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Cues for communal egg-laying in lizards (*Bassiana duperreyi*, Scincidae)

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ABSTRACT

Animals may aggregate either because the presence of conspecifics provides information about habitat suitability, or because the presence of conspecifics directly enhances individual viability. For a female lizard, the advantage of laying her eggs in a communal nest may entail either information transfer (hatched eggshells show that the site has been successful in previous seasons) or direct physiological benefits (recently-laid eggs can enhance water ~~transfer from~~availability to other eggs). We tested the relative importance of these two mechanisms in the three-lined alpine skink (*Bassiana duperreyi* Gray, 1838) by offering gravid females a choice between sites with hatched eggshells versus freshly-laid eggs. Females selectively oviposited beside fresh eggs. In this species, early-nesting females use information transfer (presence of old eggshells) as a nest-site criterion, but later nesters switch to a reliance on direct benefits of conspecific presence (presence of freshly-laid eggs).

ADDITIONAL KEYWORDS: aggregation – oviposition site choice – proximate cues – reproduction.

INTRODUCTION

In many animal species, individuals aggregate even when resources are widely distributed. The costs and benefits of ~~actively~~ selecting sites that contain ~~high densities of~~ conspecifics have attracted considerable research, much of it oriented around antipredator tactics (Magurran & Higham, 1988; Magurran, 1990). Selecting an already-occupied site may enhance fitness of the newly arriving individual either because of information transfer (evidence that the site confers specific benefits, because of the traits of conspecifics already there) or because of a benefit to conspecific presence *per se* (e.g., predator detection or satiation: Brown, 1978; Krause & Ruxton, 2002; Uetz *et al*., 2002). The same two ~~broad categories of~~ explanations can be applied to many other cases of animal aggregation. For example, communal egg-laying is very common in reptiles (known in > 480 species: Doody, Freedberg & Keogh, 2009; Pike *et al*., 2010). This behaviour reflects active maternal preference rather than being an accidental by-product of limited nest-site availability (Brown & Shine, 2005; Radder & Shine, 2007). Although many adaptive advantages for communal oviposition have been suggested (Graves & Duvall, 1995; Doody *et al*., 2009), most can be divided into either information transfer (i.e., the presence of already-hatched eggs suggests that this is an appropriate nest-site) or direct benefits (e.g., predator satiation~~;~~, metabolic heating from other eggs~~;~~, hydric exchange between ~~adjacent~~ eggs).

~~We can experimentally test between t~~These two hypotheses ~~because they~~ make different predictions about the proximate cues stimulating communal oviposition. If information transfer is most important, empty eggshells (which document successful hatching in a previous season) should be more attractive than freshly-laid eggs; whereas if benefits of physical exchange or simultaneous hatching are most important, freshly-laid eggs (which may influence hatchling viability directly) should be more attractive than empty ~~egg~~shells. We

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conducted an experimental study to distinguish between these alternatives~~, using a montane scincid lizard~~.

MATERIAL AND METHODS

STUDY SPECIES AND COLLECTION

Bassiana duperreyi is a medium-sized (to 175 mm total length) scincid lizard ~~that is widely distributed~~found in ~~cool-climate~~ montane ~~areas of~~ southeastern Australia (Cogger, 2000). In the Brindabella Range 40 km west of Canberra, Australian Capital Territory (148°50'E, 35°21'S; 1000–1800 m asl), females produce a single clutch of 3 to 9 eggs each year, during early summer (late November/early December: Shine, Elphick & Harlow, 1997; Shine, 1999; Shine & Elphick, 2001). Oviposition is ~~highly~~ synchronous and concentrated in sun-exposed ~~sites, typically~~ clearings within the eucalypt forest (Shine, Barrott & Elphick, 2002; Telemeco *et al.*, 2010). Nests are laid beneath rocks and logs, making it possible for investigators to locate natural nests (Shine *et al.*, 2002; Du ~~and~~& Shine, 2010). Communal nesting is common; in the field at least 64% of nests are laid in communal nests, and in the laboratory 77% of females selected nest-sites containing hatched eggs as opposed to alternative identical, but empty, nest-sites (Radder & Shine, 2007).

