

Courtship and mating behaviours of two syntopic species of skink (*Plestiodon anthracinus* and *Plestiodon fasciatus*)

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Abstract. Courtship and mating behaviours are widely conserved throughout the scincid lizard genus *Plestiodon*, and ethological isolation between closely related species depends heavily on differentiating chemical cues. The purpose of this study was to determine whether or not subtle, as yet undetected differences are present in the mating behaviours of two syntopic, distantly related skinks, *P. anthracinus* and *P. fasciatus*. Observed courtship and mating behaviours were similar between the two species and to those reported for other species within this genus. However, survival analysis determined significant interspecific differences in the duration of pre-coital behaviours. Investigation of such subtle differences between species more closely related than these two may generate further insights into the evolution of ethological isolating mechanisms in lizards.

Introduction

Differences in courtship and mating behaviours are important in providing ethological barriers to gene flow among closely related species (Mayr, 1963). Among lizards, differences in morphology (Cooper, 1984; Echelle et al., 1971; Losos, 1985), behavioural displays (Hunsaker, 1962; Martins, 1993; Tokarz, 1995), and chemical cues (Cooper and Vitt, 1986a, 1987a; Fitch, 1954) all contribute to the recognition and discrimination of potential mates. Chemical recognition by both sexes appears to be particularly important in skinks of the genus *Plestiodon* (Cooper, 1996; Cooper and Vitt, 1987b). For example, ethological isolation is functionally complete among three potentially syntopic members of the *P. fasciatus* species group (*P. fasciatus*, *P. inexpectatus*, and *P. laticeps*) in the southeastern United States, even though they lack the complex behavioural displays common in iguanids (Cooper and Vitt, 1987a). High levels of sexual isolation in species possessing visually-indistinguishable courtship and mating behaviour patterns have

also been recorded in plethodontid salamanders (Kozak, 2003).

Based on published descriptions and photographs, courtship and mating appear to be very similar in a number of *Plestiodon* including *P. fasciatus* (Cooper and Vitt, 1987a; Fitch, 1954), *P. laticeps* (Cooper and Vitt, 1987a; Goin, 1957), *P. inexpectatus* (Cooper and Vitt, 1987a), *P. egregius* (Mount, 1963), *P. multivirgatus* (Everett, 1971), *P. anthracinus* (Mount, 1975), and *P. tetragrammus* (Lieb, 1985). Considering that these include members of several divergent species groups (Schmitz et al., 2004), mating behaviours, like other aspects of *Plestiodon* reproductive life history (Vitt and Cooper, 1986), appear to be highly conserved. This lack of divergence in courtship and mating behaviours may be related to the dependence on chemical communication to effect species recognition in these lizards.

The mating sequence of skinks generally begins with a male's investigation and recognition of a potential female. This may include his trailing her pheromonal cues (Cooper and Vitt, 1986b; Fitch, 1954) as well as his chemosensory investigation of her cloacal region, the location of an important pheromone-producing gland (Trauth et al., 1987). If the female is receptive, the male bites her anteriorly with a hold that may be necessary to effect coitus (Cooper and Vitt, 1987a). The male curls his body to bring

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their respective vents into contact, at which time he inserts a hemipenis. Once the female chooses to accept the male's advances, her role appears to be largely passive throughout.

Although chemical communication is of primary importance to mate recognition, and courtship and mating behaviours are similar among skinks, subtle behavioural differences may exist. One potential difference that can be measured is the amount of time species spend in each discrete behaviour. Survival analysis is a statistical tool specifically developed to test for differences between groups in the duration of a specific event. Kaplan-Meier is a non-parametric procedure that generates a cumulative survival function, which gives the probability of persistence of a given event until a specific time. Significant differences between survival functions can then be tested using a logrank test, which generates a χ^2 value (Fox, 2001).

The purpose of this study was two-fold: 1) to determine whether subtle differences in mating behaviour are present between two syntopic species of skink known to be isolated by other physiological mechanisms and 2) to investigate whether survival analysis could be an effective tool for examining divergent behaviour patterns. The two species chosen, the Coal Skink, *P. anthracinus* (Baird), and the Five-Lined Skink, *P. fasciatus* (Linnaeus), are not closely related (Lieb, 1985; Schmitz et al., 2004), *P. anthracinus* belonging to a clade of fossorial North American skinks basal to the lineage containing *P. fasciatus* and its closest relatives. Moreover, the two study species have non-overlapping mating seasons, February to April for *P. anthracinus* (Hotchkin et al., 2000; Trauth, 1994) and May or June for *P. fasciatus* (Cooper and Vitt, 1987a; Fitch, 1954). Therefore, there was no *a priori* reason to expect differences between them in mating behaviors, particularly in light of the overall conserved nature of reproductive traits in the genus. These two species, however, were available in sufficient numbers to conduct an appropriate number of mating trials for analysis, and we reasoned that if measur-

able differences exist between them, then this may prove a useful technique in studying the potential role of behavioural isolation in species that are more closely related and in which differences would seem more likely.

