

Spawning behaviors of two congeneric brittle stars

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ABSTRACT: Broadcasting gametes for external fertilization is a common reproductive strategy of many marine invertebrates; however, little is known about adult behaviors because of the brevity and unpredictability of spawning. We studied the spawning of 2 shallow-water brittle stars—the subtidal *Ophiocoma dentata* and the intertidal *O. scolopendrina*—in southern Taiwan by inducing males to spawn after exposure to conspecific ovary suspensions. Laboratory results indicated that male *O. dentata* preferred to cohabit with females, but that male-male antagonism did not occur. In the field, males with female companions were more inducible to spawn than other males. In contrast, *O. scolopendrina* did not pair; instead, males rapidly approached released eggs before spawning. Despite this, 53% of simulated egg releases in tide pools did not induce any male to spawn. Male spawning capacities in both species were relatively limited; spawning inducibility rates and disc-pumping frequencies decreased with repeated inductions. Evidence of sexual competition was observed during the spawning induction of *O. scolopendrina* in tide pools. Only males that arrived first were likely to spawn, whereas late individuals had low rates of spawning. The fitness of female *O. scolopendrina* may be limited by the shortage of spawning males; however, at the same time, males face sexual competition in addition to sperm limitation. This is due to the female-biased sex ratio, and the limited spawning capacity of unevenly distributed males.

KEY WORDS: Sperm limitation · Broadcast spawning · Echinoderm · Sperm competition · Reproduction · Spawning induction

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INTRODUCTION

Broadcast spawning is the prevalent mode of reproduction in marine invertebrates (Thorson 1946). Yet, many reports on reproduction are inferred from indirect data, e.g. change of gonad indices or egg sizes (see Hendler 1991), rather than from direct observation of broadcast spawning in nature. Spawning time may be synchronized within a species, thus presenting only a very short period to encounter spawning events. Nevertheless, there are exceptions such as in a Japanese crinoid (Kubota 1980), the palolo worm *Palola viridis* (Caspers 1984), and many reef corals (e.g. Harrison et al. 1984) in which the annual spawning time is well-known and even anticipated by local people. For most other marine invertebrates, information about behavior around the spawning time is very limited.

Behaviors at spawning time may be under strong selective pressure (e.g. Levitan 2005). One challenge that must be overcome in the marine environment is the dilution of gametes (e.g. Pennington 1985, Denny & Shibata 1989), which may potentially disperse sperm before fertilization occurs. Predators that are attracted to emerging adults engaged in spawning, or to eggs and zygotes may cause significant mortality rates and thus lower fitness (e.g. Rawlings 1994, Smale et al. 1995). Additionally, finding potential mates and locations for appropriate dispersal of fertilized eggs may require behavioral adaptations at this stage. In general, these may involve temporal modifications in behavior (e.g. in timing and/or synchronization of activities), as well as spatial adjustments (e.g. aggregation and/or movement of adults) (Harrison et al. 1984, Tyler et al. 1992, Young et al. 1992, Levitan & Petersen 1995, Yund 2000). From an individual perspective, there may

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also be sexual competition at the crucial moment of spawning when behaviors of conspecifics are critical for reproductive success (e.g. Levitan 2004, 2005).

Brittle stars are widespread in marine environments, occurring from the intertidal zone down to thousands of meters in depth, and occupying both hard and soft substrates (Sanders & Hessler 1969, Gage & Tyler 1991, Hendler et al. 1995). Reproductive strategies also differ widely among species, from broadcasting gametes for external fertilization to brooding larvae (Hendler 1991). Much of the information about their reproduction, however, is from preserved specimens. Nevertheless, some spawning behaviors have been observed in nature or in the laboratory; individuals may emerge at nighttime, go to a high ground, and raise their discs before releasing gametes for a short period (e.g. Hendler & Meyer 1982, Manfred & Van Veghel 1993, Selvakumaraswamy & Byrne 2000, Morgan & Jangoux 2002, Hagman & Vize 2003, Himmelman et al. 2008).

Two brittle star species in shallow waters are known to use pheromones, presumably contained in released eggs, to induce male spawning (Soong et al. 2005). Additionally, the males of *Ophiolepis elegans* and the burrowing ophiactid *Hemipholis elongata* can be stimulated to spawn (presumably by pheromones), and the latter was observed to move towards egg-releasing females (S. Stancyk pers. comm.). This trait of being inducible to spawn allows us to study male behaviors, since their spawning behavior can be observed repeatedly under replicable conditions. For example, males of the intertidal *Ophiocoma scolopendrina*, but not of the subtidal *O. dentata*, could be induced to spawn only at the time of low tides, which may be controlled by an endogenous clock (Soong et al. 2009).

