

An unusual copulation of *Dipsas alternans* (Fischer, 1885) in Atlantic Forest, Southeastern Brazil

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Snakes are rarely found in the field (Fitch, 1987), thus their reproductive behaviour is often poorly documented (Almeida-Santos and Marques, 2002). Consequently, many questions about the reproductive behaviour of Neotropical snakes remain unclear such as prolonged mating searches, male-male combat and explosive mating aggregations. Herein, we report on an unusual copulation involving one female and two males of *Dipsas alternans* in nature and discuss the efficiency of the sexual attractiveness mechanism favouring the occurrence of reproductive aggregations in the subfamily Dipsadinae.

On the morning of December 14th, 2012, we sighted a female *D. alternans* (from 1–2 m away) mating with two males. The locality is within the Atlantic ombrophilous dense forest (23°20'16"S; 45°6'4"W, 1,650 m elevation). The vegetation is mostly characterized by primary forest (nearly 60% of the area), and the remainder consists of secondary forest, native field, reforestation areas (*Pinus* spp. and *Eucalyptus* spp.), grass, and scattered shrubs (Hartmann et al., 2009). The weather was partly cloudy.

The copulation occurred on the branches of a tree, about 1.5 m high (Figure 1A). The female was undergoing ecdysis, and it was found partially coiled and showing a defensive display (characterized by triangulation of the head), probably due to the approach of the observer. The

hemipenes of both males were fully introduced into the female cloaca (Figure 1B). The female's cloacal mucosa showed a swelling, and some blood was observed on the vegetation around, probably due to the insertion of two hemipenes (Figures 1B, C). After approximately 45 minutes of observation, the female moved through the branches in an apparent fleeing behaviour. The males remained on the branches, with their tails attached to the female by the hemipenis (Figure 1D). The swelling and hardening of the increasingly exposed cloacal mucosa revealed moderate bleeding in this region, probably because of the female movement while the copulation was still occurring (Figures 1D, E). Voucher digital 100_LEEV_HN.png photographs of this behaviour are housed on file at the Laboratório de Ecologia e Evolução, at Instituto Butantan.

As in other Neotropical arboreal and nocturnal snakes (Cechin and Hartmann, 2001; Marques et al., 2004), mating in *D. alternans* can also occur during the daytime. Little is known about the reproductive strategies and behaviour of the genus *Dipsas*. The only copulation of *Dipsas* observed in nature was described for *D. neivai* in southeastern Bahia, occurred in the summer) and observations for *D. neivai* and *D. alternans* (reported here) occurred in the late spring (December 14th) indicating that the mating season occurs during the highest temperatures of the year - late spring and summer for the genus *Dipsas* (Table 1, Alves et al. 2005).

Reproductive aggregations have been reported in many species from different lineages (Feio and Santos, 1999), including other Dipsadinae (Table 1). Individuals of *Imantodes cenchoa* have been observed in courtship and mating, being interrupted by dispute combat between rival males (Table 1; Doan and Arriaga, 1999; Santos-Costa and Prudente, 2005). Reproductive aggregations were also recorded for *D. neivai* (Table 1). All of these aggregations occurred during the breeding season (summer or early spring) and involved disputes and agonistic interactions between males (Table 1). Females

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Figure 1. Mating of *Dipsas alternans* in Serra do Mar, southeastern Brazil: (A) Individuals of *D. alternans* copulating; (B) Ventral view of the hemipenes inserted into the female's cloaca; (C) Tails intercrossed, swelling of the female's cloaca, and bloodstains on the tails and surrounding vegetation; (D) Males hanging on the branch, stuck to the female in a probable flee behaviour (E) Cloacal mucosa of the female exposed, showing moderate bleeding.

likely use chemical signals as a strategy to attract males for mating since location and recognition of potential sexual partners are critical for the reproductive success of snakes (Shine and Mason, 2012). Taken collectively,

our observation and literature data suggest that aggregation is a conservative trait among dipsadines (see Pizzatto *et al.*, 2008). This type of reproductive aggregation behaviour observed in *D. alternans* can lead

Table 1. Mating and reproductive aggregation in the subfamily Dipsadinae.

Tribe	Species	Mating season	Reproductive Behavior	Substrate	Author
Dipsadini	<i>Dipsas neivai</i>	Summer	1 ♀ and 1 ♂ Copulation	Branches	ALVES et al. 2005
		Summer	1 ♀ and 3 ♂ Aggregation	Branches	
	<i>Dipsas alternans</i>	Late Spring	1 ♀ and 2 ♂ Aggregation	Branches	This report
Imantodini	<i>Imantodes cenchoa</i>	Spring	1 ♀ and 3 ♂ Aggregation	not mentioned	DOAN & ARRIAGA 1999
		Spring	1 ♀ and 2 ♂ Aggregation	Branches	SANTOS-COSTA & PRUDENTE 2005

to the simultaneous insemination of the female by the sperm of different males. Thus, it may result in sperm competition, multiple paternity, and, consequently, greater genetic variability of the litter (Madsen et al., 2005; Wusterbarth et al., 2010), ultimately conferring an evolutionary advantage. More studies are needed to elucidate whether this is an accidental mating or a mating strategy of the Dipsadinae.

Penetration depends on the permission of the female being courted, and it consists of the insertion of a non-rigid hemipenis into the female's cloaca (Hardy, 1998). The hemipenis of *D. alternans* is single, with a bulbous shape (Passos et al., 2004). In our observation, the opening of the female cloaca allowed the concomitant insertion of the hemipenes of both males. After penetration, the hemipenes probably became rigid and turgid inside the cloaca (Murphy and Barker, 1980), which may have caused injuries to the cloacal tissue and, consequently, the observed bleeding.

The female *D. alternans* was in the initial process of ecdysis, which may be part of the estrus signal (Aldridge and Duvall, 2002). During this period, chemical signalling becomes more pronounced because part of the vitellogenin that is being deposited in the ovarian follicles disseminates as epidermal lipids through the scales from the bloodstream (Aldridge et al., 2005). Therefore, the vitellogenesis process functions as a signal of the female reproductive status by spreading chemical cues throughout over the environment as they move through the substrate (Mason and Parker, 2010). The reproductive aggregation behaviour reported here may have been elicited by the pheromones released during ecdysis, as reported for *Chironius bicarinatus* by Marques et al., 2009.

Here we report, for the first time, the aggregation behaviour with simultaneous insertion of hemipenes, from two different males into the female cloaca, expanding the understanding of the possibility of multiple paternity. This finding increases knowledge about the reproductive strategies of snakes in nature.

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