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Male Courtship Behavior of *Tylototriton (Echinotriton) andersoni* Boulenger under Laboratory Conditions

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Abstract: We observed male courtship behavior of *Tylototriton (Echinotriton) andersoni* in the laboratory. In some males, the cloaca swelled and became wet with mucous secretions from December to May. A male in this condition crept around a female, sniffing around her body and drawing a thread of mucus from his cloaca, so that the female was surrounded by spiderweb-like string of mucous attached to the substrate. The male then deposited a spermatophore by rubbing his cloaca against the substratum while swaying his body to and fro. The male behaved similarly towards females from different localities, though we could not observe any female reaction to the male. Under a phase-contrast microscope, the gross morphology of sperms from a male from Amamioshima Island was similar to that reported for males from Okinawajima.

Key words: Male courtship behavior; *Tylototriton (Echinotriton) andersoni*; Spermatophore deposition; Sperm morphology; Amamioshima; Tokunoshima; Okinawajima

INTRODUCTION

Since its original description by Boulenger (1892) on the basis of a specimen from Okinawajima, the Anderson's alligator newt, *Tylototriton andersoni*, has been viewed as a rare (Tago, 1931), relict species. Reports on this species were thus mostly limited to collection records or brief notes on a few biological aspects for a very long time (Ichikawa, 1941; Momma and Makino, 1941; Koba, 1955, 1956). Sato (1943) reviewed biological data accumulated by that time, but details of distribution, precise habitats, and breeding ecology were totally unknown.

One of us (Utsunomiya, 1973) first observed in the laboratory that the female of this species lays eggs in the soil, unlike other Japanese urodeles. Subsequently, Utsunomiya et al. (1978) and Matayoshi et al. (1977, 1978) reported detailed distribution and several ecological aspects, especially egg-laying habits in natural habitats, of this species. Through these more recent studies, a gross outline of the breeding habits of this species was provided. For example, it was shown that this newt never enters the water after metamorphosis and lays eggs in the soil under leaf litter that could be kept moist during incubation. The oviposition site is located near the water, but is never flooded. The female rolls her eggs with her snout and buries them deep in the soil, sometimes as deep as eight cm. It is also

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known that the male is not present at oviposition, and that the female lays fertilized eggs, even after isolation from males for several months. Actually, males rarely visit the breeding sites, as shown by the female-biased sex ratio at the sites (15 males per 237 females: Tanaka, 1994).

As described below, we found spermatophores deposited in the laboratory more than twenty years ago (Utsunomiya, 1982). It was puzzling, however, how the male deposits and transfers his spermatophores to females without entering the water, unlike many other newts that are aquatic at least in the breeding season (Salthe, 1967). In this article, we summarize male courtship behavior observed in the laboratory, together with gross morphology of the spermatophore. Part of the observations described below have been briefly reported (Utsunomiya, 1982) or cited as a personal communication (Sparreboom et al., 2001).

Materials and Methods

Generic classifications of the Oriental alligator newts (*Tylototriton* Anderson, 1871, sensu lato) differ from one author to another. Current herpetologists tend to follow Nussbaum and Brodie's (1982) idea of assigning several species including *andersoni* to a separate genus *Echinotriton* Nussbaum and Brodie, 1982. However, because representatives of *Tylototriton* and *Echinotriton* sensu Nussbaum and Brodie (1982) are genetically very close (Hayashi and Matsui, 1989), the validity of *Echinotriton* as a full genus needs further taxonomic studies. We herewith treat *Echinotriton* as a subgenus of *Tylototriton*.

Nine specimens of *T.* (*E.*) *andersoni* were used for behavioral and spermatic observations: one male collected at Naze-shi, Amamioshima Island, on 12 April 1980; one female also from Naze-shi on 13 April 1980; two males and one female from Amagi-cho, Tokunoshima Island, on 6 April 1972; one female also from Amagi-cho on 4 January 1981; three females collected as eggs on 8 February 1978 at Gushikawa-shi, Okinawa-jima Island, reared in the laboratory, and attained sexual maturity within four years. The population from Okinawa Prefecture has been protected by the prefectural government as a natural monument since 9 November 1978.

