Reproductive Behaviors of the Sexually Dimorphic Brittle Star *Ophiodaphne formata* (Koehler, 1905)  
(Echinodermata: Ophiuroidea)  

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We investigated *Ophiodaphne formata*, an exceptional sexually dimorphic ophiuroid, *in situ* using scuba, and in the laboratory. Solitary males, solitary females, and pairs of coupled individuals were found associated with the irregular echinoid sand dollar *Astriclypeus manni*. We observed that coupled females, solitary males, and solitary females always hold their aboral surface against the oral surface of the host. Coupled males and females with disk diameters > 0.63 mm and > 3.7 mm, respectively, are considered to be ripe for reproduction. In order to couple, a solitary male approaches the margin of a female's disk, quickly inverts, interdigitates its arms with the female's, and keeps its mouth pressed against that of the female. At first, solitary males and females attach to their host singly and, secondly, couple with each other in order to engage in reproductive behavior. Solitary and coupled individuals are capable of changing their positions on the host, and transferring from one host to another. Coupled individuals separate after a period of attachment on the host.

**Key words:** sexually dimorphic ophiuroid, solitary and coupled ophiuroids, coupling behavior, behavioral experiment, behavioral interaction

**INTRODUCTION**

Males and females of three ophiuroid species that are sexually dimorphic, *Ophiopsphaera insignis* Brock, 1888, *Amphiura scripta* Koehler, 1904, and *Ophiodaphne formata* (Koehler, 1905), differ both morphologically and in body size. Females are larger than males, and differ so much in appearance that the two sexes were once thought to be different species. Coupling involves the male grasping the larger female and pressing its oral surface against that of the female (Brock, 1888; Koehler, 1904, 1905, 1930; Clark, 1967; Devaney, 1974; Guille, 1981; Guille and Vadon, 1986). The sexes of *Amphipholis linopneustes* Stühr, 2001 exhibit only weak size dimorphism, and the males have enlarged arm spines (Stühr, 2001). However, it is not known whether they couple with females. Although it is known that all four sexually dimorphic species are epizoic on other echinoderms, including regular echinoids, regular echinoids, and a crinoid (Devaney, 1974; Clark and Courtman-Stock, 1976; Cherbonnier and Guille, 1978), only limited ecological and behavioral observations of ophiuroids have been made to date.

The discovery of populations of *O. formata* at several Japanese localities (Irimura, 1981, 1995, 2002; Tominaga, 1998; Irimura et al., 2001; Fujita, 2006; Kiyomoto pers. comm.; Kohtsuka pers. comm.) accessible to scuba divers has facilitated studies of its ecology, including a description of its entire developmental process (Tominaga et al., 2004). Although spawning behavior of *O. formata* has been observed only for couples in the breeding season (Tominaga et al., 2004), the overall pattern of coupling behavior by the male and female remains unclear. Metamorphosed larvae of *O. formata* settled on the sea bottom are free-living. Juveniles lacking hooked-arm spines may grow “solitarily” during the development of sexual dimorphism. It is unknown whether male and female ophiuroids attach to their host *Astriclypeus manni* Verrilli, 1867, including their movement behaviors on the host's oral surface, in “coupled” or “solitary” form.

In the present study, we integrated quantitative information on the size frequency distribution of solitary and coupled *O. formata* individuals, their size at sexual maturity, observations of their spawning behavior, and analyses of interactions between male and female individuals and between ophiuroids and their host, to clarify the nature of the species' unusual reproduction.

**MATERIALS AND METHODS**

**Collection and processing of specimens**  
*Ophiodaphne formata* and its echinoid host, the keyhole sand dollar *A. manni*, were collected using scuba at a depth of about 5 m on the sandy bottom of Tsuruga Bay, Fukui Prefecture (35°44'N, 136°03'E), Japan. The behavior of the symbionts, and the incidence of association between *O. formata* and its host were observed from 1999 to 2009.
Ophiuroids associated with their hosts were classified as either solitary or, in the case of females clasped by males, coupled. Ophiuroids were removed from the oral surface of their hosts, fixed in 70% ethanol, and measured using a dissecting microscope with an ocular micrometer. Size was measured in terms of disk diameter (dd), i.e., the distance from the base of the arm to the opposite margin of the disk on the aboral surface.

Reproductive biology

To characterize the gonadal development of sexually mature individuals, histological sections of males \( (n = 20; \text{dd} \ 0.5–1.1 \ \text{mm}) \) and females \( (n = 14; \text{dd} \ 1.6–3.7 \ \text{mm}) \) were analyzed following the methodology described in Tominaga et al. (2004). A series of \( O. \text{formata} \) pairs \( (n = 10) \) were placed in a 250 ml glass vessel immediately after removal of every single pair from \( A. \text{manni} \), and their spontaneous spawning behavior was observed in the laboratory without stimuli such as light and temperature changes. In July 2008, a previously coupled male was introduced to a large solitary female in a glass vessel containing seawater to allow the male to ascertain her coupling and spawning potential.

