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First Observations of Mating Behavior in Captive Kuroiwa's Ground Gecko (*Goniurosaurus kuroiwae*)

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Abstract: Captive breeding programs are used worldwide to elucidate the natural history of endangered species and to recover and conserve their field populations. Kuroiwa's ground gecko, *Goniurosaurus kuroiwae* (Namiye, 1912) is an endangered species occurring in southern Japan; however, its mating behavior has not yet been described. We thus observed and quantified mating behavior of captive *G. kuroiwae*. Breeding trials with two males and three females resulted in 12 successful matings. Mating included precopulatory, copulatory, and postcopulatory stages, with 13 distinct mating behaviors observed. Behaviors indicative of successful copulation included the male preferentially biting the female's neck and the female approaching the male to initiate copulation. Mating behavior was initiated more than 10 h at night after cohabitation in some instances. These results suggest that at least one night of cohabitation is required for captive breeding of this species. Our findings thus contribute to conservation efforts by informing captive breeding programs.

Key words: Eublepharidae; Ex situ conservation; *Goniurosaurus kuroiwae*; Mating behavior

Introduction

Wild populations of Kuroiwa's ground gecko, *Goniurosaurus kuroiwae*, have declined because of habitat loss caused by development, predation by alien species such as mongoose, *Urva auropunctata*, and illegal collection (Toda and Tanaka, 2017). The species is currently listed as "vulnerable" on the IUCN Red List of Threatened Species (Kidera and Ota, 2017) and is in CITES Appendix III (CITES, 2023). Conservation guidelines advocate ex situ methods such as captive breeding to supplement wild populations temporarily, which may be crucial for species preservation (Ministry of the Environment, Government of Japan, 2011). A thorough understanding of reproductive behaviors, including mating, is fundamental for the efficient implementation of captive breeding programs; however, no previous studies on the breeding behavior of *G. kuroiwae* exist. This species is nocturnal and solitary, complicating observing breeding behavior in situ. This study was conducted to observe and quantify the mating behaviors of *G. kuroiwae* in captivity to improve the understanding of its reproductive biology to inform and improve captive breeding techniques.

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Materials and Methods

Study animals

Five geckos (two males and three females) were captured at the Ocean Expo Park (Motobu, Okinawa Prefecture, Japan) in August and September 2020 and May 2021. Only sexually mature individuals (snout-vent length >77 mm) were used in this study (Tanaka and Nishihira, 1987) (Table 1). Each individual's sex was determined based on the presence or absence of hemipenal bulges (Kurita and Toda, 2013). All experiments were performed in accordance with the ethical guidelines for animal exhibition and research of the Japanese Association of Zoos and Aquariums (Japanese Association of Zoos and Aquariums, 2017).

Experimental conditions and behavioral observations

Each gecko was kept in a separate plastic container (21.5×36.5×15.0 cm) containing peat moss, red ball soil, a plastic shelter, and a water dish. Rearing conditions were maintained at 18–31°C and >80% humidity; under a 10L:14D photoperiod. Every two days, geckos were fed crickets dusted with vitamin supplements. For mating trials, the geckos were placed in larger containers (34.0×43.0×28.0 cm) containing peat moss and red ball soil. For each trial, one male and female among the five captured individuals were randomly selected and were simultaneously placed together in a breeding container at 21.5–29.8°C. Mating behavior was observed between May and September. Four combinations of male-female pairs were tested during the trials (Table 1). Each trial lasted 16 hours, from 1800 to 1000 h, with 40 lx illumination. Behaviors were recorded using a digital video camera (Hero7 Black, GoPro Inc.) and were categorized into precopulatory, copulatory, and postcopulatory stages. In total, 15 trials were conducted.

Table 1. Pairing of male and female Kuroiwa's ground geckos (*Goniurosaurus kuroiwae*) during mating trials. Black circles indicate the successful insertion of the male hemipenis into the female cloaca.