We also expected these 2 species to have different spawning behaviors for several reasons. First, the water flow regime, which is considered to be the main factor in gamete dilution, differs greatly between intertidal and subtidal areas (Denny & Shibata 1989, Serra?o et al. 1996, Engel & Destombe 2002, Marshall 2002). Second, the possible effect of predators may also differ between the species because the subtidal *Ophiocoma dentata* is more cryptic than the intertidal *O. scolopendrina*. Third, sexual selection is likely because both species are mobile and local densities are often high in *O. scolopendrina* (see Levitan 2004, Andersson & Simmons 2006).

During the spawning seasons of the intertidal *Ophiocoma scolopendrina* and the subtidal *O. dentata*, we studied male–female pairing, the spawning inducibility of paired vs. unpaired males, the behaviors of males upon detection of egg suspensions, and the behaviors of males in the presence/absence of others in the field, as well as their spawning capacities with repeated inductions.

MATERIALS AND METHODS

Study sites and habitats. Fieldwork was carried out at Wanliton (22° 00' N, 120° 42' E) in southern Taiwan, between March and July (the reproductive season) in both 2005 and 2006. The study area consists of fringing reefs, with the intertidal zone being protected from waves at low tide. *Ophiocoma scolopendrina* live on reef flats with their arms extending from the crevices they inhabit. *O. dentata* inhabit the same area, but at depths of 2 to 5 m in the subtidal zone. During the late spring reproductive season of both species, only males could be induced to spawn with suspensions made from conspecific ovaries (Soong et al. 2005). Thus, only male spawning behavior was easy to study. For the same reason, male–male antagonism was investigated, but female preference and the other possibilities of sexual selection were not studied. All individuals were caught just before each experiment, and were not reused. They were kept in an outdoor shaded tank with running water at ambient light and temperature. There was no apparent sexual dimorphism in either species. Gender was determined by pressing the discs and observing the color of the gonads in the slits between the arms and discs; males had white spermaries and females had pink to dark-red ovaries.

Pairing behavior. To examine whether there was pairing behavior and if it differed with sex, we tested all combinations, i.e. male–male, male–female, and female–female, for both species using a 27 × 14.8 × 15 cm (L × W × H) testing chamber in the laboratory. Each brittle star was initially isolated at opposite ends of the chamber by 2 removable dividers. A horizontal black plate (14.8 × 10 cm) placed in the center 5 cm above the bottom simulated crevices where the brittle stars could hide. Then, both dividers were removed, allowing the 2 tested brittle stars to move freely in the 5 cm deep water of the chamber. After they became stationary, usually under the black plate, we recorded the extent of disc overlap of the tested individuals by looking through the side of the tank. A total of 40 *Ophiocoma dentata* (20 males and 20 females) and 48 *O. scolopendrina* (24 males and 24 females) were used. Each individual was paired with same-sex and opposite-sex individuals that were chosen haphazardly. Fifty percent of the individuals were paired with same-sex individuals first, while the remaining individuals had the reverse sequence.

We further tested the combination of 2 males and 1 female to clarify if there was antagonism between males in the presence of females. In this case, a female was placed under the dark plate, and the males were placed at the 2 ends of the chamber before removing the dividers. The extent of association of these 3 individuals was recorded after they became stationary.

Spawning inducibility of males. We categorized males into 2 groups based on the presence or absence of females in the same refuge in the field during collection. Immediately after collection, each male was tested for inducibility to spawn by the application of an ovary suspension. This experiment was run only with *Ophiocoma dentata* because no pairing was apparent in the other species. A standard testing method was developed. Individuals were put into 1 l beakers with ~300 ml of seawater. An ovary suspension was prepared by dissecting 5 to 10 mature females and collecting their gonads. The gonads were then homogenized in filtered seawater to prepare 0.1 g ovary ml⁻¹ of seawater stock solution, which was then stored at 5°C and diluted upon use. For an induction test, 2 ml of conspecific ovary suspension at a concentration of 0.01 g ml⁻¹ was added to each beaker with a test individual. Spawning males usually released sperm within seconds by pumping their discs. Non-spawning individuals were monitored continuously for 30 s and reexamined after 2 min. This test was done twice at a 2 wk interval, with 69 males being used in the first test, and 54 males being used in the second.

Egg-approaching ability. The egg-approaching ability test was designed to determine if males could detect and approach an ovary suspension. We used a round plastic tray of 33 cm diameter to examine if an individual placed in the center could find an ovary suspension placed arbitrarily along the periphery of the tray. The tray was marked with 2 concentric circles of 13 and 23 cm diameter and 8 equally-spaced radii, thus dividing the tray into 24 areas. This allowed us to assign a 'distance index' (DI) to each of the 24 subdivided areas based on the approximate distance from the point of introduction of the ovary suspension. Two ml of ovary suspension (0.01 g ml⁻¹) was poured into a tube and the tube was then placed in 1 of the 8 outer sections of the tray. Five seconds after placing the tube, a conspecific test individual was released at the center of the tray. The position of the individual was recorded once it started to spawn or after 2 min, which corresponds with the time usually required for the brittle stars to settle in a location. A total of 25 males were tested for each species.

