Ek107 ( $n = 18$ )	Inferred by no. of paternal alleles	Inferred by no. of paternity analysis	
2	1	2	1	2	2	2	2	No	Yes
6	2	1	3	1	3	2	3	Yes	Yes
7	2	3	3	1	2	3	3	Yes	Yes
21	2	1	1	1	2	3	3	Yes	Yes
23	—	2	2	2	2	2	3	No	No
34	—	2	2	1	3	2	5	Yes	Yes
43	2	1	2	2	2	2	3	No	Yes
70	2	2	2	2	2	3	3	Yes	Yes
71	1	1	3	2	2	2	3	Yes	Yes
76	1	1	3	1	3	2	4	Yes	Yes
79	2	2	2	2	2	2	4	No	Yes
83	1	1	3	1	3	2	4	Yes	Yes
98	2	1	2	1	1	2	2	No	No
99	1	1	3	2	2	1	3	Yes	Yes
100	1	1	1	1	2	2	3	No	No
101	2	1	2	2	3	3	3	Yes	Yes
102	1	1	3	1	2	2	3	Yes	Yes

SVL and measure of male overlap was not significant but there was a trend for number of female neighbours to increase as male SVL increased (Spearman's;  $r_s = 0.77$ ,  $N = 31$ ,  $P = 0.057$ ). So as male SVL increases so do percentage overlap, overlap pressure and encroachment by females. A similar relationship was not found between increasing female SVL and measures of overlap pressure, percentage overlap and encroachment.

#### Paternity Analysis

Of the 21 potentially gravid females caught (representing 21 of the 23 resident females), 17 gave birth (*Eulamprus tympanum* females sometimes skip years of reproduction (Schwarzkopf 1992)). Litter size ranged from 2 to 5 offspring (mean  $\pm$  SE =  $3.2 \pm 0.2$ ) with a total of 55 offspring from the 17 litters. All offspring, their mothers and

potential sires were scored at each locus with the exception of offspring number 23.3 (from mother/litter number 23) and 34.5 (from mother/litter number 34) at locus Ek8 (see Scott *et al.* 2001 for details).

Loci were examined separately and allele numbers determined (Table 3). In 11 of the 17 litters, at least three paternal alleles were identified at a locus, confirming certain multiple paternity in these litters (Table 3). At least two loci determined paternity in five of these litters, suggesting that mutation is unlikely to explain the multiple paternal alleles in these litters. From the six litters where single paternity was suggested by the allele number method, two comprised only two offspring allowing a maximum of two paternal alleles at any locus. This makes it impossible to assign single or multiple paternity satisfactorily with the allele number method. In three other of these six litters, including a litter of two, genotyped fathers

Identified parents (F-M)	Distance between nearest sightings (m)	Pair observed as basking partners (females always bigger)	Difference between male and female SVL (mm)
2-1	0	Y	5
6-81	11	N	8
21-38	0	Y	16
34-38	38	N	15
34-66	0	Y	13
43-60	0	Y	6
70-81	45	N	14
71-68	0	Y	1
76-48	30	N	2
76-68	28	N	2
79-68	0	Y	3
83-9	57	N	6
98-12	0	Y	0
102-82	0	Y	1

**Table 4** Summary of behavioural observations between adult males and females (our lizard identification number used, resident males in bold). Only males that obtained certain paternities are included

assigned to one or more of the offspring could be excluded as the father of the remaining offspring. This suggests multiple paternity for litters 2, 43 and 79, but it is not possible for us to exclude the possibility that an unsampled male was the sole father of those litters. Single paternity was confirmed for the remaining three litters (23, 98 and 100, Table 3). Assignment of multiple paternity by comparison with our genotype database resulted in 10 of the litters corresponding to that inferred by the number of paternal alleles at a given locus.

Thus our data suggests that this species displays extreme levels of multiple paternity given the small mean clutch size of three. We are able to confirm multiple paternity in 11 (64.7%) of 17 clutches and three other clutches (for a total of 82.4%) also may display multiple paternity. In one case there were three sires in a clutch of four offspring. There is no relationship between litter size and the number of loci at which multiple paternity was detected ( $r = 0.275$ ,  $P = 0.29$ ).

