Host specialization of an obligate sponge-dwelling brittlestar

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ABSTRACT: On coral reefs off the Florida Keys (USA), the obligate sponge-dwelling brittlestar *Ophiothrix lineata* lives almost exclusively in the tube sponge *Callyspongia vaginalis*. We examined chemotactic recognition by *O. lineata* to assess sponge host preferences using a Y-tube assay chamber. *O. lineata* preferentially selected seawater conditioned by *C. vaginalis* relative to seawater controls and showed no preference for seawater conditioned by the infrequent host sponge *Niphates digitalis* or the non-host sponge *Aplysina archeri*. When offered seawater conditioned by *C. vaginalis* and *N. digitalis*, *O. lineata* preferentially chose *C. vaginalis*. Field manipulations examined growth of *O. lineata* confined to live in all 3 sponge species and the impact of habitat size on growth of *O. lineata* living in *C. vaginalis*. Growth of *O. lineata* was significantly greater in longer (12 cm) tubes of *C. vaginalis* than in shorter (6 cm) tubes, and while *O. lineata* was able to survive in *A. archeri*, growth was greater for brittlestars living in *C. vaginalis* and *N. digitalis*. Surveys of *O. lineata* in *C. vaginalis* revealed that 74.3% of sponges had at least 1 male and female brittlestar, with 37.1% of sponges having a greater proportion of males, which may increase brittlestar fertilization success. Abundance of *O. lineata* increased with sponge size and did not differ based on the presence of brooded larvae in sponge tissue. The obligate association of *O. lineata* with *C. vaginalis* likely evolved as a consequence of some combination of host abundance, enhanced food availability, and greater probability of mating success in the multi-tubed sponge.

KEY WORDS: Host specificity · Chemotaxis · *Ophiothrix lineata* · *Callyspongia vaginalis*

INTRODUCTION

The development and maintenance of host specificity has long been recognized by evolutionary ecologists as an important means of speciation in terrestrial plant–herbivore and host–parasite interactions (Hufbauer & Via 1999, Tompkins & Clayton 1999, Giorgi et al. 2004). Multiple factors can increase host specificity, including limited dispersal or encounters with potential hosts, adaptive specialization resulting from some fitness gain from an association, adaptations resulting in lower fitness on alternative hosts, or increased probability of finding a mate in a specific area (Combes 1991, Timms & Read 1999, Sotka 2005, Agosta 2008). The importance of host specificity in marine systems is less understood (Sotka 2005), and ecologists have re-

cently increased their focus on the foregoing mechanisms to describe the impact of host specialization on speciation in marine systems (Faucci et al. 2007, Johnston & Miller 2007, Sotka 2007).

Studies of host specificity in marine systems frequently cite plant–insect analogs, described as associations between small invertebrates with limited mobility or dispersal that associate with larger hosts for refuge and food (Hay et al. 1987, Sotka 2005). One of the best described examples of host-mediated speciation in a marine system is that of alpheid shrimp that live in association with sponges (Duffy 1996a,b, Macdonald et al. 2006). In a collection of 27 *Synalpheus* spp. from inside sponges on coral reefs in Belize, 55% were found in only 1 species of sponge (Macdonald et al. 2006). The high level of host specialization in *Synal-*

pheus is most likely a function of space partitioning based on sponge morphology, competitive interactions between shrimp species, the eusocial behavior of several species, and the direct development and short dispersal distances of larvae (Duffy 1992, 1996b, Macdonald et al. 2006).

The pattern of host use observed in alpheid shrimp is probably one of many, considering the abundance and diversity of sponges and their associated fauna (reviewed by Wulff 2006). The sponge-dwelling brittlestar *Ophiothrix lineata* is a species-specific obligate living in association with the tube sponge *Callyspongia vaginalis* (Henkel & Pawlik 2005). Although *O. lineata* has been observed living in association with other sponges at low frequency (Kissling & Taylor 1977, Hendler et al. 1995, Henkel & Pawlik 2005), surveys of sponges in the Florida Keys, USA, recorded *O. lineata* living in *C. vaginalis* 99% of the time, despite the presence of similarly shaped tube sponges that could provide refuge (Henkel & Pawlik 2005). The association between *O. lineata* and *C. vaginalis* has been described as a cleaning mutualism, with *O. lineata* deposit-feeding on the surface of the sponge (Hendler 1984). Comparisons of growth and larval output of *C. vaginalis* with and without associated *O. lineata* demonstrated that the sponge does not benefit from *O. lineata* (T. P. Henkel unpubl. data). Furthermore, *O. lineata* is capable of consuming larvae released by *C. vaginalis* in lab-feeding experiments and may be a larval parasite on the sponge (T. P. Henkel unpubl. data). The association between *O. lineata* and *C. vaginalis* provides an example from the marine benthos for examining mechanisms driving selection for an obligate association.