Behavioral experiments

The responses of male and female \( O. \text{formata} \) to one another were examined in the laboratory using ophiuroid pairs. Immediately after the individuals had been drawn apart with fine forceps, they were placed at a distance of 7 cm from each other in a glass vessel containing standing seawater. In four trials conducted in March 2002, the vessel contained only seawater. In another four trials, the vessel contained seawater and sand from the collection site. In six trials conducted in July 2003 and August 2009, the ophiuroids were placed at a distance of 7 cm from each other on the oral surface of \( A. \text{manni} \) in a water tank. In July 2003, individuals of \( O. \text{formata} \) were also placed in a water tank at a distance of 30 cm from a single individual of \( A. \text{manni} \) to ascertain their responses to a potential host. Responses were recorded for coupled ophiuroids (two trials), a solitary female (23 trials), and a solitary male (10 trials).

From August 2004 to February 2005, the movement of \( O. \text{formata} \) on and between two \( A. \text{manni} \) individuals (hosts #1, #2) was evaluated using a pair of coupled ophiuroids and a solitary female held in a water tank. During these observations, associated ophiuroids were not removed from \( A. \text{manni} \) and their positions on their host were checked and recorded on each observation day.

All experiments carried out in the water tank were recorded during daylight hours using digital cameras mounted above and below a 60-liter, circular, temperature-controlled water tank with a sandy bottom. The water temperature was adjusted to match the temperature at the collection site, and varied from 12°C to 30°C, depending on the season.

RESULTS

Size standard for reproduction of \( O. \text{formata} \)

We were able to sex all individuals on the basis of their morphology (Tominaga et al., 2004). Differences in the shape of the jaw, the number of disk scales and arm spines, the presence or absence of grooves and tentacle scales distin-

The sex ratio was approximately 1:1 (128 females, 120 males). There were differences in the size distributions of coupled and solitary individuals of both sexes (Fig. 1). The mean dd of coupled ophiuroids was 1.5 times greater than that of solitary individuals of the same sex (Table 1), and the

![Fig. 1. Size frequency distributions of coupled and solitary female and male \( O. \text{formata} \). Circles and diamonds indicate females and males, and filled and clear symbols indicate coupled and solitary individuals, respectively. Disk diameter (dd) in mm.](https://bioone.org/journals/Zoological-Science/)

![Fig. 2. Horizontal sections of ripe gonads in both sexes of \( O. \text{formata} \). (A) Testis filled with spermatocytes (SC) and sperm (S) in August, 2001. (B) Ovary filled with vitellogenic oocytes (V: arrowheads) and fully grown oocytes (O) in June, 2002. Scale bars: (A) 20 \( \mu \text{m} \), (B) 60 \( \mu \text{m} \).](https://bioone.org/journals/Zoological-Science/)
dd differed significantly between coupled and solitary individuals (t-test for both sexes, \( P < 0.001 \)). Males with a dd exceeding 0.63 mm accounted for 56 (98.2%) of the 57 coupled males, but only 33 (52.4%) of the 63 solitary males. Females with a dd exceeding 3.7 mm accounted for 52 (91.2%) of the 57 coupled females, but only 18 (25.4%) of the 71 solitary females.

Coupled males that had spawned in the laboratory had a dd of at least 0.63 mm (\( x = 0.91 \pm 0.15 \) mm). During the spawning season, their testes changed from 100 μm long, spheroidal structures to 300 μm long, pear-shaped structures. The lumen of each ripe testis was filled with mature sperm (Fig. 2A). Coupled females that had spawned in the laboratory had a dd of at least 3.7 mm (\( x = 4.80 \pm 0.71 \) mm). The body cavity in these animals was filled with ovaries that were packed with fully-grown oocytes, 60 μm in diameter.