Materials and methods

We collected adult lizards during the peak times of the respective mating seasons for the two species, March for *P. anthracinus* and May for *P. fasciatus* (Fitch, 1954; Hotchkin et al., 2000; Trauth, 1994). We collected 10 adult male and 10 adult female *P. anthracinus* during February and March of 2002 and 2003. We collected 14 adult male and nine adult female *P. fasciatus* during May of the same years. Lizards were collected from the campus of Piedmont College and an adjoining mesic hardwood forest in Habersham Co., Georgia, where the two species are syntopic (Hotchkin et al., 2000). The two species are the only generic representatives at the study site.

Lizards were individually housed at 21 C in separate glass aquaria and regularly fed mealworms (*Tenebrio molitor*). Experimental mating trials were conducted within one week of collection. Each male was randomly paired with a conspecific female. Although some individuals were paired with multiple partners to increase the number of successful matings, the average number of pairings for each individual was no more than two. Because of the need for the independence of data points, we used only data from the first successful mating of each individual for statistical analyses. Lizards showed no reduced tendency to mate in further trials, but changes in successive matings of the same individuals were not investigated as different partners were present in each encounter. The experimental chamber consisted of a plastic terrarium measuring 22 × 20 × 18 cm, whose floor had been lined with moist paper toweling. The terrarium was cleaned, and the toweling was replaced between trials. Each trial lasted 30 min and was filmed with a video-cassette camera-recorder mounted on a tripod. Following mating trials, all lizards were released near their initial site of capture. During the first year, all lizards were individually marked by toe-clipping prior to their release to ensure that the same individuals would not be used the following season.

Individual behaviours (table 1) on video were scored and timed in s. Behaviours were grouped into three categories for analysis. These were 1) investigation and recognition; 2) pre-coital biting; and 3) cloacal contact and coitus. Individual members of mating pairs separated following coitus and appeared to show no further interest in each other. Therefore, we did not include post-coital behaviours in the analyses. We assessed interspecific differences in the duration of each of these categories by the Kaplan-Meier survival analysis using Statview®, version 4.1.

Table 1. Definitions of behaviors observed in laboratory-based mating trials of *Plestiodon anthracinus* and *Plestiodon fasciatus*.

Behavioural types	Description
Investigation and Recognition	
Pre-contact Investigation	Male shows interest by moving head toward female; male moves toward female
Contact and Recognition	Male tongue-flicks female's tail base, then moves up her body, tongue-flicking her torso
Pre-coital Bite	
Bite	Male bites female on torso and holds her by skin fold, may adjust bite for firmer grip
Bite Progression	Male shifts bite hold up female's torso until he holds her by the nape of her neck
Head Shake	Male lifts female's anterior end off of ground and shakes her back and forth in a regular motion; both lizards may also be walking forward during this behavior
Cloacal Contact	
Vent Rub	Male curls tail base under that of female and rubs his vent against her until hemipene is inserted
Coitus	Both lizards lie in position with male's hemipene inserted in females cloaca; terminates when female walks forward

Table 2. Duration of individual behaviors of *Plestiodon anthracinus* and *Plestiodon fasciatus* during laboratory-based mating trials. Data are reported in s as mean \pm 1 standard error.

Behaviour	<i>E. anthracinus</i>	<i>E. fasciatus</i>
Pre-contact Investigation	2.13 \pm 0.49	4.15 \pm 0.88
Contact and Recognition	4.13 \pm 1.03	40.08 \pm 9.43
Total Investigation and Recognition	6.27 \pm 1.30	44.23 \pm 9.95
Bite	7.73 \pm 1.31	11.92 \pm 1.11
Bite Progression	3.53 \pm 0.79	14.39 \pm 2.10
Head Shake	41.53 \pm 3.67	55.54 \pm 7.70
Total Pre-coital Bite	52.80 \pm 4.13	81.85 \pm 7.62
Vent Rub	5.80 \pm 1.44	6.85 \pm 0.85
Coitus	449.33 \pm 79.63	409.15 \pm 39.94
Total Cloacal Contact	455.13 \pm 79.61	416.00 \pm 36.17

Results

We recorded 15 successful matings of *P. anthracinus* and 13 for *P. fasciatus*. Both species exhibited the same recognizable behaviours (table 2). Nine of the *P. anthracinus* matings and seven of *P. fasciatus* matings were first matings of independent individuals. These were the pairings used in the analyses, and they revealed significant differences between the species in duration of behaviours. *Plestiodon anthracinus* spent significantly less time in both the investigation/recognition phase ($\chi^2 = 5.093$, $df = 1$, $P = 0.024$; fig. 1) and the pre-coital bite phase ($\chi^2 = 4.427$, $df = 1$, $P = 0.035$; fig. 2). The two species did not differ in the amount of time spent during cloacal contact and coitus ($\chi^2 = 0.152$, $df = 1$, $P = 0.696$; fig. 3).