Date	Male ID	Female ID	Insertion of genitalia
11 May 2021	4	2	●
24 May 2021	5	3	●
29 May 2021	5	1	●
5 Jul 2021	4	1	●
21 Jul 2021	5	3	●
5 Aug 2021	4	2	●
15 Aug 2021	5	3	●
20 Aug 2021	4	2	
22 Aug 2021	4	1	●
26 Aug 2021	5	3	
7 Sep 2021	4	2	●
9 Sep 2021	5	3	●
12 Sep 2021	5	3	●
14 Sep 2021	5	1	●
19 Sep 2021	5	1	

Results and Discussion

Insertion of the male genitalia into the female cloaca (defined as mating success) was observed in 12 (80%) of the 15 trials (Table 1; Fig. 1). The male and female precopulatory, copulatory, and postcopulatory behaviors are described in Fig. 1, Table 2; photographs of the behaviors are shown in Fig. 2.

The first step in precopulatory behavior is the sex identification of the mating partner. Previous studies indicate that in many gecko species, mating behavior is typically initiated by males (Brillet, 1993; Regalado, 2003; Todd, 2005). In *Eublepharis macularius*, *Hemidactylus mabouia*, and *Sphaerodactylus vincentii*, males lick females to obtain chemical signals for sex identification (Regalado, 2003; Marcum et al., 2008; Mason and Parker, 2010); however, in the present study, *G. kuroiwae* males licked females in only five cases (33%), suggesting the possibility that they recognize sex via other cues than non-volatile chemicals on the body surface of females. Additionally, males frequently initiated neck biting (67%; 10

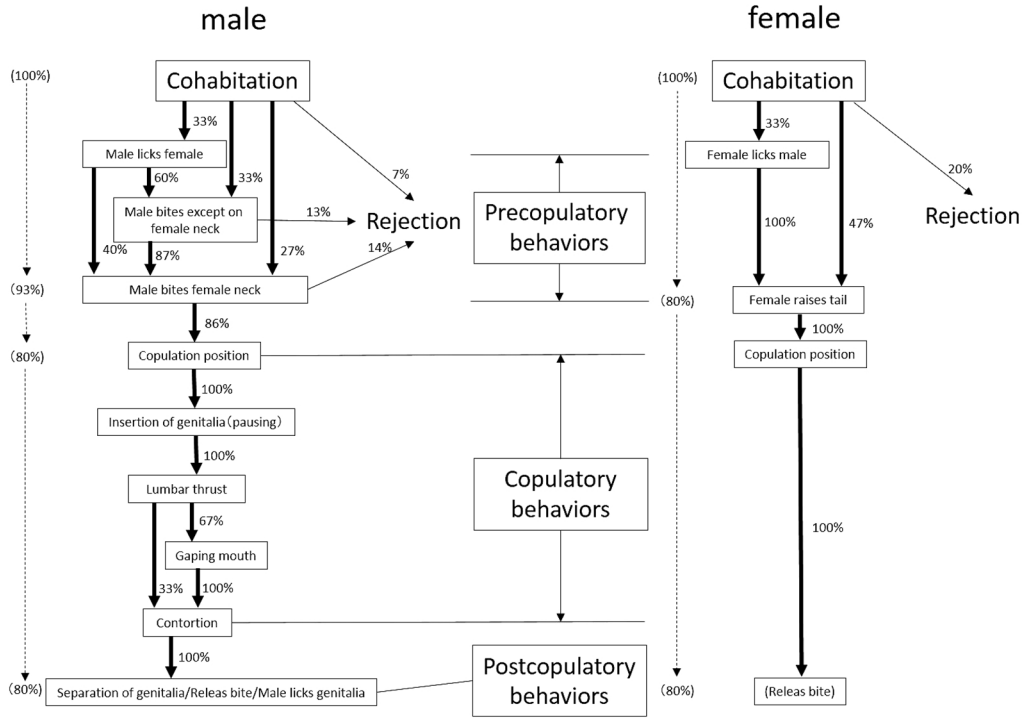

FIG. 1. Mating behavioral sequence in Kuroiwa's ground gecko (*Goniurosaurus kuroiwae*) were categorized into precopulatory, copulatory, and postcopulatory patterns for both sexes. The behaviors are presented in the chronological order as displayed during mating.