Fig. 3. Spawning, coupling, and attachment behaviors of O. formata. (A) Spontaneous shedding posture of coupled ophiuroids. Spawned eggs are visible (arrow). (B) The overall style of coupling behavior between a female (arrows) and a male (arrowheads), viewed from below the glass vessel. The dwarf male approaches a solitary female (B1), becomes attached to the female's disk, and rotates his disk vertically (B2). The male holds the larger female, with the oral surfaces of the two sexes closely appressed (B3). Note the male's position, with five arms situated at the internradius of the female (B4). (C) Coupling behavior between a female (arrow) and a male (arrowheads) on sand, viewed from above. The dwarf male approaches the female (C1), climbs onto the female’s disk (C2), and rotates his disk vertically (C3-4) in the same way as shown in (Fig. 3, B1-2). (D) Coupling behavior between a female (arrow) and a male (arrowheads) on the oral surface of their host, the sand dollar Astreliclypeus manni, in a water tank viewed from below. The dwarf male waits for a female at the external edge of the lunule (asterisk) of his host, approaches (D1), and becomes attached to the female’s oral surface (D2-3) in the same way as in (Fig. 3, B2-3). Oral views (female) and aboral views (male). (E) Aboral view of the hook-shaped arm spines at the tips of a female’s arm. Note that four pairs of spines (E2, asterisk) are directed aborally at the base of the rectangular part surrounded by dotted lines (E1). (F) The water tank equipment used for observing the attachment behavior of O. formata. An ophiuroid was dropped onto the bottom (asterisk and dashed arrow), and its attachment behavior to the host was then recorded. Arrow (solid line) indicates the direction of movement of the host A. manni (double asterisks). (G) Position of ophiuroids on a host. Note the pair of coupled ophiuroids (G1), a solitary female (G2), and a solitary male (G3) attached to the margin of the lunule and the oral surface of the host, respectively. Arabic numerals on the upper right (B1-B3) indicate the elapsed time (minutes and seconds). Scale bars: (A, B1-4, C1-4, D1-3, G1-2) 5 mm, (E1, G3) 1 mm, (E2) 500 μm, (F) 10 cm.
Solitary individuals with ripe gonads were not observed to spawn in the laboratory. However, solitary females spawned after coupling with a previously coupled male.

### Spawning and coupling behavior

Spontaneous spawning behavior of *O. formata* was observed on the day after coupled individuals had been removed from *A. manni*. A newly coupled pair, comprising a previously coupled male and a solitary female, also spawned. The female raised her disk and the basal portion of her arms off the substrate, and both sexes released gametes at the same time within 30 seconds while coupled with each other (Fig. 3A). A single pair did not release gametes all at one time, but two or three times at intervals of a few days. After spawning, coupled individuals separated from each other and shed their arms, resulting in death. They spawned spontaneously in the laboratory on 10 October 1999, 16 July and 23 August 2000, and 17 June, 5 and 14 July, and 5 August 2001.

When paired individuals were separated and placed in vessels containing seawater, the male quickly moved toward the margin of the female’s disk within 20 seconds, and keeping his oral surface in contact with the female, rotated beneath her disk (Fig. 3B-1-2). Within seven minutes, the male aligned his mouth with that of the female (Fig. 3B-3-4). The coupling process was identical in experimental vessels containing sand (Fig. 3C-1-4, Fig. 5B). Solitary males and females placed on the oral surface of *A. manni* took up to a few minutes to pair together (Fig. 3D-1-3, Fig. 5A). The female remained stationary and contracted her disk several times during the coupling. In these experiments, males always succeeded in rejoining their female partners.

### Occurrence of *O. formata* on *A. manni* and behavior on the host

The newly metamorphosed juveniles (dd 0.41 mm) had no arm spines, while all individuals attached to *A. manni* with a dd of > 1.1 mm for females and > 0.48 mm for
males had hook-shaped arm spines (Fig. 3E₁₋₂). The tips of the hooked spines were directed aborally. It was difficult to remove the ophiuroids from the oral surface of A. manni, as they attached themselves firmly using their hook-shaped arm spines to grasp the host’s spines.

Between 1999 and 2002, solitary ophiuroids were somewhat more common on A. manni than paired ophiuroids, comprising 54% of the 248 associated O. formata (Table 1). Coupled and solitary ophiuroids were found on the margin of the lunules or elsewhere on the oral surface of A. manni in the field and in the laboratory, but never on the aboral surface of the host. In the laboratory, we found that coupled O. formata (Fig. 4B, 5B₁) solitary females (Fig. 4A, 5A₁) and solitary males (Fig. 5A₁) moved toward, and climbed onto A. manni when placed in a water tank with the echinoid (Fig. 3F). Ophiuroids approached the ambitus of the echinoid and crawled beneath its disk quickly before bringing their aboral surface in contact with the host. The mouths of coupled females and solitary individuals of both sexes were always turned away from the oral surface of the host (Fig. 3G₁₋₂).

Coupled and solitary O. formata were observed to change position on a host, and to transfer from one host to another (Fig. 4A, B). In one instance, after a coupled pair had changed hosts twice (Fig. 4B₁₋₂), two individuals that had been previously coupled separated (Fig. 4B₁₋₂, Fig. 4B₁₋₂), and the female left the host.