Individuals were reared at the room temperature and under natural light conditions in Hiroshima, Honshu, Japan, except for the winter season when the air temperature was kept at 15 C. Each terrarium used measured 36×22×25 cm and one-third each of the bottom was covered with shallow water, pebbles, and sand. Pieces of slate were laid on the pebbles for shelters, and leaf litter from the natural habitat was placed on the sand (Utsunomiya, 1973).

We periodically checked each individual, and when some of them showed notable bahaviors, we made intensive observations. To examine sperms, we took male cloacal secretions or the white part of deposited spermatophores, smeared them on glass slides, and examined the slides with a phase contrast microscope. The male secretions were obtained by gently pressing his belly. We also examined these slides with a light microscope after fixig them with either aceto-orcein or Navasin's solution and staining them with Heidenhain's hematoxylin.

Results

Morphological change in the breeding season

Three adults from Tokunoshima could not be sexed at the time of collection on 6 April 1972, but at the time of observation on 14 February 1973, two of them had a swollen cloaca. The remaining one that did not have a swollen cloaca proved to be a female because it laid fertile eggs on 9 May 1973. Thus, the possession of a swollen cloaca by the two individuals also proved to be an indication that they were males.

Male courtship behavior

On 16 April 1982, we found the cloaca of

the male from Amamioshima to be swollen and wet with secretions. Because the female from the same island kept with him showed no response, we put him in another terrarium containing a female from Okinawajima. Soon after this manipulation, the male tried to sniff at the female with his snout close to her body (Fig. 1A, B). The female also once reacted to the male in such a way as to seem to be trying to sniff at him, but soon stopped. The male sniffed at her in the order of head, belly, cloaca, and tail tip, and repeated this behavior several times. The female, however, showed no response to his behavior.

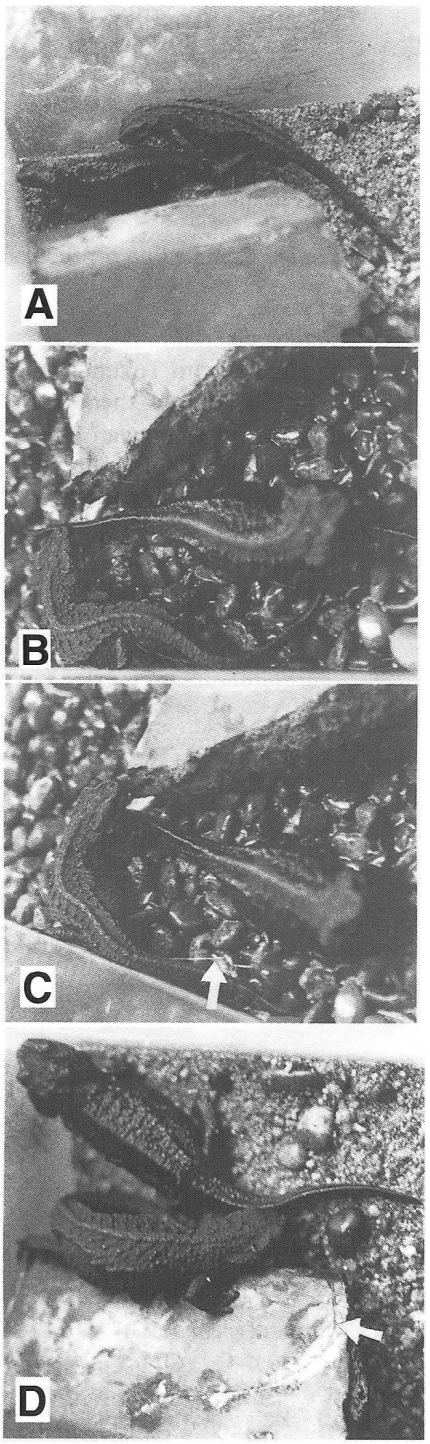

FIG. 1. A male *Tylototriton* (*Echinotriton*) *andersoni* sniffing at a female (A, B) and a string of mucus from the male cloaca (white arrow, C, D).