For data analysis, we calculated the total area with the same distance index on the tray and used the proportion of this to the total tray area to estimate the probability of an individual randomly settling there. The excess frequencies (here defined as the percentage of total observations appearing at a given position beyond that expected by chance) at a given position with a particular DI was calculated by subtracting the expected frequencies, based on area on the tray, from the observed frequencies.

Spawning capacity. To test the ability of the brittle stars to spawn repeatedly with several inductions, we

analyzed data from an experiment designed to test the role of biological clocks in Soong et al. (2009). In this experiment, we collected ~50 mature males of each species 1 d before the test. They were isolated in individual beakers that were placed in a large water tank of circulating seawater in a shaded environment. We then tested each individual, using the standard spawning induction method above, at the times of both high and low tides; the water depth in the laboratory did not reflect tidal changes. The experiment lasted for 2 d and included 8 tests ind.⁻¹. The spawning inducibility data were analyzed previously to detect spawning rhythms (Soong et al. 2009). Here, we analyzed the spawning frequencies of an individual and the disc-pumping frequencies in each spawning episode to see if males could repeatedly spawn and if there was any declining trend in pumping frequencies.

On-site induction. During a preliminary field test of egg-approaching abilities, we noticed that an ovary suspension could attract >1 ind. at a time. This provided an opportunity to study interactions between individuals in nature. In the present test, the concentration of the ovary suspension introduced was increased to 0.1 g ml⁻¹ and applications were 2 ml of ovary suspension; therefore, 0.2 g of ovary, which is the approximate gonad size of a median-sized female (pers. obs.), was the test dose. Sixty intertidal pools were chosen, each ~0.5 × 0.5 m and <25 cm deep and containing at least 10 brittle stars. We removed 1 female and dropped the egg suspension solution in her original position near the bottom. Then, we recorded the number and behavior of responding individuals, their starting points, and the nearest distance to the egg suspension for each individual. A video camera recorded the process, although this was not useful in all cases due to reflection of light from the water surface. This experiment was possible only for *Ophiocoma scolopendrina* because *O. dentata* is more cryptic.

G-tests were used for tests of independence except in cases when *G*-statistics could not be calculated; chi-square tests were then used in these cases. The software Statview was used for all statistical calculations.

RESULTS

Pairing behavior

In the laboratory, pairing behavior differed between the 2 brittle star species (Fig. 1). In the subtidal *Ophiocoma dentata*, pairing behavior depended on gender ($p < 0.01$, *G*-test). Male *O. dentata* were much more likely to overlap discs with females (95%) than with other males (10%); females overlapped discs with

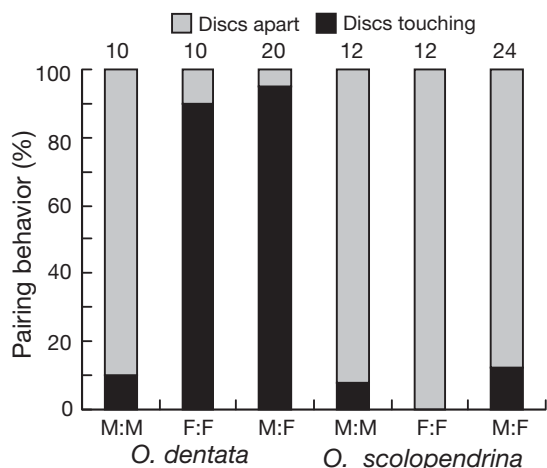


Fig. 1. *Ophiocoma dentata* and *O. scolopendrina*. Pairing behavior of brittle stars in various combinations of sexes in laboratory experiments. *G*-test for *O. dentata*, $p < 0.01$; chi-square test for *O. scolopendrina*, $p = 0.44$. M: male; F: female. Numbers above bars are numbers of pairs

either sex (90 to 95%). In the intertidal *O. scolopendrina*, all sex combinations had similar behavior ($p = 0.44$, chi-square test) and neither sex was likely to associate with others (<15%).

In combinations of 2 males and 1 female *Ophiocoma dentata*, the 3 ind. aggregated with discs touching or overlapping in 8 of the 10 tests; in both of the remaining 2 tests, 1 female and 1 male paired and the second male remained free. In contrast, the 3 tested individuals of *O. scolopendrina* remained separate in all 10 tests.

