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Multimale breeding aggregations by "many-friends" snakes: courtship behaviours by Malagasy Pseudoxyrhophiine snakes, *Dromicodryas bernieri* and *D. quadrilineatus*, and their sexual size dimorphism

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Abstract

When several male snakes dispute over one female in the breeding season, two major male-male rivalries are known to occur: male-male combat and multimale breeding aggregation. The roles of male body size and the degree of sexual size dimorphism are different between these rivalries. We report field observations of mating behaviour including a multimale breeding aggregation of pseudoxyrhophiins, *Dromicodryas bernieri* and *D. quadrilineatus*, in northwestern Madagascar, which have a local name, "Maro longo", meaning "many friends". To examine the relationships between sexual size dimorphism and mating strategies of males, we also analysed the body size of the two species of *Dromicodryas* and two other pseudoxyrhophiins, *Leioheterodon madagascariensis* and *L. modestus*, which are known to exhibit the male-male combat. Our data obtained during a long-term field study showed that *D. bernieri* and *D. quadrilineatus* have female-biased sexual size dimorphism, whereas *L. madagascariensis* and *L. modestus* have male-biased sexual size dimorphism. This result conforms to the general tendency of the relationship between body size and male-male rivalry in snakes.

Key Words

Lamprophiidae, *Leioheterodon madagascariensis*, *Leioheterodon modestus*, Madagascar, mating aggregation, mating ball

Introduction

When several male snakes dispute over one female in the breeding season, two major male-male rivalries are known to occur: male-male combat and multimale breeding aggregation (Rivas and Burghardt 2005; Senter 2022). Male-male combat is a ritualised behaviour between two males, which appears to be a contest for superiority or dominance (Carpenter 1977). In this case, mating is usually observed between a female and a male that won the combat (Rivas and Burghardt 2001). On the other hand, multimale breeding aggregation involves one female and several males. For example, *Thamnophis sirtalis parietalis* is known to form a "mating ball". The males align their bodies with the female and wrap the anterior part of their tail around the female's tail immediately posterior to the cloaca. The males engage in tail wrestling, and copulation occurs within this mating ball (Shine et al. 2000).

Shine (1978) and several subsequent studies (e.g., Shine 1994; Almeida-Santos and Marques 2002) demon-

strated that male-male combat of snakes tends to occur in the species of which males have larger body size than females, strongly supporting the hypothesis that larger male size is an adaptation to intrasexual competition. On the other hand, the majority of snake species, which are not known to show male-male combat, tend to have the opposite tendency: females have larger body size than males (Shine 1978, 1994). In the case of species that show multimale breeding aggregation, females have larger body size than males (e.g., Shine 1986; Madsen and Shine 1993). In these species, the large body size of males is considered to have both advantages (Madsen and Shine 1993; Weatherhead et al. 1995; Luiselli 1996; Shine et al. 2000) and disadvantages (Joy and Crews 1988; Rivas and Burghardt 2001), or have no effect on the mating success (Shine 1986).

Therefore, the roles of male body size and the degree of sexual size dimorphism (SSD) are different between species showing male-male combat and those showing multimale breeding aggregation. Particularly, in the case of multi-male breeding aggregation, the effects of body size seem to vary. To fully understand why this variation occurs, it is necessary to compare more cases of multimale breeding aggregation. Nonetheless, available studies are meagre, and most of them are conducted with natricine snakes in a temperate zone.

Here we report field observations of mating behaviour including a multi-male breeding aggregation in two species of pseudoxyrhophiins, *Dromicodryas bernieri* and *D. quadrilineatus*. To examine the relationships between SSD and male mating strategy, we also analysed the body size of these species collected during a long-term study. In addition, we analysed SSD of two other pseudoxyrhophiins, *Leioheterodon madagascariensis* and *L. modestus*, which are the only species known to show the male-male combat in this subfamily (Shine 1994; Glaw and Vences 2007), to compare the relationships between SSD and patterns of male-male rivalry in Pseudoxyrhophinae.