Enhanced fitness is often inferred as the selection mechanism for a high degree of host specificity, although limited interactions with alternate hosts can also result in the appearance of host preferences (Tompkins & Clayton 1999). The presence of a species-specific chemical cue used in host recognition is evidence of a specialized adaptation that may reflect a fitness advantage. Echinoderms rely on chemical cues to recognize prey items (Sloan & Campbell 1982) as well as associated hosts (Clavico et al. 2006, Fourgon et al. 2007). In the Florida Keys, *Ophiothrix lineata* lives primarily in association with *Callyspongia vaginalis*, and also lives at low frequency (<1%) in the tube sponges *Niphates digitalis* (Henkel & Pawlik 2005) and *Verongula (Aplysina) lacunosa* (Kissling & Taylor 1977). We hypothesize that the brittlestar should distinguish *C. vaginalis* from other sponge hosts if the observed preference for *C. vaginalis* results in enhanced fitness for *O. lineata*.

Host specificity imposes potential population constraints if host availability or size is limited. Size-specific shelter requirements limit crustacean popula-

tions through mortality, emigration, or stunting of the affected size classes (Caddy 1986, Caddy & Stamato-poulos 1990, Beck 1995). Alpheid shrimps chose sponge habitat based on the size of suitable channels in the sponge tissue (Duffy 1992), and sponge host size can constrain the size, abundance, and diversity of the associated shrimp community (Hultgren & Duffy 2010). In the Florida Keys, 69% of large *Ophiothrix lineata*, >5 mm disk diameter, lived in sponge tubes with >70 cm² inner tube surface area (T. P. Henkel unpubl. data). Large *O. lineata* live inside sponge tubes as a refuge from fish predators (Hendler 1984), and brittlestar abundance may be limited by available sponge habitat (Kissling & Taylor 1977, Henkel & Pawlik 2005). Food availability, including surface area for deposit-feeding and predation on sponge larvae, is a function of sponge size; therefore, limited food resources from smaller sponges also may restrict the distribution of large *O. lineata* to larger tubes.

In the present study, we explored both specific adaptations and fitness advantages to host sponge exploitation by the brittlestar *Ophiothrix lineata*. A choice assay was used to determine whether *O. lineata* was capable of chemotaxis in response to chemical cues from 3 species of tube sponge: *Callyspongia vaginalis*, *Niphates digitalis*, and *Aplysina archeri*. The last of these was chosen because it has a similar oscular diameter to *C. vaginalis*, but *O. lineata* has not been found living in *A. archeri* (Henkel & Pawlik 2005). Controlled laboratory experiments are often employed to track growth and reproduction of species living on alternate hosts over multiple generations (Sotka 2005) because fitness advantages to host specificity are often difficult to determine. However, sponges are difficult to maintain in the lab; therefore, we used a field-based approach to examine the fitness impacts of different sponge hosts by measuring the growth of *O. lineata* confined to living in each of the 3 sponge species. Focusing on the association between *O. lineata* and *C. vaginalis*, we also examined the potential fitness constraints of habitat size by comparing the growth of *O. lineata* living in 2 different-sized tubes of *C. vaginalis*. Finally, we compared and surveyed the sex distribution of *O. lineata* living in *C. vaginalis* for insights into reproductive constraints for an obligate commensal.

MATERIALS AND METHODS

Host choice experiments. Three species of tube sponges, *Callyspongia vaginalis*, *Niphates digitalis*, and *Aplysina archeri*, were collected using SCUBA from 2 shallow reefs off Key Largo, Florida: North Dry Rocks, (25° 07.850' N; 80° 17.521' W) and Pickles Reef (24° 59.286' N; 80° 24.6' W). Sponges were cut under-

water, placed in mesh cages attached to the substratum, and allowed to heal for at least 2 d in the field. Cut sponges then were transported in containers of seawater to the laboratory and kept in a large recirculating 100 l holding tank. Water changes were conducted daily, and sponges were used in experiments before any deterioration in sponge health was observed (<7 d). Individual *Ophiothrix lineata* were collected from *C. vaginalis* on the reef and held in perforated plastic containers submerged in a separate aquarium using the same recirculating seawater as the sponges.