Spawning inducibility of males

In the first test on May 14, 2005, male *Ophiocoma dentata* with females in the same refuge at the time of

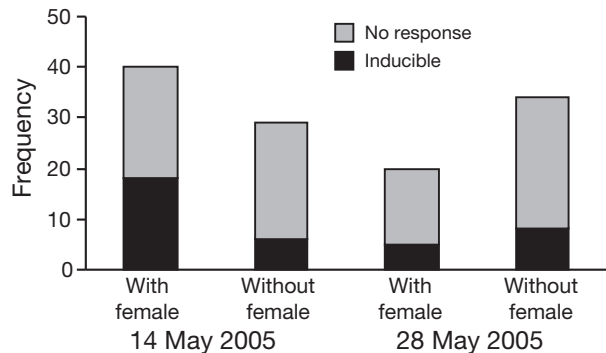


Fig. 2. *Ophiocoma dentata*. Frequency distribution of male responses to ovary suspensions in the laboratory depending on the presence or absence of female companions when collected. Tests of independence (*G*-tests) for 14 May 2005, $p = 0.03$, for 28 May 2005, $p = 0.90$

collection had a much higher probability (45%) of being induced to spawn than males without female companions (21%, $p = 0.03$, *G*-test, $n = 40$ and 29, respectively). These 2 groups of males did not differ in disc diameters ($p = 0.17$, *t*-test). In the second test on May 28, 2005, however, no such pattern was found; both groups of males had low rates of spawning inducibility (25 vs. 24%, $p = 0.90$, *G*-test, $n = 20$ and 34, respectively; Fig. 2). Comparison of the males in these 2 tests showed that a significantly higher proportion was associated with females in the first test than in the second (58 vs. 37%, $p = 0.02$, *G*-test, $n = 69$ and 54, respectively).

Egg-approaching ability

Whether male brittle stars approached the egg suspension or not differed between species. In the laboratory, most male *Ophiocoma scolopendrina* that spawned approached the ovary suspension before releasing sperm, whereas only few males that did not spawn approached the ovary (Fig. 3). Individuals took from 8 to 130 s to start spawning and 18% (2 of 11) spawned within 10 s. For this species, excess frequencies of spawning males at DI = 0 (the same position as the ovary suspension) was nearly 60%, but it was near 0% for non-spawning males (Fig. 4).

Male *Ophiocoma dentata*, whether spawning or not, did not show such tendency to approach the ovary suspension (Figs. 3 & 4). Individuals took 3 to 85 s to release sperm and 58% (7 of 12) spawned within 10 s. The proportion of males that spawned within 10 s depended on species ($p = 0.04$, *G*-test), being higher in *O. dentata* (58%) than in *O. scolopendrina* (18%). There were high proportions of non-spawning males of both species that remained far from the introduced egg suspension (Fig. 4).

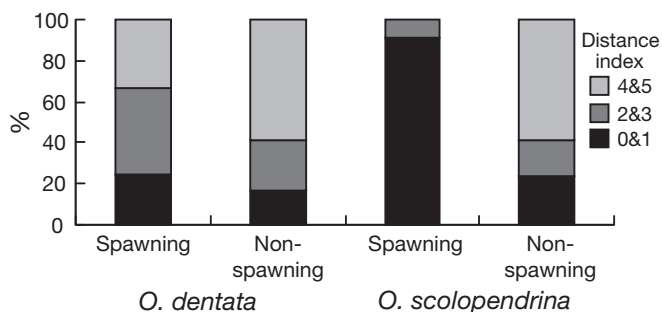


Fig. 3. *Ophiocoma dentata* and *O. scolopendrina*. Comparison of egg-approaching abilities of spawning and non-spawning males in the laboratory. A small distance index indicates a closer approach to released eggs. *G*-test for *O. dentata*, $p = 0.57$, $n = 24$; chi-square test for *O. scolopendrina*, $p < 0.01$, $n = 28$

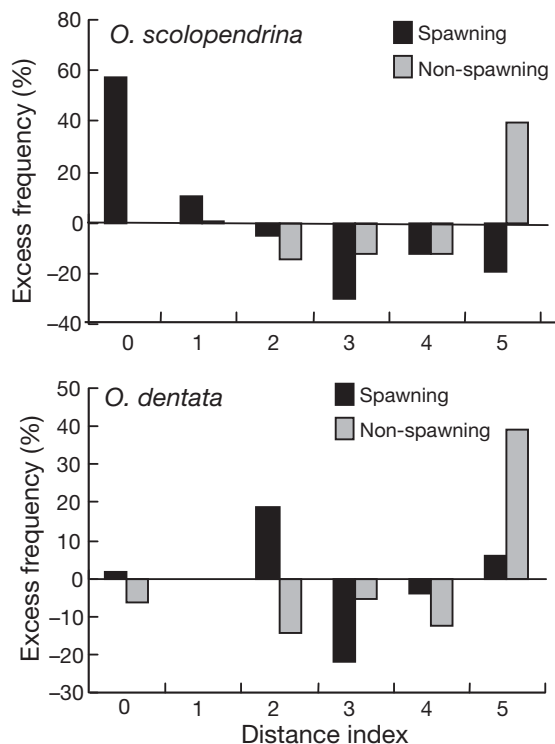


Fig. 4. *Ophiocoma dentata* and *O. scolopendrina*. Comparison of the ability of males to approach the ovary suspension in a tray. Excess frequency (%) = observed frequency – expected frequency, $n = 24$ for *O. dentata*, $n = 28$ for *O. scolopendrina*