**DISCUSSION**

The present study revealed for the first time the reproductive features, the overall style of coupling behavior, and the actual interactive movements of O. formata. The behavior of male and female O. formata is related to individual body size. There is a statistically significant tendency for the larger females and males to mate, and for smaller individuals of both sexes to be solitary. Judging from the results related to the size (dd) of spawned ophiuroids, coupled individuals with a dd larger than 0.63 mm (male) and 3.7 mm (female) are considered to be competent partners for reproduction. Some solitary individuals that did not spawn had ripe gonads with mature gametes. Our behavioral experiments showed that coupled ophiuroids are able to separate, suggesting that such individuals may mate with more than one partner. Therefore, solitary individuals with a dd exceed 0.63 mm for males and 3.7 mm for females can breed under conditions of pair formation.

Ophiuroid coupling occurred throughout the year, not only during the breeding season (Tominaga et al., 2004). This suggests that solitary females and males may couple as soon as they encounter one another, and may remain paired even in the nonbreeding season. The coupling behavior of this species appears to be necessary for spawning, as solitary females and males were never observed to spawn during the breeding season (Tominaga et al., 2004). If this is the case, the tendency for males and females to couple upon encountering each other may serve to enhance the rate of fertilization and increase reproductive success (Tominaga et al., 2004), as well as ensuring access to a partner during the breeding season.

In a glass vessel containing seawater and sand, a male first climbs onto the female’s disk, and moves to the oral side, reversing his disk. This manner of coupling is the same as that observed on the oral surface of the host sand dollar, A. manni. Males and females of O. formata may release some chemical substances such as pheromones to assist encounters and coupling. A female exhibited shrinkage of her disk several times while a male was in contact, and maintained this behavior until a male had finished coupling. This shrinking motion of the female appears to be a sign of partner recognition.

In both the laboratory and the field, solitary males and females become attached to A. manni by pressing their aboral surface against the oral surface of the host. In this position, they may be able to ingest nutrients from the benthos. Coupled females also become attached to the host in the same way as solitary males and females. In the case of coupled individuals, it may be the case that the male and female establish a symbiotic or parasitic relationship in order to obtain food. However, male–female coupling cannot represent commensal parasitism, since males and females of O. formata have jaws and digestive organs, and have a solitary period in their life cycle.

It appears likely that the hook-shaped arm spines are specialized for attachment to the host. However, we have not excluded the possibility that these spines may also play a role in stabilizing the animals or assisting their locomotion on the host. Hook-shaped arm spines occur in other dimorphic ophiuroids, such as O. insignis and A. scripta (Tominaga unpubl.). Orally directed hook-shaped arm spines are clearly adaptations for attachment in adult, free-living ophiuroids belonging to several families including Ophiomyxidae, Gorgonocephalidae (Lawrence, 1987), and Ophiotrichidae (Tominaga unpubl.). In some Amphiuridae, Ophiactidae, Ophiolepididae, and Ophiuroidae, which have hook-shaped spines as juveniles, but not in the adult stage, the hooks are directed aborally and their precise function is not known with certainty (Hendler and Turner, 1987; Hendler et al., 1999).

Ophiocomina formata has a planktonic larval stage (Tominaga et al., 2004). Although the behavior and ecology of newly metamorphosed juveniles has not been studied, they may be recruited to sandy benthic substrates before developing into dimorphic males and females. Thus, individuals of O. formata may be recruited to a host through three different mechanisms (Type A, B, C) as shown in Fig. 5: as solitary males and females (Type A), as coupled males and females (Type B), or as post-metamorphic juveniles (Type C). Solitary males and females may couple after becoming attached to A. manni, and pairs of males and females may attach to A. manni after coupling on the benthic substrate. Solitary individuals outnumber coupled individuals on A. manni. Furthermore, young, solitary individuals show a consecutive change from a small to a large dd. Our observations strongly suggest that young, solitary males and females, which become attached to their hosts singly, grow without showing coupling behavior. This concept supports a Type A, rather than a Type B recruitment mechanism. Type C recruitment seems highly unlikely, as juveniles were never found on the oral surface of A. manni in the natural habitat. Particularly for solitary males and females, changing position on a host and transfer to another host may be associated with exploratory behavior in the search for a partner. It seems unlikely that solitary O. formata males and
females, which are rather small, would frequently encounter each other on the extensive sandy plains where they occur. However, by associating with large individuals of *A. manni*, which are highly motile and common in Tsuruga Bay, they would probably increase their chance of encountering members of the opposite sex. We suggest that in this species, and perhaps in other small, sexually dimorphic ophiuroids, associations with large mobile hosts may increase the likelihood of locating a mate. Furthermore, coupling behavior, especially between dwarf males and females, might increase the efficiency of fertilization and improve reproductive success. Moreover, it is certainly of interest that a uniform suite of adaptations, including symbiosis, coupling, and dwarf males, seems to have evolved independently among all the known species of sexually dimorphic ophiuroids.

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