Meanwhile, the male pressed his cloaca on a substrate pebble and secreted transparent mucus. Then he moved forward for 5–10 cm and again pressed his cloaca to another pebble. By this action, the mucus was extended like a string (Fig. 1C, D). The male continued this action around the female, keeping his snout close to her, and the string of mucus was stretched around the female like a spider's web. Unfortunately, most portions of the mucous strings soon disappeared on the pebbles that were always kept wet to maintain moisture in the cage.

After continuing this behavior for about three minutes, the male placed his cloaca on a pebble, swaying his waist. Then he moved his body to and fro and slowly lifted his pelvic region from the pebble. Below his cloaca, a conical spermatophore could be seen tightly attached to the pebble. There were white portions in and at the top of the otherwise transparent spermatophore. After this first spermatophore deposition, the male resumed pursuing the female, pulling the mucous strings. About ten minutes later, the female hid under a slate, and we had to cease observation.

We put the active male into another cage containing a female from Tokunoshima, but the male did not react toward the female during ten minutes of observation.

Then we put the male into still another cage containing the two remaining females from

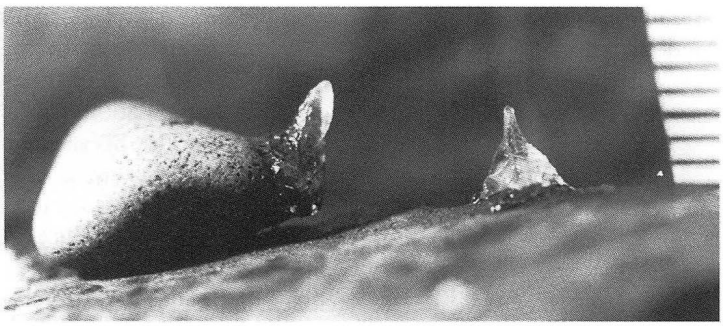

FIG. 2. Two spermatophores of *Tylototriton* (*Echinotriton*) *andersoni* deposited on a pebble and a shelter slate. Scale indicates 1 mm.

Okinawajima that were smaller sibs of the female mentioned above. The male soon reacted to one of them and resumed the same behavior as described above. He pressed his cloaca to a shelter slate and left a clear mucous string on it. When the forelimb of the female became tangled in the mucus, she vigorously tried to remove it by rubbing her hand in the substrate sand and tried to escape from the male. Notwithstanding her action, the male persistently tried to move around the female drugging mucous strings from his cloaca. We failed to continue observation, but, ten minutes later, we found two spermatophores, one on a pebble and another on the slate. They were connected by mucous strings and thus seemed to have been deposited one after the other (Fig. 2).

Spermatophore morphology

On 10 December 1981, we found two spermatophores attached to the surface of a slate shelter in a cage containing the male and the female from Amamioshima. Both spermatophores were nearly conical in shape (Fig. 2), transparent like crystal, and partially spotted with white. Their material was hard but somewhat jelly-like.

On 23 January 1982, the male from Amamioshima again deposited a spermatophore on a slate. It was 4.2×4.0 mm in base diameter and 7.1 mm in height. As in the previous case, the spermatophore was hard, jelly-like, and transparent with white portions on top and inside.

Sperm morphology

When one female from Tokunoshima laid eggs on 9 May 1973, we took them out of the soil and washed them in a small amount of water. We observed the water thus obtained under a light microscope, but could not find any sperms.