cases) rather than tail biting, and neck biting was observed in all successful matings. This behavior, also observed in other geckos (e.g., *He. mabouia* and *Hoplodactylus maculatus*) (Regalado, 2003; Todd, 2005), has been described as a mechanism for female restraint during copulation. Therefore, neck biting by males likely contributes to successful copulation and mating success in *G. kuroiwae*. Furthermore, females approached males in five cases (33%), all of which resulted in successful copulation. This behavior, coupled with females raising their tails, would be a reliable indicator of female readiness for copulation. Although the time between introduction to the container and the onset of interaction varied in the present study (147–38, 117 s), mating was successful even in the cases where the outset of interaction was delayed. This suggests that if mating behavior does not begin immediately, cohabitation at least overnight is required for captive breeding of *G. kuroiwae*.

Among copulatory behaviors, successful mating occurred in all cases that progressed to the "copulatory position." Additionally, "gaping mouth" and "contortion" were observed, which, to our knowledge, have not been reported in other geckos. Among postcopulatory behaviors, licking of genitalia, as observed after copulation in the present study, may be a further indicator of ejaculation; similar behaviors have been observed in various gecko species (e.g., *E. macularius*, *Thecadactylus rapicauda*, *He. maculatus*, and *He. mabouia*) (Regalado, 2003; Todd, 2005; Quesnel, 2006; Huang, 2013). Further studies with larger sample sizes are needed to evaluate the interspecific differences in the reproductive behavior and the functional significance of licking of genitalia between this and the other gecko species.

TABLE 2. Definitions and quantitative data on mating behaviors observed in Kuroiwa's ground geckos (*Goniurosaurus kuroiwae*) presented in chronological order.

Behavior classification	Behavior type	Definition	Quantitative data (mean±SD; range, n)
Precopulatory behaviors	Female licks male	The female licks the male body surface before the male bites her.	6.8±9.8 times (1–24, n=5)
	Male licks female	The male licks the female body surface before biting her.	8.2±15.5 times (1–36, n=5)
	Male bites female, except on the neck	The male bites the lower trunk region of the female (e.g., lateral body, hind limb, or tail).	—
	Time from the start of cohabitation to the neck bite	—	2,619.5±4,162.5 s (387–12589 s, n=8)
	Male bites female's neck	Male bites female's neck while copulating.	—
	Time from the start of cohabitation to the neck bite	—	5,033.0±10,082.1 s (147–38117 s, n=14)
	Female raises tail	The female raises its tail in a copulatory position.	—
	Time from the start of the neck bite to the female raising tail	—	102.2±35.9 s (42–176 s, n=12)
Copulatory behaviors	Copulatory position	After the female raises its tail, the male assumes a position where they contact each other's cloacal regions at the ventral side of the female.	—
	Insertion of genitalia (pausing)	The male inserts his genitalia into the female cloaca and pauses briefly.	—
	Pausing	—	8.2±2.4 s (6–15 s, n=12)
	Lumbar thrust	The male thrusts its lumbar region after intromission.	4.4±1.3 s (2–6 s, n=12), 2.0±0.7 times/s (1.0–3.3 times/s, n=12)
	Gaping mouth	After the lumbar thrust, the male pauses and gapes its mouth, followed by a re-bite on the female's neck.	2.9±0.6 s (2–5 s, n=8)
	Contortion	The male shakes his lower trunk region very slightly after lumbar thrust.	23.7±2.9 s (18–28 s, n=11), 3.2±0.5 times/s (2.4–3.9 times/s, n=11)
	Time from insertion of the genitalia to the end of contortion	—	42.7±6.7 s (34–57 s, n=11)
Postcopulatory behaviors	Separation of genitalia	Separation of male's hemipenis from the female's cloaca.	—
	Time from the end of contortion to separation	—	90.1±81.9 s (40–325 s, n=11)
	Releases bite	Terminating copulation, the male releases the female neck.	—
	Male bite	—	305.4±105.0 s (194–529 s, n=12)
	Male licks genitalia	Terminating copulation, the male licks its genitalia before retracting the hemipenis into the hemipenal bulges.	—

