

Prolonged copulation and possible mating plug formation in the Ryukyu Greensnake, *Ptyas semicarinata* (Hallowell, 1861), on Okinawa Island

Hiroaki Yanagi^{1,*} and Akira Mori¹

Copulation is one of the fundamental reproductive behaviours in animals with internal fertilization. The duration of this activity may reflect the degree of sperm competition and predation pressure, and it has long been of interest to behavioural ecologists (Magnhagen, 1991; King et al., 2009; Barbosa, 2025). Snakes are considered one of the animal groups with the longest copulation duration, and the longest duration was approximately 32 h in a boid snake and 28 h in a rattlesnake (Olsson and Madsen, 1998; King et al., 2009). In some snake species, gelatinous mating plugs are formed during copulation, physically blocking the female's cloaca (Shine et al., 2000). The plugs are thought to function both to prevent the leakage of sperm and to hinder subsequent copulation attempts by rival males (Friesen et al., 2013). Here, we report an exceptional case of prolonged copulation in *Ptyas semicarinata*. We also describe the formation of a substance at the female's cloaca after copulation, which may represent a mating plug.

Ptyas semicarinata is a medium-sized colubrid species with a maximum snout–vent length (SVL) of 1000 mm found only in Japan, on the Okinawa and Amami Islands as well as on Takara and Kodakara Islands. Its mating is known to occur in spring (April–May) and late summer (August–September) (Moriguchi et al., 1987; Ota, 1993; Matsui and Mori, 2021). On 31 August 2024 at around 16:30 h we found a mating pair of this species on a mountain trail in Ogimi Village on Okinawa Island (Fig. 1A). The pair was collected for body size measurements. Because mating continued even after we brought the snakes to the laboratory, they were transferred to a plastic cage (30 × 18 × 20 cm) and kept until copulation ended. Unexpectedly, they continued mating for two more days

after capture. The last time we confirmed copulation was at 23:15 h on 2 September, and separation was confirmed at 09:50 h on 3 September. A minimum of 54.45 h had elapsed from capture to the final observation of hemipenial insertion. To the best of our knowledge, this is the longest documented duration of continuous genital insertion (true copulation) among all vertebrates. Previous reports by Nakaima and Iraha (1983) and Moriguchi (1988) described copulation of *P. semicarinata* under captive conditions. In both cases, the copulating pairs remained firmly joined even when handled, suggesting strong physical attachment during mating. Our observation is consistent with these reports, as the pair remained coupled despite being transported and confined in a small enclosure. However, it should be noted that the process of capture may have played a role in prolonging copulation, and the narrow space in the cage may have limited the female's ability to reject the male.

During copulation, the posteriormost part of female's body (approximately eight ventral scales anterior to the cloaca) was noticeably inflated, with stretched skin visible between the body scales (Fig. 1B). This appearance resembled the neck of snakes after swallowing large prey. It is likely that the male's hemipenis was significantly enlarged during copulation and that the female's cloacal region has high elasticity to accommodate it. After separation, a solid red substance was observed at the female's cloaca (Fig. 1C), which may represent a mating plug, a structure not previously reported in *P. semicarinata*. Devine (1975) suggested that mating plugs are gelatinous, cause swelling of the female's cloacal region, and can be detected by palpation as a hard mass. In our case, although the female's cloacal region appeared swollen after copulation, we did not specifically examine it by careful palpation and therefore we cannot determine whether a firm structure was present. However, the substance attached around the cloacal region did not appear gelatinous; rather, it was a more solid material.

¹ Department of Zoology, Graduate School of Science, Kyoto University, Sakyo, Kyoto, 606-8502, Japan.

* Corresponding author. E-mail: yanagi.hiroaki.82k@st.kyoto-u.ac.jp



Figure 1. (A) A pair of *Ptyas semicarinata* encountered while copulating on Okinawa Island, Japan. The left individual is the female, the right the male. (B) The right hemipenis is inserted and the posteriormost part of the female body is inflated. (C) The female's cloaca seen in close-up in caudal view after mating had ended. A red substance, seemingly a mating plug, was observed. Photos by Hiroaki Yanagi.

Measurements taken after copulation showed that the male's SVL was 949 mm with a body mass of 244 g, while the female's values were 760 mm and 110 g, respectively. The snakes were marked by ventral scale clipping and released at the site of capture.

Prolonged copulation may serve to increase sperm transfer, promote mating plug formation, and physically prevent subsequent copulation by other males, all of which are advantageous under high sperm competition (Olsson and Madsen, 1998). *Ptyas semicarinata* inhabits island environments with poor predators, where such extended mating may have evolved under intensified intraspecific sexual selection. Additional knowledge on various aspects of mating behaviour in this species would provide further insight into the evolutionary pattern of their reproductive ecology.

Acknowledgements. This survey was conducted with approval of the Animal Experiment Committee of Kyoto University (Approval No. 202404). We would like to express our sincere gratitude to Mamoru Toda for valuable suggestions during the preparation of this manuscript. This study was supported by

JST SPRING (grant number JPMJSP2110) and the Sasakawa Scientific Research Grant from The Japan Science Society (2024).

References

- Barbosa, F. (2025): The value in the details: copulatory courtship behaviour in soldier flies and bean beetles. *Animal Behaviour* **221**: 122825.
- Devine, M. (1975): Copulatory plugs in snakes: enforced chastity. *Science* **187**: 844–845.
- Friesen, C.R., Shine, R., Krohmer, R.W., Mason, R.T. (2013): Not just a chastity belt: the functional significance of mating plugs in garter snakes, revisited. *Biological Journal of the Linnean Society* **109**: 893–907.
- King, R.B., Jadin, R.C., Grue, M., Walley, H.D. (2009): Behavioural correlates with hemipenis morphology in New World natricine snakes. *Biological Journal of the Linnean Society* **98**: 110–120.
- Magnhagen, C. (1991): Predation risk as a cost of reproduction. *Trends in Ecology & Evolution* **6**: 183–186.
- Matsui, M., Mori, A. (2021): *Amphibians and Reptiles of Japan*. Hikone, Japan, Sunrise Publishing.
- Moriguchi, H. (1988): Mating of Ryukyu green snakes in captivity. *Akamata* **5**: 2. [in Japanese]
- Moriguchi, H., Tanaka, S., Hinoue, M. (1987): A wild sighting of

- Ryukyu green snakes mating. Akamata **4**: 7–8. [in Japanese]
- Nakaima, H., Iraha, T. (1983): An example on the mating of the Ryukyu green snake *Opheodrys semicarinatus* in captivity. Akamata **1**: 11. [in Japanese]
- Olsson, M., Madsen, T. (1998): Sexual selection and sperm competition in reptiles. In: Sperm Competition and Sexual Selection, p. 503–577. Brikhead, T.R., Møller, A. P., Eds., San Diego, California, USA, Academic Press.
- Ota, H. (1993): Observations on the mating of *Cyclophiops semicarinatus* (Colubridae: Squamata) in the field. Akamata **8**: 3–4. [in Japanese]
- Shine, R., Olsson, M., Mason, R. (2000): Chastity belts in gartersnakes: the functional significance of mating plugs. Biological Journal of the Linnean Society **70**: 377–390.