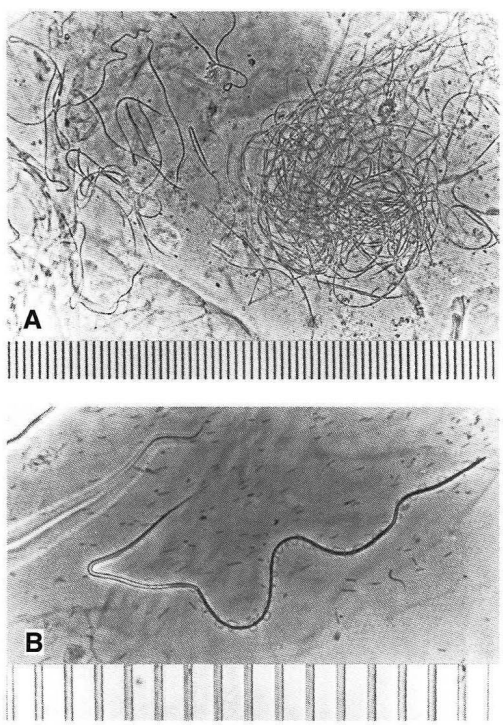

FIG. 3. Clumps of sperms of *Tylototriton* (*Echinotriton*) *andersoni* in the spermatophore (A) and a spermatozoon observed under a phase-contarst microscope (B). Scale indicates 10 μm.