Spawning capacity

The number of spawning episodes over 2 d in the laboratory were limited in both species. None of the 48 intertidal *Ophiocoma scolopendrina* tested spawned >2x; most spawning individuals spawned only once (Fig. 5). Of the 26 spawning episodes, 77% occurred on the first day.

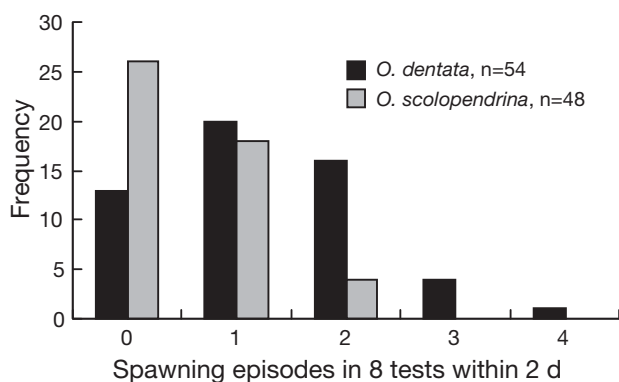


Fig. 5. *Ophiocoma dentata* and *O. scolopendrina*. Frequency distribution of spawning episodes of individuals in the laboratory

In the subtidal *Ophiocoma dentata*, 1 ind. was induced to spawn 4x, whereas most (67%, $n = 54$) were induced to spawn only once or twice during the 2 d experiment (Fig. 5). Of the 68 spawning episodes, 88% occurred on the first day of the 2 d experiment.

Pumping frequency differed between species. For the intertidal *Ophiocoma scolopendrina*, males pumped their discs only once in each spawning episode in the 2 d experiment. The disc-pumping frequencies of the subtidal *O. dentata* varied greatly among individuals. In their first episode of spawning, *O. dentata* pumped their discs from 1 to 9x. In the second episode, the maximum number was 5x; in the 3rd and 4th episodes, the maximum recorded pumps were 2 and 1, respectively (Fig. 6). Clearly, the number of pumps per episode declined with repeated spawnings.

We recorded higher variation, i.e. from 1 to 4 pumps (2.3 ± 0.8 SD) episode⁻¹, when the intertidal *Ophiocoma scolopendrina* brittle stars were in their natural habitat during 'on-site induction' (see below). No field data of pumping frequencies were collected for *O. dentata* due to its cryptic behavior.

On-site induction

Multiple individuals of *Ophiocoma scolopendrina* approached the released ovary suspension in 33% (20) of the intertidal pools; 1 ind. approached the ovary suspension in 27% of the pools and no individual responded in the remaining 40% (24 pools) (Fig. 7). Of all 60 pools tested, 53% had either no males approaching or no males spawning. Spawned individuals could be identified as males, whereas the sex of non-spawning individuals was not identified. In the 20 pools

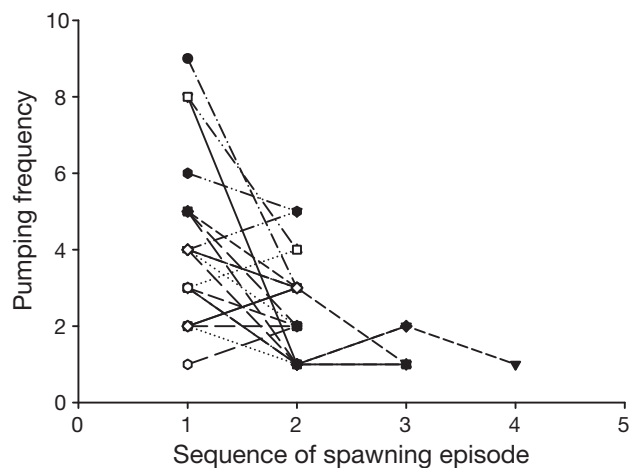


Fig. 6. *Ophiocoma dentata*. Disc-pumping frequencies of males in subsequent spawning episodes. Lines connect the performance of each individual (indicated by the same symbols and lines). Male *O. scolopendrina* always pumped only once per spawning episode in this laboratory test