Discussion

Although the similarity of behaviours between *P. anthracinus* and *P. fasciatus* further verifies the highly conservative nature of mating behaviours in *Plestiodon*, our results support the hypothesis that subtle, significant differences exist between the two species. There are at least three distinct explanations for these differences. The first is that the evolution of significant temporal differences in the pre-coital behaviours of the two species represents reproductive character isolation to prevent interspecific hybridization. Significant interspecific differences in mating behaviour have also been reported in the plethodontid salamander genus *Desmognathus* (Mead and Verrell, 2002). However, as in scincid lizards, reproductive isolation in the Pletho-

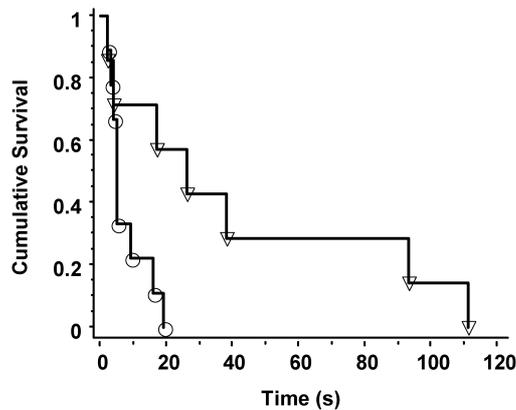


Figure 1. Event times from survival analysis of contact and recognition in skinks. Circles represent event times for *Plestiodon anthracinus*, and inverted triangles represent event times for *Plestiodon fasciatus*. See text for explanation of analysis.

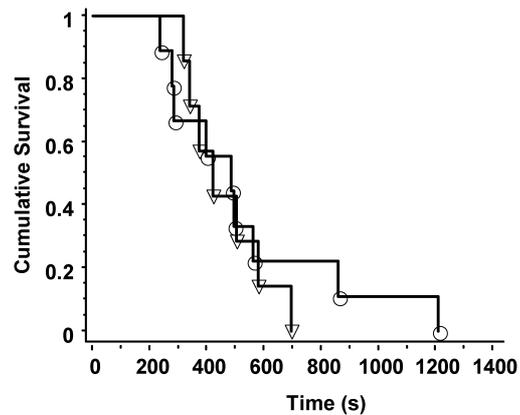


Figure 3. Event times from survival analysis of coitus in skinks. Circles represent event times for *Plestiodon anthracinus*, and inverted triangles represent event times for *Plestiodon fasciatus*. See text for explanation of analysis.

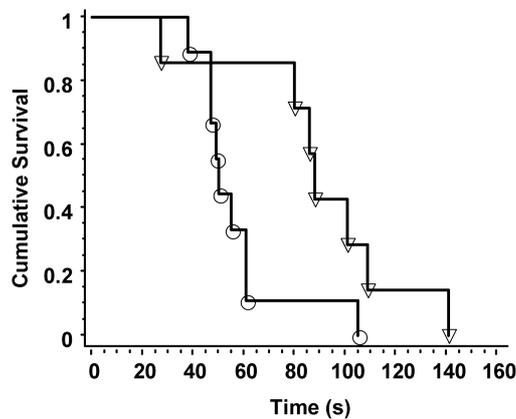


Figure 2. Event times from survival analysis of pre-coital biting in skinks. Circles represent event times for *Plestiodon anthracinus*, and inverted triangles represent event times for *Plestiodon fasciatus*. See text for explanation of analysis.

dontidae is driven by phomonal systems utilized in mate recognition. This suggests little need for selection driving behavioural reproductive character displacement given the functional isolation of chemical cues and other non-behavioural aspects of lizard and salamander physiology (Cooper and Vitt, 1987a; Kozak, 2003; Mead and Verrell, 2002). Indeed, *P. anthracinus* and *P. fasciatus* are isolated temporally with very little, if any, overlap in mating seasons. This combination argues against a significant role of reproductive character displace-

ment in determining behavioural differences. However, we cannot rule out the possibility that such differences persist as relicts of past character displacement associated with the original divergence of the respective lineages to which *P. anthracinus* and *P. fasciatus* belong.

Alternatively, ecological factors such as differential utilization of microhabitat by the two species or energetic constraints related to divergent aspects of the timing of the mating seasons may be responsible. In the case of these lizards, the phylogenetic distance and the obvious temporal isolation between the two species suggest that such differences may be ecological in nature, e.g., *P. anthracinus* perhaps mates in more open, potentially vulnerable situations and, therefore, spends less time in pre-mating behaviours.

Another possibility is that these differences coincidentally reflect phylogenetic divergence between the two species (Shmitz et al., 2004) as a result of genetic drift or linkage. The development of ethological isolation as a byproduct of genetic divergence has been shown in other vertebrate species (e.g., Tilley et al., 1990).

The divergence of a subset of courtship behaviours in our study organisms cannot be fully explained as yet. Whatever the cause of such differences, however, they do exist as measur-

able entities. This raises the possibility that overt mating behaviours may influence the respective reproductive biologies of skinks more than their behaviourally conservative nature would otherwise suggest. If these behaviours do indeed play a role in fostering and maintaining reproductive isolation, then differences would be expected to be more pronounced in species with few, potentially tenuous reproductive barriers. We suggest that the investigation of subtle differences through the use of such statistical techniques as survival analysis may be fruitful in providing further insights into the nature of ethological isolation in lizards.

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