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EXPERIMENTAL PROTOCOL

We hand-collected gravid female skinks from the Brindabella Range in early December over three austral summers (November-December 2008, 2009, 2012; *n* = 40 females in total), and brought them back to the laboratory. One day later, the lizards were measured (snout-vent length, SVL) and individually marked using a non-toxic paint pen for identification. We then randomly assigned skinks to identical experimental enclosures (*n* = 8), with two females per tub. These large tubs (60 x 40 x 20 cm) contained a sand substrate (2 cm deep), two water dishes, and three "shelters" (inverted square flower pot drip trays 12 x 12 x 2.5 cm, with a small "door" cut out of one side to allow lizard ingress or egress). Shelters were evenly spaced within each tub, and they were randomly assigned to three treatments: (1) "no eggs" shelters contained a mound of moist vermiculite (-200 kPa) only, (2) “hatched eggs" shelters contained a mound of moist vermiculite plus four hatched eggshells from the previous year's hatchlings, and (3) "fresh eggs" shelters contained a mound of moist vermiculite plus four recently-laid eggs (each marked to distinguish them from eggs laid by females in the experimental tubs). Fresh eggs were obtained from gravid females that were not used as part of this experiment, and eggs were replaced every few days to ensure that they were fully hydrated and viable. Tubs were placed beneath mercury vapour bulbs set to a 14 hr on:10 hr off light cycle that provided an average temperature in each tub of 32°C during the day, falling to 20°C overnight. Temperatures were similar among the three types of shelters (repeated-measures ANOVA on vermiculite temperatures beneath each shelter collected at 30-min intervals over a 24-h period; treatment effect: $F_{2,21} = 0.39$, $P = 0.68$; time * treatment interaction: $F_{94,987} = 0.94$, $P = 0.63$). Gravid females were offered crickets twice weekly and water was always available.

Tubs were checked twice daily for newly-laid eggs. We recorded which female laid the eggs (based on maternal mass loss) and where the eggs were laid. Every day we rebuilt the vermiculite mounds and re-moistened them. All females were released at their point of capture within a month of collection.

Data analysis

Data were examined using the software program JMP 9.0 (64-bit edition; SAS Institute, Cary, NC). We used a contingency table analysis to test the null hypothesis that females would lay their eggs randomly with respect to available nest-site cues (i.e., no eggs, hatched eggshells, or fresh eggs), and ANOVA to compare maternal body sizes between treatments. All data conformed to the relevant assumptions of the statistical tests that were used. All ~~*p*~~*P*-values are two-tailed; we used an alpha level of 0.05 throughout.

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RESULTS

All females laid their eggs beneath the shelters within nine days of commencing the experiment. Gravid lizards selected nest-sites non-randomly (n = 40, χ^2 = 10.85, d.f. = 2, P < 0.01); most oviposited in the shelter containing freshly-laid eggs (Fig. 1a). If we restrict this comparison to females that laid with freshly-laid eggs vs. hatched eggs, females nested significantly more often with freshly-laid eggs (χ^2 = 5.12, d.f. = 1, P < 0.05; Fig. 1b). Maternal body size did not significantly influence nest-site selection ($F_{2,39}$ = 1.38, P = 0.26; mean SVLs per treatment range from 69.2 cm [with freshly-laid eggs] to 72.4 mm [in empty shelters]), suggesting that maternal age (and hence, prior experience) did not influence these results.

DISCUSSION

Radder and Shine (2007) showed that nesting *Bassiana duperreyi* prefer to lay their eggs in sites with old hatched eggshells rather than similar sites lacking such cues; but the current study shows that sites with freshly-laid conspecific eggs are even more attractive. Hence, fitness benefits from the proximity of conspecifics may outweigh the advantages of information transfer about previous events, if both cues are available.