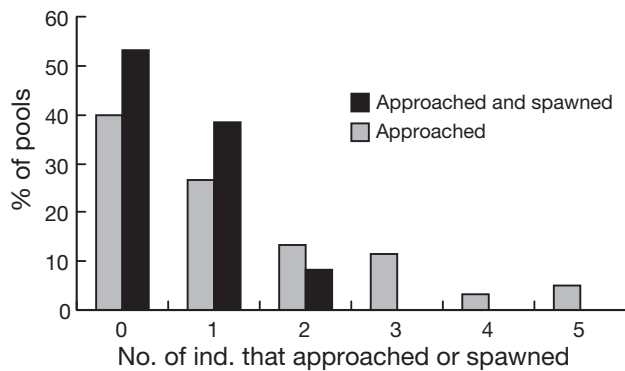


Fig. 7. *Ophiocoma scolopendrina*. Percentages of tidal pools in which males approached sites of simulated egg release. 60 pools were tested

where multiple individuals approached the ovary suspension, 50% had 1 spawning male, 25% had 2 spawning males, and 20% had none. In comparison, in the 18 pools with a single individual approaching, 13 ind. (72%) spawned and 5 ind. (28%) left without spawning. For all pools (36) in which at least 1 ind. approached the ovary suspension, 22% (8) had no males spawning.

In this field experiment, 73 ind. approached the ovary suspensions, but only 34 of them spawned. In each pool, those arriving first had a high probability of spawning than those arriving later (80 vs. 16%, $n = 35$ and 38, respectively, $p < 0.01$, G -test). We observed that male brittle stars sometimes blocked others from approaching the ovary suspension by using their arms to push or entwine others. A contingency table analysis indicated that spawning and blocking behavior were dependent on each other ($p < 0.01$, G -test). Spawned individuals were usually the ones blocking others, and blocked individuals usually did not spawn (Fig. 8).

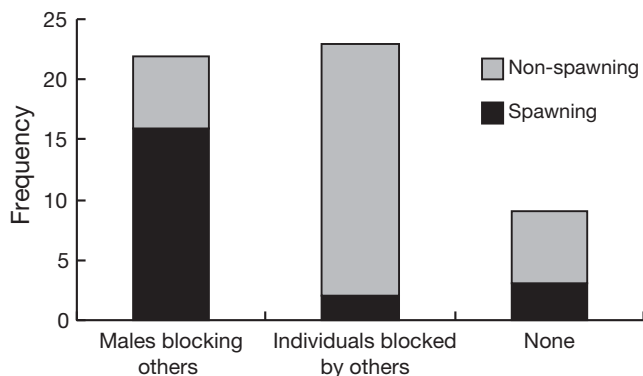


Fig. 8. *Ophiocoma scolopendrina*. Frequencies of behaviors of brittle stars when multiple individuals approached egg homogenates in tidal pools. Test of independence (G -test), $p < 0.01$, with the 'None' group being excluded from the test

The behavior of spawned males also depended on whether other individuals approached the ovary suspension ($p < 0.01$, G -test). About half of the spawned males (7 of 13) not approached by others moved away after releasing gametes; but if others approached, all spawners but one (16 of 17) remained after releasing gametes. The disc-pumping frequency of males that left after spawning, however, did not differ from that of males remaining near the egg suspension ($p = 0.39$, Kruskal-Wallis Rank test).

In this experiment, spawning individuals arrived first and were 0 to 4 cm from the egg suspensions when they started to release gametes. Approaching but non-spawning individuals were 1 to 7 cm from the egg suspensions. Spawning males were always closer than the blocked, non-spawning individuals in each pool. Individuals were 5 to 21 cm from the egg suspension when they started their approach.

DISCUSSION

Our study showed contrasting spawning behaviors in the 2 species of congeneric brittle stars studied (Table 1). The pairing behavior of the subtidal *Ophiocoma dentata* effectively shortens the distance between males and females before gamete release (Fig. 1). Because multiple males may pair with a female, any antagonism between males must be small, if any. Thus, the pairing behavior is more likely an adaptation for sperm limitation rather than for sexual selection. It is, nevertheless, hard to imagine that neighboring males do not compete for eggs. Fertilization rates may be low in *O. dentata*, so males may suffer more from adverse physical factors than from the presence of neighboring males.

We expect that sperm limitation would impact male as well as female fitness and that there would be strong selection to compensate for this limitation. Our laboratory tests, however, suggest that female *Ophiocoma dentata* do not distinguish males from females, and only males discriminate when forming pairs (Fig. 1). There may be other mechanisms causing this sexual dichotomy.