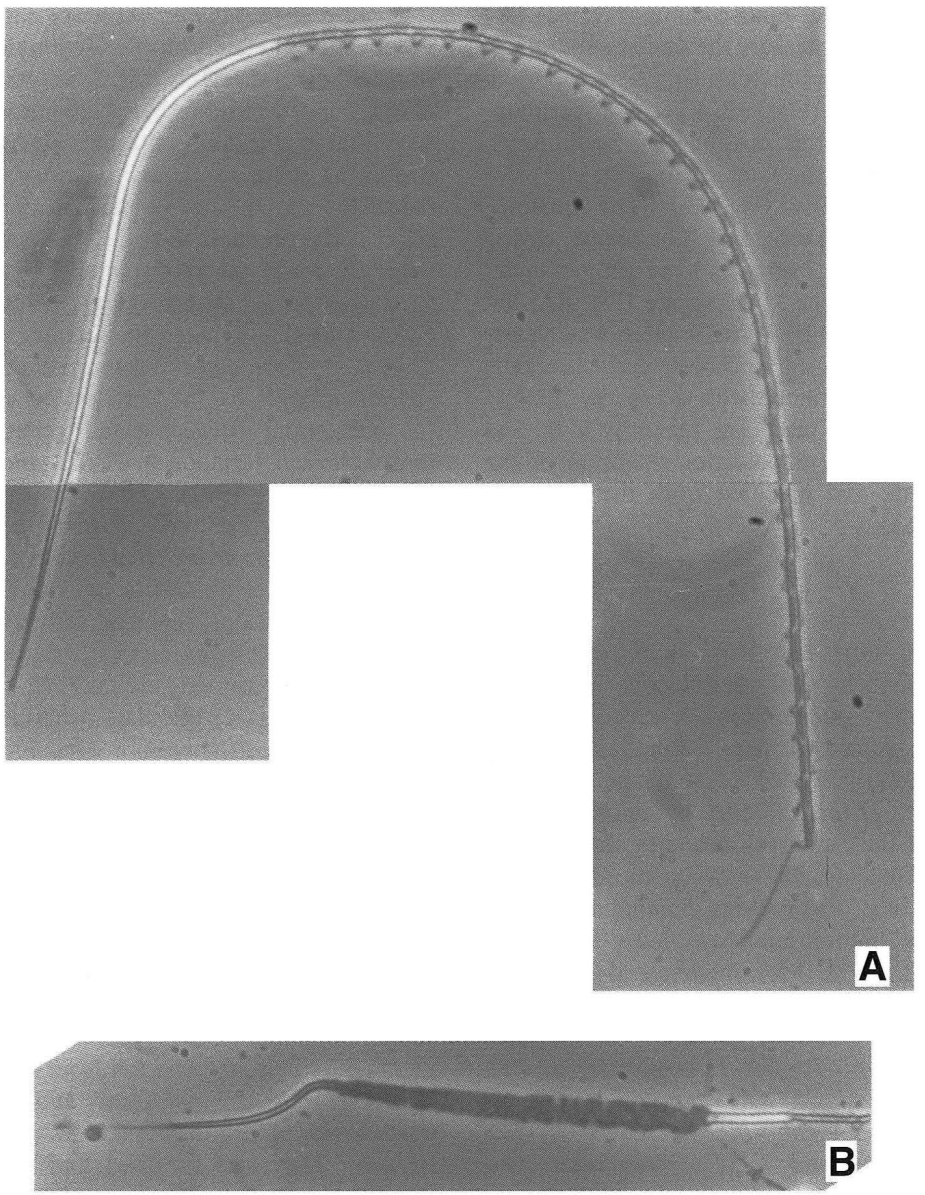

FIG. 4. A spermatozoon of *Tylototriton* (*Echinotriton*) *andersoni* observed under a phase-contarst microscope (A) and the head and neck region observed under a light microscope after staining with aceto-orcein (B). ×ca. 1300.

For the spermatophores deposited on 10 December 1981 by the male from Amami-oshima, we continued observation for ten days, in order to witness the way the female took up these spermatophores. Unfortunately, however, the female showed no particular response, and the spermatophores became gradually smaller day by day. Therefore, we gave up further observation and observed these spermatophores under a phase-contrast microscope. In the white portion of the spermatophore, we could observe numerous sperms

moving slowly, forming a clump (Fig. 3A).

We failed to measure the size of a spermatozoon exactly, but its total length was approximately 250 μm (Figs. 3B, 4A). In a spermatozoon, in order, the acrosome, the head proper, the neck piece, the axial rod, and the end piece could be identified (Fig. 4A). The acrosome and the neck piece were not always clearly distinguishable from the head proper under the phase contrast microscope (Fig. 4A, but see Fig. 3B), but the neck piece was clearly recognized by its thickness and chromatic nature when stained with aceto orcein (Fig. 4B). The end piece was relatively long, and was formed by a posterior extension of the flagellum, and was free from the axial rod (Fig. 4A).

DISCUSSION

Newts of the family Salamandridae are highly deiversified in their reproductive habits and quite a few studies have been made on their courtship behavior (e.g., Salthe, 1967; Halliday, 1977). Males of some genera (e.g., *Cynops, Triturus*) court by tail-fanning in front of their mates (Kawamura and Sawada, 1959; Halliday, 1977), while those of other genera (e.g., *Pleurodeles, Salamandra*) clasp females before spermatophore deposition (Arnold, 1977). Most members of this family generally breed in the water, and of the genus *Tylototriton*, all four species of the subgenus *Tylototriton, T.* (*T.*) *taliangensis*, *T.* (*T.*) *kweichowensis*, *T.* (*T.*) *shanjing* (Fei et al. 1999: *T.* (*T.*) *shanjing* treated as *T.* (*T.*) *verrucosus*), and *T.* (*T.*) *verrucosus*, also breed in the water (Annandale, 1908; Smith, 1924; Fei et al. 1999).

On the other hand, all members of the subgenus *Echinotriton*, *T.* (*E.*) *andersoni*, *T.* (*E.*) *chinhaiensis*, and *T.* (*E.*) *asperrimus*, lay eggs on land (Utsunomiya, 1973; Fei et al. 1999). The paucity of detailed relevant information on these terrestrial breeders, however, has been an obstacle to inferring evolutionary trends in the courtship behavior of the Salamandridae (Sparreboom et al., 2001).

Because *T.* (*E.*) *andersoni* never enters the water after metamorphosis, spermatophore transfer in this species is enigmatic. As shown above, our observations in the laboratory were successful in elucidating the male behavior for depositing spermatophores, but we failed to detect the way the female receives spermatophores. It is probable that the females we used were not ready for receiving sperms due to their age (too old or too young). Also, differences in locality between the females and the male might have prevented acceptance of the spermatophores by the females, considering the substantial between-island genetic and morphological variation in *T.* (*E.*) *andersoni* (Hayashi et al., 1992). However, the male indiscriminately exhibited courtship behavior toward females from different localities.