Methods

We observed mating behaviours of the two species of *Dromicodryas* in Ankarafantsika National Park, northwestern Madagascar, in July and August, which is the dry season of western Madagascar (Jury 2003). A series of behaviours of *D. bernieri* was video-recorded using an action camera, GoPro HERO7 Black (GoPro Inc., San Mateo, CA). We set the action camera on the tip of a 1.5–2 m selfie-stick to take a distance between the observer and the snakes. A series of behaviours of *D. quadrilineatus* was directly observed and was not video-recorded, and they were captured and measured after the observation. We classified behavioural characters of the snakes according to Senter (2022) to compare with other species. The following behavioural characters were recognised in our observations: Bite, Breeding aggregation, Head raise (Type 1) and Jerk body (Senter 2022).

Based on the data collected between 1999 and 2019 in Ankarafantsika National Park, we compared snout-vent length (SVL) of the four species, *D. bernieri*, *D. quadrilineatus*, *L. madagascariensis* and *L. modestus*. We collected these snakes, measured their SVL, marked, and released them at the site of capture after the measurement. When the same individuals were collected several times, we only used the last data for the analysis. Because there is no information on maturation size of any of the four species, we plotted SVL in histograms for each species and sex to visualise cohorts of young generations, and removed the smaller cohorts to exclude the data of juveniles. To compare SSD, we used an index recommended by Lovich and Gibbons (1992). Mean adult SVL of the larger sex was divided by the mean adult SVL of the smaller sex. The result was arbitrarily defined as positive when females were larger and negative in the converse situation. We used Welch's t-test for testing the differences of adult SVL between sexes. We used $\alpha = 0.05$ to determine statistical significance. All statistical analyses were carried out using R version 3.6.0 (R Core Team 2020).

Results

On 24 July 2019 at 1140 h, we sighted two males of *D. bernieri* chasing one female on a sunny forest floor covered with dead leaves in the north-eastern corner of Jardin Botanique A (JBA) in Ampijoroa Forestry Station (16°19'03.7"S, 46°48'35.2"E) (Suppl. material 1). At 1141 h, the two males were positioned behind the female, and all of them were pausing and raising their heads (Fig. 1A). When we brought the camera close to the snakes, one male (Male 1) approached the camera with tongue-flicking, turned back, and approached the female from behind the other male (Male 2). When Male 1 approached the female, Male 2 followed Male 1 with tongue-flicking at first, and immediately turned back and rose the head to the opposite direction of the female's head. When Male 1 approached the female, the female exhibited a sudden, staccato jerk with a large part of its body (Fig. 1B). At 1142 h, the female began to rapidly crawl forward, and the two males chased her. The female stopped at approximately three meters distance from the first point, and Male 1 caught up with her and bit the posterior body of the female from her right side (Fig. 1C). The female struggled and rotated its body immediately after the bite, and Male 2 joined the entangling pair and formed the breeding aggregation. The female moved violently at first, but after approximately 15 seconds from the bite, she almost calmed down. The two males aligned their bodies along the female's body and wound their tails around her cloaca (Fig. 1D). At 1143 h, we confirmed that a hemipenis of Male 2 had been inserted into the female. At this point, Male 1 had wound his tail around her immediately in front of the area where Male 2 had wound his tail around her. After that, there was basically no conspicuous movement, except that the female jerked her body