Seawater for chemical cue assays was collected over shallow coral reefs (~10 m deep) from 2 to 3 m below the surface using a submersible pump, then filtered through a 500 μm filter-fiber bag. Sponge-conditioned seawater was prepared by placing 1 l of living sponge tissue in 6 l of filtered seawater in a bucket with an aerator for 4 h. Sponge volume was determined by volumetric displacement of seawater. Control seawater was prepared in the same manner, without the addition of sponge tissue. During the seawater incubation process, brittlestars were held in an aquarium containing filtered seawater that had not been exposed to sponges.

An assay chamber was constructed using tygon tubing with an inner diameter of 2.54 cm. Two pieces of tube 14 cm in length were connected to a 120° PVC Y-connector, with a shorter piece of tubing 6 cm in length attached to the base of the Y that served as a brittlestar entrance. An L-shaped fitting with a 6 cm piece of tubing on one end was attached to each 14 cm tube, and positioned perpendicular to the plane of the Y-connector, and the openings of these tubes served as inlets for treatment and control seawater. Two MityFlex peristaltic pumps (Model 907-014) with 6.3 mm diameter peristalsis tubing delivered treatment and control seawater at a rate of 48 ml min^{-1}. The assay chamber was placed in a shallow pan of control seawater with the inlets for each L-shaped fitting projecting vertically above the water surface, allowing control and treatment seawater to flow through the chamber as each was added by the peristaltic pumps.

Three choice experiments were conducted using filtered seawater and seawater conditioned with 1 of the following sponge species: *Callyspongia vaginalis*, *Niphates digitalis*, and *Aplysina archeri*. Additional choice assays were conducted using seawater conditioned with *C. vaginalis* and *N. digitalis*. Choice experiments were performed in the dark, as brittlestars are negatively photo-sensitive. A single

Ophiothrix lineata was placed into the assay pan and allowed to acclimate for 2 min. The brittlestar was placed inside the entrance tube after the acclimation period and the pumps were activated. A choice occurred when the oral disk of the brittlestar passed beyond the PVC Y-connector into 1 of the 2 treatment arms. Each brittlestar was given 10 min to make a choice between the 2 treatments, and was used only once in any paired experiment. The assay chamber was flushed with control seawater between replicate assays, and the treatments were alternated between the 2 pumps and treatment arms to control for any slight differences between them. Preferences for a particular treatment were determined using a chi-squared goodness of fit to test for deviation from an expected equal probability of selecting either treatment. Only replicates in which a brittlestar made a choice were used in the analyses. A right-tailed Fisher's exact test was used to determine if the probability of *O. lineata* making a choice was greater when *C. vaginalis* was present than when it was absent.

Choice experiments were further used to isolate the chemical cues from *Callyspongia vaginalis* responsible for host choice by *Ophiothrix lineata* (Table 1). Assays were conducted in Wilmington, NC, using *O. lineata* collected from Key Largo, FL, and kept in aquaria with artificial seawater (Red Sea brand) prepared to a salinity of 35 ppt. Crude organic extracts were prepared from *C. vaginalis* tissue collected from Key Largo using standard extraction techniques (Pawlik et al. 1995). Paired choice experiments were conducted following the same choice protocol, using artificial seawater and treatment-conditioned seawater. Treatments included crude organic extracts incorporated into gel matrix (as

Table 1. *Ophiothrix lineata*. Choice experiments using chemical cues isolated from *Callyspongia vaginalis* and presented to brittlestars using different techniques and at various concentrations. Crude organic extracts of sponge tissue were incorporated into carrageenan gels from which they would diffuse when soaked in seawater. Alternatively, crude extracts (10 ml sponge tissue dissolved in 10 ml methanol) were added directly to seawater. NC: natural concentration

Treatment	Seawater (l)	Soak time	No. of choices made		
			Sponge	Control	No choice
Carrageenan gels					
80 ml, NC	5	4 h	0	4	1
40 ml, NC	5	4 h	0	3	0
20 ml, NC	5	4 h	0	3	6
20 ml, 50% dilution	5	20 h	0	0	6
50 ml, 20% dilution	5	4 h	0	1	7
Crude extract					
10–50 drops (n = 12 trials)	1	–	0	0–2	1–5
Frozen sponge					
5–10 ml tissue (n = 33 trials)	1–2	1 s to 5 min	0–4	0–4	0–3

per Engel & Pawlik 2000) and extracts directly added to seawater. Seawater conditioned with frozen whole sponge tissue was used in experiments when choice experiments with extract treatments were unsuccessful (Table 1).