A total of 30 offspring from 12 litters were assigned to genotyped males. Five complete clutches and a total of 25 out of the 55 offspring could not be positively assigned to any male surveyed as part of the study and were attributed to floater males or resident males adjacent to our study site that had not been genotyped. Out of the 32 genotyped males captured on the study site, only 10 were assigned paternities and only five of these males held home ranges on our study site. In eight cases, paternities were attributed to males that had been observed basking with the female, with only three of these males the home range holders for the area (Table 4). The distance between nearest sightings for the remaining six pairings resulting in paternities ranged from 11 to 57 m (Table 4). While samples sizes are small, our data show no relationship between the number

of paternities obtained and either male SVL, number of females fertilized or male home range ownership.

## Discussion

We have demonstrated that female *Eulamprus heatwolei* occupy small resource based home ranges, and males overlap these areas of female activity to a higher degree than they do other male's core areas. We also have shown that this species displays high levels of multiple paternity. Although the mean clutch size was only three, multiple paternity was confirmed in 11 of 17 clutches and evidence of multiple paternity is strong for three other clutches. These data suggest a polygynous mating system where females position themselves around resources while males position themselves relative to females (Andersson 1994; Abell 1997; Jenssen & Nunez 1998).

Home range size and position can be included in the suite of factors influencing reproductive success and providing information on the observed mating system. In many lizard species, differential fighting ability leads to territory ownership which then can lead to increased access to females and reproductive success (Tokarz 1995; Olsson & Madsen 1998). In our study, males had considerably larger home ranges than females and these home ranges do not extensively overlap with other males. However, home range overlap is extensive between females. This is probably due to females congregating around high quality logs inducing extensive overlap between individuals.

While sample sizes are small, neither males nor females with calculated home ranges were significantly larger than the rest of the adult population. However, female SVL was significantly correlated with home range size, a finding that is most likely attributed to larger females requiring

more resources (Hews 1993; Nunez *et al.* 1997; Olsson & Madsen 1998). There was no correlation between SVL and home range size in males, however, male SVL was significantly correlated with all three measures of overlap. This demonstrates that males shared more space with a greater number of females and also encroached upon more females as their SVL increased.

Overlap and similar proximity measures may be correlated with estimates of male mating, and consequently reproductive success (M'Closkey *et al.* 1990; Censky 1995; Jenssen & Nunez 1998). Several studies on lizard taxa using genetic paternity assignment have demonstrated a correlation between proximity and male reproductive success (Abell 1997, 1998; Gullberg *et al.* 1997; Lewis *et al.* 2000). In our study the offspring successfully assigned to genotyped males were from the litters of 12 females. In seven of the cases, females had been observed basking next to or within five metres of males obtaining paternity, but in only two cases did overlap predict paternity.

We suggest that smaller exclusive areas may be defended within overlapping home ranges in this species. Males share a similar amount of space with females as females do with each other. Lower overlap values also were found between males and for males on females. This supports the idea that females base themselves independently of males and males arrange themselves to overlap females. As females tend to be clustered and their overlap measures are high, males should not be constrained by number of obtainable mates as they would in instances of widely distributed females (Abell 1999). This gives females ample opportunity to mate multiply and our data demonstrate very high levels of multiple paternity in this species.

The high incidence of multiple paternity in *E. heatwolei* is remarkable considering that *E. heatwolei* has a mean clutch size of only three. Although studies on other reptiles have demonstrated a significant positive correlation between litter size and the number of loci at which multiple paternity is detected (McCracken *et al.* 1999), there was no such relationship in our study which showed that multiple paternity is found in both small and large clutches. Multiple paternity is evidence that multiple mating occurs within a population and if identified can serve to highlight mating strategies otherwise overlooked in field studies (Abell 1997; Gullberg *et al.* 1997; Roberts *et al.* 1999). In reptiles, multiple paternity has been characterized in a number of different groups including snakes (Madsen *et al.* 1992; McCracken *et al.* 1999), lizards (Olsson & Madsen 1995; Abell 1997; Gullberg *et al.* 1997; Bull *et al.* 1998; Ciofi & Bruford 1998; Lewis *et al.* 2000; LeBas 2001) and turtles (Kichler *et al.* 1999; Valenzuela 2000) and it often is associated with high rates of multiple matings (Valenzuela 2000). In some species, such as the European adder, *Viprus berus*, and the sand lizard, *Lacerta agilis*, observations show that females readily accept copulations from almost any courting

male (Madsen *et al.* 1992; Luiselli 1995; Olsson *et al.* 1996). DNA analyses of clutches belonging to both species indicate that multiple matings usually lead to multiple paternity.