Laying beside other eggs provides a direct fitness benefit~~ in this species~~, because an egg's physical contact with other eggs during incubation modifies water exchange and hence enhances offspring viability (Radder & Shine, 2007). This direct link between oviposition choice and offspring fitness ~~thus ~~may favour a stronger maternal response to freshly-laid eggs than to old eggshells. Hence, communal oviposition in our study species may have arisen as a result of both of the advantages/processes outlined in the Introduction. In the absence of other cues (as must be the case for females ovipositing early in each annual nesting season), a reproducing female selectively oviposits beside already-hatched eggshells (Radder & Shine, 2007), and hence benefits through information transfer (that site was successful in previous years, so is likely to be successful again). Once some females have laid, however, their eggs confer a direct physiological benefit to the eggs of any later-ovipositing female (because of modified hydric dynamics within the nest: Radder & Shine, 2007) and thus, the proximate cues from newly-laid eggs become more important than cues from hatched shells.

Communal oviposition might incur costs as well as benefits. For example, later-arriving females might disturb earlier-laid clutches, or the resultant high densities of hatchlings in small areas might exacerbate intraspecific competition (Doody *et al.*, 2009). Also, communal nests might be easier for egg-predators to locate, especially if they are laid in the same sites year after year. Another potential cost involves the build-up of pathogens and parasitoids that may accumulate in the soil and attack eggs laid in subsequent years (by fungi in sea turtle nests - Patino-Martinez *et al.*, 2012; by beetles in snake nests - Blouin-Demers & Weatherhead, 2000). However, hatching success of eggs is high in our study system (pers. obs.), suggesting that these potential disadvantages of communal oviposition are too weak to outweigh the advantages.

~~Communal oviposition in the same sites year after year might incur costs as well as benefits. LOOK AT DOODY'S REVIEW – ARE THERE OTHER COSTS IDENTIFIED??? For example, the resultant high densities of hatchlings in small areas might exacerbate competition among those animals. In practice, high dispersal abilities probably minimize any impact of this factor for *Bassiana*. Also, local egg-predators might learn the locations of nests, thereby increasing egg mortality. We have not recorded predation by vertebrates on *Bassiana*~~

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~~eggs however, so this is unlikely to be important in our study species. A third potential cost involves the build-up of fungal pathogens, that could accumulate in the soil and attack eggs laid in subsequent years. This scenario has been documented in sea turtles, especially in repeatedly-used hatcheries (Patino-Martinez *et al*. 2012). ***DIDN'T WEATHERHEAD RECORD THIS IN SNAKE NESTS TOO IN CANADA?? However, fungal infection of eggs appears to be rare in our study system (pers. obs.), again reducing the potential disadvantages of communal oviposition.~~

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Future work could usefully explore the proximate basis for maternal discrimination between empty nests *versus* those with old eggshells *versus* those with recently-laid eggs. Female insects can use chemical cues to detect the presence of freshly-laid eggs (e.g., Laurence & Pickett, 1985; McCall, 1995), and female reptiles may have similar abilities. The lesser response to eggshells than eggs seen in the present study may simply reflect a disparity in the magnitude of stimulus, with eggs retaining a strong chemical signature for some period after oviposition. The diversity of reptile taxa that exhibit communal egg-laying suggests there will be equal diversity in both the proximate cues and adaptive forces that result in this behaviour (Doody *et al*., 2009). Maternal decisions about communal oviposition thus may offer an excellent model system in which to explore the relative importance of information transfer *versus* conspecific presence in eliciting animal aggregations.

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FIGURE LEGEND

Figure 1. A gravid lizard (*Bassiana duperreyi*) laying her eggs with the fresh eggs already present beneath the experimental shelter (A), and the percentage of female lizards laying in each shelter type (B). Dashed line at 33% indicates the percentage of females expected to lay eggs in each treatment under the null hypothesis that females do not actively select nest-sites. Females nested significantly more often in sites with freshly-laid eggs than in sites with no eggs, or older eggshells (see text for analyses).