Effect of habitat size on growth of *Ophiothrix lineata*. Growth of *O. lineata* was monitored from 11 March to 12 July 2005 on a shallow patch reef at North Dry Rocks using SCUBA. The mass of 143 towel-dried *O. lineata* was determined and compared with disk diameter using linear regression to determine the relationship between disk diameter of *O. lineata* and overall body size. Although both metrics can be obtained non-lethally, disk diameter is not impacted by loss of arms due to handling damage or sub-lethal predation.

Forty tubes of *Callyspongia vaginalis*, ~2 cm in oscular diameter, were cut for 2 habitat-size treatments. Long tubes were 12 cm and short tubes were 6 cm in length, representing ~35 and 75 cm^2 inner tube surface area, respectively. Long and short sponge tubes were cable-tied upright to bricks that were secured to coral pavement and placed haphazardly on the reef, ≥2 m apart at 10 m depth and allowed to heal for 1 wk. *Ophiothrix lineata*, 5.5 to 8.0 mm disk diameter, were collected from nearby sponges and brought back to the laboratory in seawater, and their oral surfaces were tagged with small dots of the histological dye Congo Red. Tagged brittlestars were kept in recirculating seawater overnight to confirm survival and quality of tags. The following day, initial disk diameter, measured as the distance from the base of 1 arm to the point on the disk directly opposite, was measured to the nearest 0.5 mm using calipers. Tagged brittlestars were then transplanted to the field and haphazardly assigned to 1 of the 2 sponge tube length treatments (n = 20 for each treatment). Brittlestars and sponge tubes were collected after 4 mo and final disk diameter was measured. Sponge tubes were dissected longitudinally into 1 cm strips, and the number of larval brood chambers per tube was counted in order to determine whether habitat size is a proxy for potential sponge larval food resources. This width ensured that brood chambers were only counted once, as brood chambers did not exceed 1 cm width. Differences in the percentage change in the disk diameter of *O. lineata* between the 2 habitat size treatments were compared using ANOVA (Sokal & Rohlf 1981). Differences in the total number of brood chambers between long and short sponge tubes were compared using ANOVA on log$_{10}$-transformed data. All statistics were performed using JMP v. 7.0 (SAS Institute).

Growth of *Ophiothrix lineata* living on alternative sponge hosts. *O. lineata* will abandon sponges other than *Callyspongia vaginalis* within 24 h (Henkel & Pawlik 2005); therefore, we devised a method to cage

O. lineata inside sponge tubes to assess growth of the brittlestar in alternative sponge hosts. We first examined potential caging effects by comparing the growth of *O. lineata* living in caged and uncaged *C. vaginalis* at North Dry Rocks. A small piece of flexible plastic 5 mm mesh was placed over the osculum of equally sized (10 cm tube height) *C. vaginalis* tubes, and tubes were cable-tied upright to bricks that were secured to coral pavement and placed haphazardly on the reef. Mesh prevented brittlestars from escaping but permitted them to extend their arms over the sponge to deposit-feed. Percentage change in disk diameter of *O. lineata* was compared between brittlestars living in caged and uncaged *C. vaginalis* (n = 20). Mesh was cleaned of epibionts every 2 wk for 2 mo.

Single sponge tubes of *Callyspongia vaginalis*, *Niphates digitalis*, and *Aplysina archeri* were collected at Pickles Reef from ~15 m depth using SCUBA in March 2005. A piece of 5 mm mesh was placed over the osculum of each tube and held in place by 2 small cable-ties through the lip of the osculum. Fifteen sponge tubes of each species were cable-tied upright to bricks on the reef and allowed to heal for 2 wk. At the end of 2 wk, *Ophiothrix lineata* were collected, brought back to the lab, tagged, and disk diameters measured. Tagged *O. lineata* were placed inside a mesh-covered sponge tube within 24 h of collection and then the tube was cable-tied upright to a brick. The sponge tubes were placed haphazardly on the reef ≥2 m apart at ~15 m depth. Mesh-covered sponge tubes were cleared of fouling organisms every 2 wk for 4 mo. The percentage change in disk diameter was calculated for all *O. lineata* remaining in sponge tubes after 4 mo.