There are a variety of explanations for why females might mate multiply (Kirkpatrick 1987; Jennions & Petrie 2000). Our data do not allow us to rigorously address these alternative explanations but our data do allow us to speculate about possible determinants of male reproductive success. Females in our population are able to resist sexual harassment and forced copulation (our unpublished data) and so the high level of multiple paternity we have observed is not simply due to females trying to avoid the costs of male attention.

In *E. heatwolei*, females obtain larger SVL and mass than males, yet at the same SVL males have longer, wider and deeper heads, longer hind-limbs, and wider tail bases than females (our unpublished data). This suggests that there should be at least low levels of intrasexual sexual selection on males in *E. heatwolei*. We frequently have observed male combat in the field and scarring on the throats, heads and necks of male *E. heatwolei*. Male-male contests also have been observed in other closely related *Eulamprus* species (Saylor Done & Heatwole 1977; Blomberg 1994).

While our samples sizes are small and not all males in the population could be sampled, we found no relationship between number of offspring sired vs. adult male body size, male head size, male territory ownership, or male proximity to the female. So both quite small (just mature) and very large males sired offspring, and both territorial and floater males sired offspring. Furthermore, almost half of the offspring from our field site were fathered by males that were never sampled and genotyped. We know that females can store sperm for at least two weeks (our unpublished data), thus we cannot discount the possibility that unassigned paternities were the result of females storing and using the sperm of unsampled resident males from adjacent areas. Finer scaled field work and manipulative experiments are need to test this possibility. However, floater males have been found to contribute paternity to clutches in a number of studies on lizards (e.g. Cooper & Vitt 1993; Olsson *et al.* 1996; Olsson & Madsen 1998). Although floaters are generally thought to be young males without territories (Abell 1997, 1998; Olsson & Madsen 1998), in at least one system they were found to be a larger size class that obtained equal levels of paternity to the territorial males (*Urosaurus ornatus*, Thompson *et al.* 1993). We found no difference in mean body size between floater and territorial males, and it is worth pointing out that the body size range in these two categories is large. So both small and large males may be floaters or residents. It may be that females actually seek out matings with floater males they are not familiar with because there may be a higher probability that these males are not as genetically closely related as neighbour males. Multiple

mating has been demonstrated to decrease the chance of receiving related sperm in inbred populations and increase offspring viability (Olsson *et al.* 1996; Olsson & Madsen 1998).

To date most studies on lizards have shown that females choose mates on the basis of resources that males defend and not necessarily by physical attributes of males (Stamps 1983, 1987; Hews 1990; LeBas 2001). However, female choice of larger males has been demonstrated in several lizard species (Cooper & Vitt 1993; Censky 1997; Lewis *et al.* 2000). While sample sizes are small, if we consider only the 10 genotyped males that obtained definite paternities on our study site, we found no difference in the number of paternities obtained by five floater and five resident males, with each category of male obtaining approximately half of the paternities. We also found no difference in the size of males that obtained paternities and those that did not. So contrary to some other studies, both small and large males obtained paternities. However, the equal largest genotyped male in the population by SVL (SVL = 91 mm, weight = 13.5 g) did father the most offspring with seven certain paternities. This male also fertilized the largest number of females, with offspring identified in three litters. However, the other equal largest male by SVL, but slightly larger by weight, obtained no paternities on our study site (SVL = 91 mm, weight = 15.0 g). Further, three males ranging in body size from only 74 mm to 83 mm (thus up to 19% smaller in SVL than the largest male), fathered four offspring each and each of these males also sired offspring in two litters. Thus, the smallest genotyped male to obtain paternities also obtained the second largest number of paternities and obtained fertilizations with two females.

If females are discriminating on male traits, these traits may be more subtle than we have been able to detect. Avenues of female choice are not restricted to male morphology or colouration and calls have been made for further research into the role of multiple paternity and sperm selection within lizard taxa (Olsson & Madsen 1998). Overall more information needs to be obtained on the function of multiple paternity within lizard systems. Sperm selection has been suggested to be a major selective factor in lizards and increased offspring viability has been identified as one potential benefit of multiple matings (Olsson & Madsen 1998). It has been hypothesized that females of 'poor quality' would have the most to gain from sperm choice and so may exhibit a higher level of promiscuity than 'high quality' females (Olsson & Shine 1997). These avenues of research remain largely unresolved and *E. heatwolei* provides a model system for their examination.

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This research was conducted as part of Suzi Morrison's honours research under the supervision of Scott Keogh. Keogh is a molecular systematist and ecologist who is exploring mating systems in several lizard and snake species. Ian Scott is a molecular systematist who specializes in phylogeography of reptiles and birds.

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