In nature, the interactions between cryptic *Ophiocoma dentata* individuals are difficult to observe. Similar pairing is known in other species: pseudocopulation was reported in 2 ophiuroid species where individuals releasing gametes can be wrapped around by others (Himmelman et al. 2008). Close associations between male and female brittle stars occur in some sexually dimorphic species in which diminutive males cling to large females even in non-reproductive seasons (Hendler 1991, Tominaga et al. 2004). In these species, males must recognize the females, as we

Table 1. *Ophiocoma dentata* and *O. scolopendrina*. Summary of behaviors at spawning

Trait	<i>O. dentata</i>	<i>O. scolopendrina</i>
(1) Pairing behavior	♂ and ♀ closely associated (Fig. 1); no ♂-♂ repelling when ♀ present	No ♂-♀ pairing (Fig. 1)
(2) ♂ spawning inducibility	♂ may refrain from spawning without ♀ companion (Fig. 2)	1st arrivals spawned more frequently than late arrivals in field test
(3) Egg approaching (♂)	None (Figs. 3 & 4)	Spawning ♂ approach eggs (Figs. 3 & 4) in the lab
(4) Male interference	–	Spawning ♂ blocked others (Fig. 8), stayed around eggs when others approaching
(5) Environmental factors	No tidal, light/dark effect (Soong et al. 2009)	♂ spawning inducible at low tides (Soong et al. 2009)
(6) Spawning capacity of ♂	Low (Fig. 5, Soong et al. 2005)	Low (Fig. 5)
(7) Disc-pumping frequency (♀)	Decline with repeated induction (Fig. 6)	Independent of other ♂
(8) Field test	–	53% of tidal pool tests failed to attract or induce any ♂ (Fig. 7)

observed for *O. dentata*. The well-known gregariousness of some brittle stars usually involves many individuals (see references in Hendler 1991), unlike *O. dentata* in which groups usually contain only 2 to 3 ind. (unpubl. data).

The likelihood of a male spawning in response to a spawning female or to the presence of eggs (spawning inducibility) seems to depend on the presence of a female companion, at least for a certain period during the reproductive season for the subtidal *Ophiocoma dentata* (Fig. 2). The low rate of spawning inducibility in both accompanied and unaccompanied males in the second test 2 wk later suggests that most were in non-spawning condition. The significantly lower proportion of female-associated males at the later date also suggests that certain temporal factors, e.g. the end of the reproductive season, may explain the difference between the 2 test results. As supporting evidence, the average gonad index dropped from a peak of ~35% in April to <20% in June, in 1998 (see Soong et al. 2009). Males gain fitness by fertilizing eggs, but detection of ovary suspensions does not guarantee that these eggs are unfertilized. A female companion ensures that the detected eggs are released nearby and are likely to be unfertilized. This suggests that males may become more inducible to spawn after association with females in order to reduce wasteful spawning. Alternatively, a male may actively associate with females only when he is ready to spawn. This latter hypothesis, however, was falsified by our pairing experiments where almost all male *O. dentata* (95%) sought to join females (Fig. 1) even though many of them could not be induced to spawn (Fig. 2).

The spawning capacity of male brittle stars is limited in both species. In *Ophiocoma scolopendrina*, spawning was likely only at low tides (Soong et al. 2009);

thus, a maximum of 4 spawning episodes was possible during the 2 d experiment. On the other hand, male *O. dentata* spawned both at low and at high tide; thus, 8 spawning episodes were possible per individual. The limited ability of both species to repeatedly spawn (Fig. 5), as well as the rapid decline in disc-pumping frequencies in each subsequent spawning episode in *O. dentata* (Fig. 6), suggest a limited capacity for sperm storage and/or replenishment. The reproductive investment by these brittle stars is large; up to 35% of the disc weight at the peak spawning season in southern Taiwan can be gonads (Soong et al. 2009). The gonad index of the population decreases slowly for 1 to 3 mo after the peak, and an extended reproductive season, rather than synchronized spawning on one evening, is likely. It is unclear how individual males allocate their sperm in different spawning episodes throughout the reproductive season. The limited tendency of both species to repeatedly spawn, however, must limit their ability to use quantity to gain fertilization advantage (see Yund 1998). In fact, the low number of pumpings per episode must also limit their ability to fertilize all eggs available, especially in their later spawning episodes. The effective range of the spawn-inducing pheromone extended to at least 21 cm, as shown in the field experiment where we placed ovary homogenates in intertidal pools. This factor also limits the number of potential mates for a male. On the other hand, the female-biased sex ratio in *O. scolopendrina* (24:45; unpubl. data) suggests that males may have little problem finding a mate. The spawning history of the collected brittle stars may contribute to the lack of a response to induction in many individuals.