Sex and size distribution of *Ophiothrix lineata*. The sex and size distribution of *O. lineata* living in multi-tubed *Callyspongia vaginalis* was examined by collecting 35 individual sponges from 2 sites, Conch Reef, FL (n = 25) and North Dry Rocks (n = 10), in May 2008. Only sponges with at least 3 tubes and 1 visible *O. lineata* were collected. Sponges were cut from the base and individually placed into large 11 l plastic bags in the field. Sponges were carefully dissected and all *O. lineata* were removed and held in seawater tanks. In addition, the presence or absence of brood chambers within 30 of the sponges was noted. Disk diameter was measured for all *O. lineata*, and sex was determined by visual inspection of the gonads under a dissecting microscope. White testes and yellow ovaries were easily identifiable through the tissue of larger gravid individuals; however, smaller individuals were dissected to determine gender.

The density of *Ophiothrix lineata* living in brooding and non-brooding *Callyspongia vaginalis* was compared using analysis of covariance (ANCOVA), with total sponge surface area as the covariate. Additional

data were added from surveys of *C. vaginalis* collected at North Dry Rocks and Pickles Reef for which the presence of brood chambers was recorded (n = 18). For these added data, *O. lineata* >7 mm disk diameter were considered mature adults based on the sex-size distribution (see Fig. 2). Both density of *O. lineata* and sponge surface area were $\log_{10}$ transformed to meet the assumptions of ANCOVA (Sokal & Rohlf 1981).

RESULTS

In total, 55, 62, and 24 choice experiments were conducted to determine the preferences of *Ophiothrix lineata* for filtered seawater or seawater conditioned by *Callyspongia vaginalis*, *Niphates digitalis*, or *Aplysina archeri*, respectively. In these experiments, *O. lineata* made a choice 23, 22, and 7 times, respectively. Given the choice of filtered seawater vs. seawater conditioned by *C. vaginalis*, *O. lineata* preferentially selected sponge-conditioned seawater (χ^2 = 9.78 p < 0.05). Brittlestars showed no preference between control seawater and seawater conditioned by either *N. digitalis* or *A. archeri* (Table 2). When presented with a choice between seawater conditioned by *C. vaginalis* or *N. digitalis*, *O. lineata* preferentially chose the former 78.1% of the time (p = 0.001; Table 2), making a choice in 32 out of 59 assays. Based on all 4 choice experiments, *O. lineata* was more likely to make a choice when *C. vaginalis* was present in the assay than when it was not included (p = 0.009). No preferences were exhibited in assays using crude organic extracts or frozen tissue samples of *C. vaginalis* (Table 1).

Disk diameter was both positively correlated with, and a good predictor of, mass of *Ophiothrix lineata* ($\log_{10}$ mass = 2.867($\log_{10}$ disk diameter) + 0.254; R^2 = 0.941). Growth of *O. lineata* after 4 mo was signifi-

cantly greater in long (12 cm) sponge tubes compared to short (6 cm) tubes of *Callyspongia vaginalis* (Fig. 1). Average change in disk diameter of *O. lineata* living in long and short sponge tubes was 2.09 ± 0.28 (SE) mm (34% increase) and 0.79 ± 0.20 mm (12% increase), respectively. The number of brood chambers in long sponge tubes was significantly greater than in shorter tubes (Fig. 1), with brood chambers occurring in 5 of the short tubes and 9 of the long tubes of *C. vaginalis*.

There was no effect of caging on the growth of *Ophiothrix lineata* inside *Callyspongia vaginalis* tubes, as growth did not differ for brittlestars living in mesh-covered sponge tubes (6.51 ± 2.92% SE, n = 17) compared to uncovered sponge tubes (3.49 ± 1.96%, n = 16; ANOVA, $F_{1,32}$ = 0.996 p = 0.326) after 2 mo. Many of the replicates from the experiment designed to test the growth of *O. lineata* living in different species of sponges were lost due to strong storm surge associated with hurricanes. After 4 mo, there were 2, 3, and 7 replicates remaining of individual *O. lineata* living in *C. vaginalis*, *Niphates digitalis* and *Aplysina archeri*, respectively. Although statistical comparisons are not advised at this low level of replication, *O. lineata* increased in disk diameter by 42.5 ± 37.5%, 21.7 ± 8.2%, and 8.8 ± 4.4% living in mesh-covered tubes of *C. vaginalis*, *N. digitalis*, and *A. archeri*, respectively. Mean growth of *O. lineata* in *C. vaginalis* was similar to that reported in the previous experiments (Fig. 1).