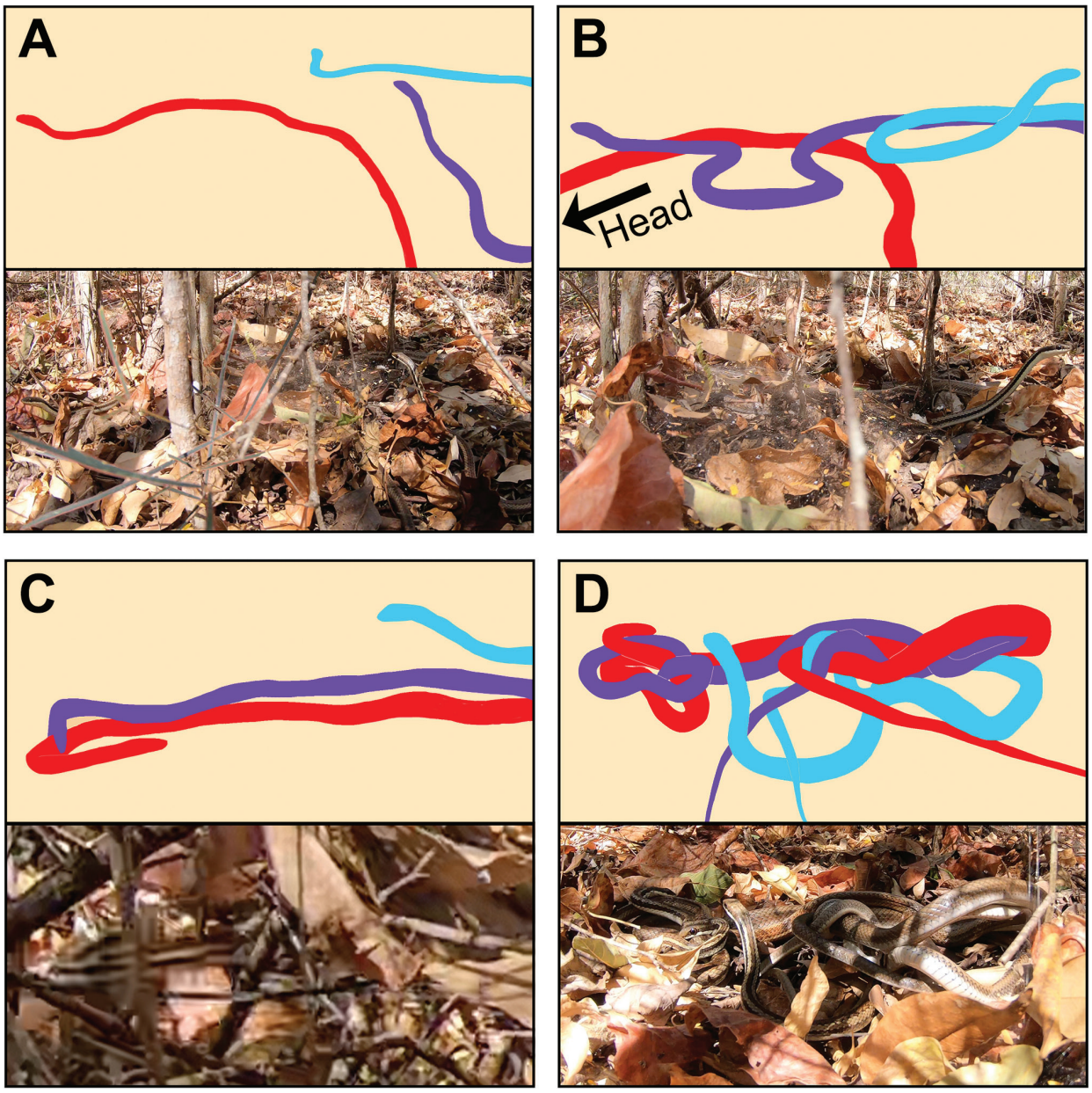

Figure 1. Mating behaviour of *Dromicodryas bernieri.* Red, purple, and blue snakes indicate the female, Male 1, and Male 2, respectively; **A.** Two males positioned behind the female while raising their heads; **B.** Male 1 approaching the female; **C.** Male 1 biting the female; **D.** Breeding aggregation formed by the female and two males.

intermittently approximately every minute. We finished the observation at 1202 h, when the snakes still formed the breeding mass. In the observation, we confirmed the following behavioural characters: Bite, Breeding aggregation, Head raise (Type 1) and Jerk body. Although we did not capture the snakes, the female seemed to be larger than the males, and the two males looked similar in size.

On 28 August 2019 at 1158 h, we sighted two males (larger male: 726 mm in SVL, 207 mm in tail length, and smaller male: 638 mm in SVL, 250 mm in tail length) and one female (778 mm in SVL, 286 mm in tail length) of *D. quadrilineatus* formed a breeding aggregation on the roadside of Ampijoroa Forestry Station (16°18'44.5"S, 46°49'01.3"E). The breeding aggregation was found in the bush of dry grass, and most parts of them were not visible. The snakes exhibited a sudden, staccato jerk with a large part of their body. When we captured all snakes at 1202 h, the males had not inserted the hemipenis into the female. We maintained them in a single mesh bag together, and when we checked them at 1300 h, the larger male inserted the hemipenis into the female. In the observation, we confirmed the following behavioural characters: Breeding aggregation and Jerk body.