The high percentage (53%) of simulated female *Ophiocoma scolopendrina* spawnings in the intertidal pools that did not attract a male or did not induce a male to spawn was not anticipated (Fig. 7). It suggests

that female reproductive investment might have a high probability of being wasted if those simulated spawnings had been real. A large volume of released eggs could conceivably improve this situation, but the effective range may not increase proportionally because of the 3-dimensional dispersal of the pheromones contained in the eggs. The female-biased sex ratio could have contributed to this phenomenon, at least partly, because the tested pools may have had few resident males. A certain proportion of females would be expected to have few or no males within the range of their chemical attractants. For example, calculations based on the sex ratio in the field and a binomial distribution show that if 8 individuals are within the effective range of a female's pheromone, only 1 male is expected to be within reach in 19% of the cases, and ≤ 2 males are expected to be present in 43% of the cases. The limited spawning ability of males is another possible cause, because nearby males may have exhausted their sperm supply during previous spawning events in the same pool. This is especially likely in tide pools with highly female-biased sex ratios. It is unlikely that mature females would approach males when they are ready to release eggs, since it would make the egg-approaching behavior of males that we have discovered here redundant. Apparently, some female spawning tactics remain to be investigated.

Egg-approaching behavior can obviously increase the fertilization rate in *Ophiocoma scolopendrina*. For this species, no pairing behavior was observed in adults either in the laboratory (Fig. 1) or in the field. If males and females do not associate with each other at the time of spawning, one of the remaining options is for males to approach the released eggs in order to shorten the distance between released eggs and sperm. In contrast, egg-approaching behavior was not found in *O. dentata*. If pairing with a female is indeed a prerequisite for male *O. dentata* to spawn (see Fig. 2), then egg approaching would not improve the fertilization rate.

The 2 ophiuroid species studied obviously tackle environmental challenges in very different ways, which may reflect the different environments they inhabit. For *Ophiocoma scolopendrina*, individuals usually expose 2 to 3 arms from the crevices they occupy, and these arms are used to feed on surface particles when the water level is low enough (Oak & Scheibling 2006). Pairing with others under such limited space may be difficult and may interfere with their feeding behavior. For *O. dentata*, hiding under large slabs or boulders with ample space may enable them to pair without exposure.

In the egg-approaching experiment in the laboratory, the egg homogenate used may have contained escape pheromones because non-spawning males of

both species seemed to avoid the egg suspensions (Fig. 4). Escape pheromones could be used by echinoderms to modify their behaviors (Campbell et al. 2001). Nevertheless, spawning *Ophiocoma scolopendrina* obviously approached egg suspensions without much hesitation, both in the laboratory and in the field. A more direct test of the presence of escape pheromones is possible if a pure spawning pheromone could be extracted for experiments. In other species of brittle stars, females might be attracted to spawning males (C. Dumont pers. comm.).

In the field test, we suggest that most of the approaching individuals are likely to be males. This is because only those males that were able to spawn approached egg suspensions in the laboratory (Fig. 4), whereas females behaved like non-spawning males (unpubl. data). The ratio of spawned males to all the approaching individuals (34:73) in this investigation is too high if both sexes are equally attracted to the egg suspension. The generally low spawning inducibility of males (<50%; Soong et al. 2005) and the female-biased sex ratio (24:45) of the population would predict a much lower spawning inducibility rate than was actually observed. Nevertheless, the possibility of females approaching the egg suspension cannot be ruled out.

The blocking behavior after spawning by the male *Ophiocoma scolopendrina* (Fig. 8) is likely a result of sexual selection if the approaching individuals are males. Neighboring males, even in non-mobile species, are known to reduce the fertilization success of other males as was found in 2 brooding species (Yund & McCarty 1994). Similar principles should apply in broadcast-spawning brittle stars. The lower spawning frequency of late individuals in the tidal pool tests suggests that blocking is an effective tactic in *O. scolopendrina*. If females also approach eggs in nature, they do not do it for reproductive reasons, since none spawned eggs in this investigation. Rather, feeding on the eggs of other females is a possibility. If this is the case, the spawning males may have stayed to block potential egg predators. No such behaviors, i.e. blocking of other males, remaining near eggs, and refraining from spawning by late arrivals, have been reported in other ophiuroids or even in other echinoderms, to our knowledge.

Why should late arrivals have a lower rate of spawning than first arrivals? Because many eggs may have been fertilized by the first arrivals, refraining from spawning by the late males would be adaptive and save their limited supply of sperm for better opportunities. Production of polyspermic embryos could be just as bad as low fertilization success (Marshall & Bolton 2007); this may be an additional incentive for late males to refrain from spawning. Other explanations are possible, but more observations are needed at this stage.

Judging from the spawning behaviors of the 2 brittle star species studied, these broadcast-spawning species must face severe challenges from sperm limitation to sexual selection at spawning time. The diverse traits evolved in these 2 species indicate the availability of a rich behavioral repertoire that is under the control of natural selection.

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