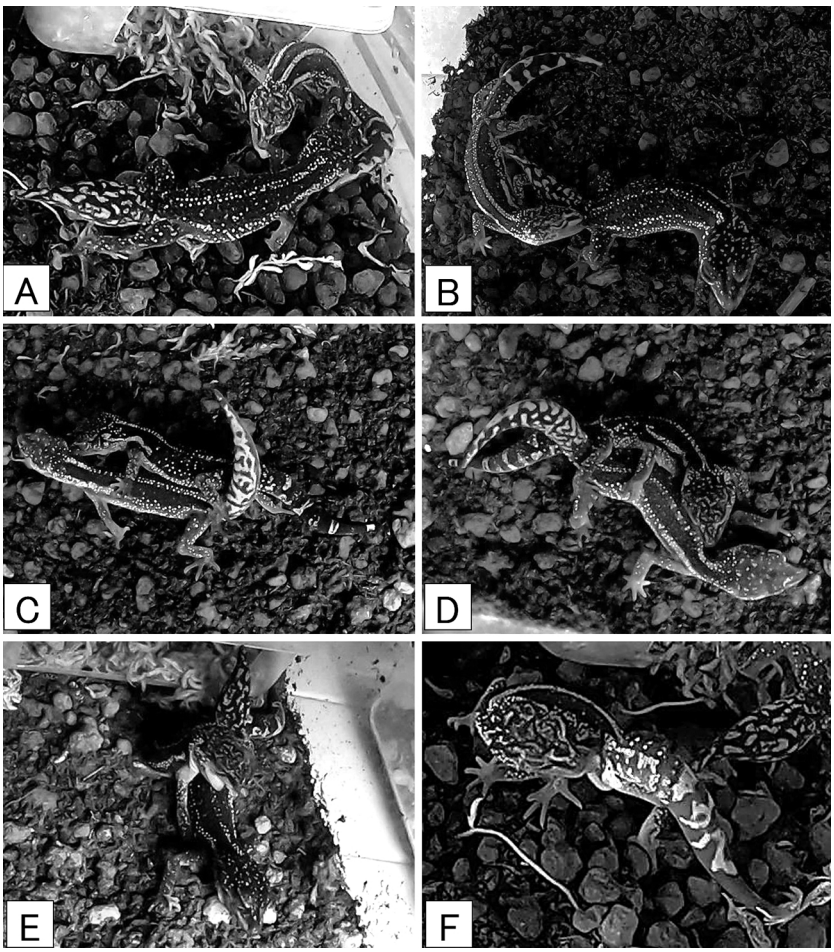

Fig. 2. Photographs depicting mating behaviors of Kuroiwa's ground gecko (*Goniurosaurus kuroiwae*): (A) male licks female, (B) male bites female, except on the neck, (C) male bites female neck and female raises its tail, (D) copulatory position, (E) gaping mouth, and (F) male licks its own genitalia.

This is the first report on the mating behavior of *G. kuroiwae* in captivity. The observed behavioral patterns and quantitative data across precopulatory, copulatory, and postcopulatory stages serve as indicators of mating success and provide valuable insights for informing captive breeding programs and improving the mating success rates. However, due to the small number of individuals used, these observations unlikely cover the entire repertoire of reproductive behavior of this species. Future studies with a larger number of individuals and populations are warranted.

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