The proportion of gravid *Ophiothrix lineata* increased with disk diameter, as all *O. lineata* <4 mm disk diameter were juveniles and all *O. lineata* ≥8 mm were gravid (Fig. 2). Immature *O. lineata* comprised 31.2% of individuals found living on *Callyspongia vaginalis* in May 2008. The density of gravid *O. lineata* within a sponge ranged from 1 to 12. Of the 35 sponges surveyed, 25.7% had either only males or only females
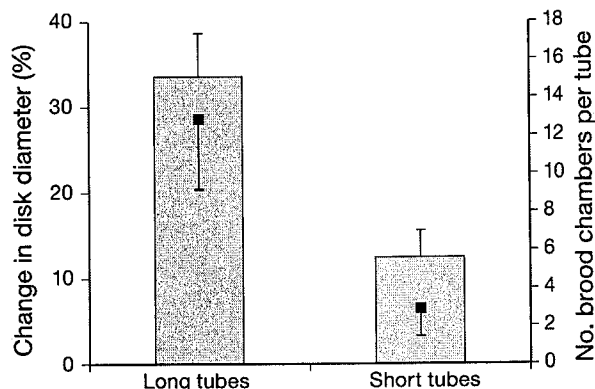
Table 2. *Ophiothrix lineata*. Results of paired chemical cue choice assays in which *O. lineata* was presented sponge-conditioned seawater vs. filtered seawater, or seawater conditioned with *Callyspongia vaginalis* vs. *Niphates digitalis*. The percentage of choices for each treatment is given relative to the number of choices made (n) out of the total number of assays. *denotes a significant difference from an expected equal probability of selecting either treatment using chi-squared goodness of fit

Paired treatments	Percentage of choices	n	Total no. of assays
Sponge seawater vs. control seawater			
C. vaginalis	82.6*	23	55
N. digitalis	50.0	22	62
Aplysina archeri	42.9	7	31
C. vaginalis vs. N. digitalis			
C. vaginalis	78.1*	32	59

Fig. 1. *Ophiothrix lineata*. Percentage change in disk diameter of *O. lineata* (gray bars: +SE) living in long (n = 11) and short (n = 12) *Callyspongia vaginalis* sponge tubes after 4 mo (ANOVA, $F_{1,21}$ = 13.0683, p = 0.0016). Black squares are mean number of brood chambers in the same sponge tubes (–SE; ANOVA, $F_{1,21}$ = 7.35, p = 0.013)

living inside, and 28.6% had an equal ratio of males to females. The majority of sponges (37.1%) had a greater proportion of males relative to females (Fig. 3). An equal proportion of brooding *C. vaginalis* (17; 56.7%) and non-brooding sponges (13; 43.3%) were observed ($\chi^2 = 0.53$ p > 0.05). There was no correlation between the presence of brood chambers and the presence of male ($\chi^2 = 0.083$ p > 0.05) or female *O. lineata*
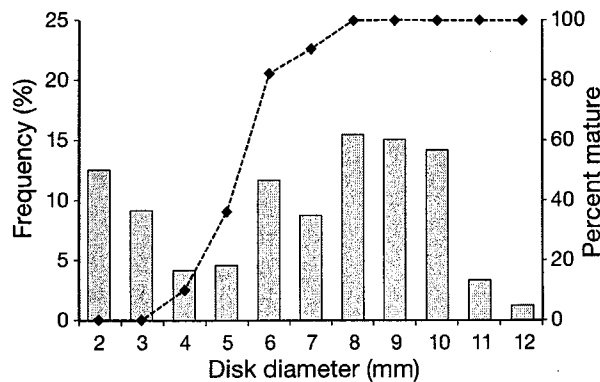
($\chi^2 = 0.14$ p > 0.05). Based on combined survey data, 18 non-brooding and 28 brooding *C. vaginalis* contained at least 1 *O. lineata*. Density of both gravid and total *O. lineata* increased with increasing sponge size, and there was no difference in the density of either gravid or total *O. lineata* between brooding and non-brooding *C. vaginalis* (Table 3, Fig. 4).