Sparreboom et al. (2001), on the basis of captive observations, reported mating behavior of *T.* (*E.*) *chinhaiensis*, which was basically similar to that reported here for *T.* (*E.*) *andersoni* with respect to the male behavior up to the stage of spermatophore deposition. In both species, for example, the male, after sniffing the female, marks a trail around her by excreting mucous from his cloaca, and finally deposits the spermatophore without clasping her. Like male *T.* (*E.*) *andersoni*, male *T.* (*E.*) *chinhaiensis* may deposit several spermatophores in one evening (Sparreboom et al., 2001).

Sparreboom et al. (2001) observed the trail-marking by mucous excretion from the male's cloaca in *T.* (*E.*) *chinhaiensis*, and assumed the behavior to be a remarkable feature of this species. They also surmised that this trail, presumably serving as orientation for the female through olfactory or tactic stimuli, has evolved as an adaptation to underground mating. But we are dubious of this view because the male *T.* (*E.*) *andersoni* observed by us marked a trail only on pebbles and slates, never on the surrounding soil. We suspect the spiderweb-like threads to be an apomorphy of *Echinotriton*, although this view needs substantial verification on the basis of observations on the other species of this subgenus.

In this context, the behavior of *T.* (*E.*) *asperrimus* should be examined, because its allocation to the subgenus *Echinotriton* is questionable (Nussbaum et al., 1995).

In our observations, females showed no particular responses to the male's courtship behavior including rejection or unresponsiveness, such as the snout-kick which Sparreboom et al. (2001) found in *T.* (*E.*) *chinhaiensis*. In that species, the female, when pairing with the male, follows him on an ellipsoid track. After deposition of a spermatophore by the male, the female is lead over it by aligning her body with the male. Finally the female shifts her pelvic region and traces the spermatophore with her cloaca (Sparreboom et al., 2001). Sparreboom et al. (2001) noted that the female's success or failure in receiving the sperm mass was ascertained by the removal of the white sperm cap from the spermatophore base, but they did not make it clear whether or not the sperm cap had been absorbed in the female's cloaca.

Most probably, a succession of similar behaviors enables the female of *T.* (*E.*) *andersoni* to receive the spermatophore. Future detailed studies of female behavior in this species, including her treatment of the sperm cap, which Sparreboom et al. (2001) failed to ascertain in *T.* (*E.*) *chinhaiensis*, are strongly desired both in the laboratory and in the field.

As briefly mentioned by Sparreboom et al. (2001) on the basis of information from one of us (Utsunomiya, pers. comm., 2000), the spermatophore of *T.* (*E.*) *andersoni* (4.2×4.0 mm in base diameter and 7.1 mm in height) was much larger than that of *T. chinhaiensis* (2.5–3 mm both in base diameter and in height), and this difference, approximately 4–7 times in volume, might lead to a difference in the frequency of spermatophore deposition. The spermatozoon of *T.* (*E.*) *andersonii* from Okinawajima was described in detail by Kuramoto and Tanaka (1997). The morphology of sperms from the Amamioshima male examined here basically fits their description well. All major characteristics observed in the spermatozoa of *T.* (*E.*) *andersonii* (e.g., much smaller size [c.a. 250 μm] than that of *Cynops pyrrhogaster* [419 μm: Mori, 1936], possession of a flagellum end which is free from the axial rod, thus forming a long end piece) agree well with those reported by Kuramoto and Tanaka (1997). We could not consistently distinguish the acrosome and the neck piece from the head proper in our observations with a phase-contrast microscope. Kuramoto and Tanaka (1997) similarly could not clearly distinguish them from head proper in their SEM observations. This indicates the necessity of employing more than one method for correct observation of sperm morphology.

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