From 1999 to 2019, we captured and measured 87, 22, 144, and 52 individuals of adult *D. bernieri*, *D. quadrilineatus*, *L. madagascariensis*, and *L. modestus*, respectively (Table 1). *Dromicodryas bernieri* and *D. quadrilineatus* showed female-biased SSD, and females are significantly larger than males in SVL ($p < 0.05$). On the other hand, *L. madagascariensis* showed male-biased SSD, and fe-

Table 1. Descriptive statistics on snout-vent length (SVL) of species of *Dromicodryas* and *Leioheterodon*. Differences of adult SVL between males (M) and females (F) were tested using the Welch's T-test.

Species	Sex	Adults only							All individuals	
		Mean	SE	n	SSD	*p*-value	Largest 10 %		Range	n
							Mean	SE		
D. bernieri	M	669	7.4	47	0.23	< 0.001	756	7.7	248–780	50
	F	823	10.6	40			917	10.2	266–942	49
D. quadrilineatus	M	658	29.3	7	0.217	0.002	726	NA	500–726	7
	F	801	26.7	15			970	0	258–970	16
L. madagascariensis	M	1306	16.9	67	-0.078	< 0.001	1537	56.8	356–1870	79
	F	1211	11.1	77			1358	20.1	532–1452	80
L. modestus	M	869	12.6	36	-0.015	0.532	996	16.8	255–1030	36
	F	856	16.5	16			953	12.5	271–940	19

males were significantly smaller than males ($p < 0.05$). In *L. modestus*, mean SVL of the adults, SVL of the largest 10% of the individuals, and the maximum SVL were slightly larger in males than in females, and SSD was slightly biased to males. However, no significant sexual difference in SVL was detected in *L. modestus* ($p = 0.532$).

Discussion

Our observations are the first detailed report of courtship behaviour in wild *D. bernieri* and *D. quadrilineatus* and also the first report of the multimale breeding aggregation in Lamprophiidae (Brusch IV et al. 2019). Courtship behaviour of Malagasy pseudoxyrhophiins, which consist of 80 species, has been reported in only three species, *Langaha madagascariensis* (Krysko 2003), *Leioheterodon madagascariensis* (Campbell and Murphy 1977), and *Madagascarophis colubrinus* (Mehta and Ford 2001), but all of these observations were conducted in captivity in pairs of a single male and a female. In pseudoxyrhophiins, the rivalries between males have been reported in only two species (*L. madagascariensis* and *L. modestus*). This small number of records of male-male rivalries is probably due to the scarce information on the natural history of Malagasy snakes. For example, Cadle (2009) reported strongly male-biased SSD in four species of *Liopholidophis* and suspected the existence of male-male mate competition in these species.

It is uncertain whether the multimale breeding aggregation is a major mating system in *Dromicodryas* or we simply observed exceptional cases. Mori et al. (2006) reported several observations of aggregation by *D. bernieri* in the same forest as our study. Although specific mating behaviour, such as mating ball, was not reported in their observations, the behaviours reported by Mori et al. (2006) are similar to the pre-copulation phase of our observations. In addition, in northwest Madagascar, *D. bernieri* and *D. quadrilineatus* are called "Maro longo", which means "many friends" in the Madagascan language (Brygoo 1982; A. Mori unpubl. data). This local name seems to reflect the mating-aggregation of these species, suggesting that the behaviour may have been recognised since a long time ago by Malagasy people. Therefore, the multimale breeding aggregation would be a major mating system of *Dromicodryas*.

Our result showed male-biased SSD in *L. madagascariensis* and *L. modestus*, supporting the previous hypothesis that male-male combat tends to occur in the species in which males have larger body size than females (Shine 1978). On the other hand, *D. bernieri* and *D. quadrilineatus* showed female-biased SSD. This result also conforms to the previous tendency that multimale breeding aggregation occurs in species in which females have larger body size than males (e.g., Shine 1994; Rivas and Burghardt 2005). Thus, the previous hypothesis concerning the relationship between body size and male-male rivalry may be also applied to pseudoxyrhophiins.

Our result provides new information on the courtship of Malagasy pseudoxyrhophiins. Generally, courtship behaviours of snakes are rarely observed in nature, and these behaviours have not been recorded sufficiently even in common species. Additional field studies and behavioural experiments would help to understand the strategies of the snake's courtship behaviour.

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Supplementary material 1

Courtship behaviour by *Dromicodryas bernieri*

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Data type: MP4 file.

Link: https://doi.org/10.3897/herpetozoa.35.e91579.suppl1