Fig. 2. *Ophiothrix lineata*. Size frequency distribution (bars) and percentage of mature brittlestars at each size class (diamonds) out of the 204 *O. lineata* living inside 35 *Callyspongia vaginalis*
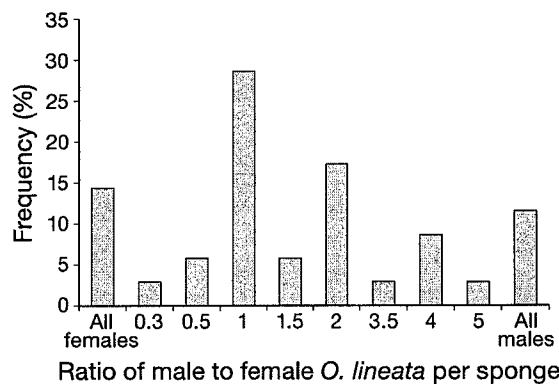

Fig. 3. *Ophiothrix lineata*. Frequency of observed ratios of males to females living in a single *Callyspongia vaginalis* (n = 35)
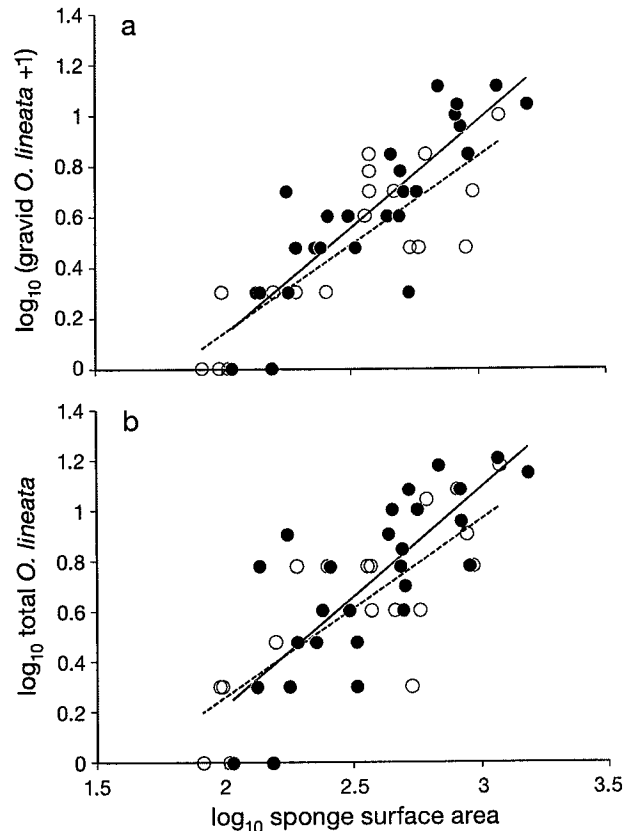

Fig. 4. *Ophiothrix lineata*. (a) Abundance of gravid *O. lineata*, and (b) total abundance of *O. lineata* as a function of surface area of *Callyspongia vaginalis* with brood chambers (filled circles, solid line; n = 28) and without brood chambers (open circles, dashed line; n = 18). Regression and ANCOVA analysis in Table 3

Table 3. *Ophiothrix lineata*. Results from linear regression analyses and analysis of covariance (ANCOVA) for number of gravid and total *O. lineata* found living in brooding (n = 28) and non-brooding (n = 18) *Callyspongia vaginalis*. ***p < 0.0001

Group	Regression		ANCOVA				
	Equation	R^2	Source	df	MS	F	p
log (gravid *O. lineata* + 1) (y) vs. log sponge surface area (x)							
Brooding	log y = 0.853x – 1.576	0.735***	Treatment	1	0.018	0.64	0.4278
Non-Brooding	log y = 0.700x – 1.263	0.686***	Slope	1	0.028	1.00	0.3242
			Covariate	1	2.880	103.09	<0.0001
log total *O. lineata* (y) vs. log sponge surface area (x)							
Brooding	log y = 0.860x – 1.495	0.610***	Treatment	1	0.023	0.49	0.4862
Non-Brooding	log y = 0.701x – 1.143	0.608***	Slope	1	0.030	0.66	0.4201
			Covariate	1	2.909	63.63	<0.0001

DISCUSSION

Host specificity of *Ophiothrix lineata*

The ability of *O. lineata* to move more often in the direction of waterborne chemical cues from the sponge *Callyspongia vaginalis* vs. control seawater or cues from other sponge species (Table 2) is evidence of host preference. Considering the overall number of experimental trials, *O. lineata* infrequently made a choice, but was more likely to do so when *C. vaginalis*-conditioned seawater was present (46 and 54% of trials) compared to when *C. vaginalis*-conditioned seawater was not present (35 and 29% of trials). The chemical cue used by *O. lineata* appears to be a species-specific signal rather than a general sponge metabolite. However, attempts to isolate the chemical signal used to detect *C. vaginalis* were unsuccessful. The sequential method extracted all organic metabolites from within the sponge cells, many of which would likely never be encountered by *O. lineata*. Freezing and thawing also would rupture sponge cells, resulting in the leakage of cell metabolites. High concentrations of intracellular metabolites may mask or dilute the cue used by *O. lineata* for chemotaxis toward its preferred host sponge. Nevertheless, the adaptation to select a single host provides a mechanism for maintaining the species-specific relationship between *O. lineata* and *C. vaginalis* and suggests some fitness advantage to the association.

Host specificity could be a function of limited dispersal to new hosts (Tompkins & Clayton 1999), and not necessarily an adaptive response to increased fitness from the association (Timms & Read 1999). Studies of emigration and immigration indicate that large (>5 mm disk diameter) *Ophiothrix lineata* remain in the same *Callyspongia vaginalis* for several months, but smaller *O. lineata* move to unoccupied *C. vaginalis*. *O. lineata* broadcast-spawn eggs and sperm, but subsequent development does not result in a larval stage (ophiopluteus), but rather direct development into a juvenile within 6 to 8 d of fertilization (Richards et al. 2007). Even with limited larval dispersal, the lack of population genetic structure along the Florida Keys reef tract (Richards et al. 2007) and observed migration of small individuals suggests that individual *O. lineata* have opportunities to encounter alternative hosts. The high level of specificity exhibited by *O. lineata* for *C. vaginalis* therefore is likely not a function of limited contact with alternate hosts, but rather an evolutionary adaptation to find a specific host as a consequence of enhanced fitness from the association with *C. vaginalis*.

The fitness advantages of associating with *Callyspongia vaginalis* include predation refuge and access to food (Hendler 1984, Henkel & Pawlik 2005), as well as increased potential to find a mate. Refuge quality is partially dictated by the oscular diameter of sponges, as *Ophiothrix lineata* is quickly consumed by fish predators when placed in sponges like *Niphates digitalis* that have wider oscula (Henkel & Pawlik 2005). We controlled for the predation limitation by placing a screen over the oscula of sponges and were able to examine growth of *O. lineata* in different sponges. Although not statistically compared because of low replication, growth of *O. lineata* was similar in both *N. digitalis* and *C. vaginalis*, but much less in the tube sponge *Aplysina archeri*. Mean growth rates of *O. lineata* living in *C. vaginalis* were comparable to other growth experiments of similar time periods (Fig. 1) and support the pattern of higher growth in association with *C. vaginalis* compared to *A. archeri*.

Unlike both *Callyspongia vaginalis* and *Niphates digitalis*, *Aplysina archeri* contains secondary metabolites that deter fish predation (Pawlik et al. 1995), and therefore should provide a better refuge habitat. However, unlike amphipods that associate with chemically defended algae for refuge and food (Duffy & Hay 1994), *Ophiothrix lineata* does not preferentially associate with a chemically defended refuge despite physical characteristics, such as oscular diameter, that are similar to those of *C. vaginalis* (Henkel & Pawlik 2005). In addition to anti-predatory effects, crude organic extracts of *A. archeri* have anti-microbial properties (Newbold et al. 1999, Kelly et al. 2003), which may reduce development of bacterial biofilms on the sponge surface, thereby reducing the nutritional quality of surface constituents compared to either *C. vaginalis* or *N. digitalis*. In addition, *A. archeri* does not regularly produce larvae (Tsurumi & Reiswig 1997) that could be consumed by the brittlestar. The food resources available from *A. archeri* are most likely less than those offered by either *N. digitalis* or *C. vaginalis*, both of which lack chemical defenses. Refuge quality plays some role in determining where *O. lineata* can survive, but food resources, both in terms of deposit-feeding and larval predation, are more likely to impact host selection by *O. lineata*. Non-preferred habitats such as *A. archeri* may serve as temporary hosts that provide protection for *O. lineata* in search of *C. vaginalis*.

Sponge larvae provide a potential food resource for *Ophiothrix lineata*, but *O. lineata* do not live solely in brooding sponges. Association with both brooding (60.9%) and non-brooding (39.1%) sponges was observed in the 46 *Callyspongia vaginalis* with associated *O. lineata* surveyed in this study. It might be expected that the added food resource provided by sponge larvae could result in a greater number of gravid brittlestars. However, although the abundance of *O. lineata* increased with sponge size, there was no

difference in relative abundance of gravid *O. lineata* between brooding and non-brooding *C. vaginalis* (Fig. 4, Table 3). Males and females were also found proportionally in both host types. Therefore, while larval predation can result in increased growth of *O. lineata* (T. P. Henkel unpubl. data), the brittlestar does not preferentially associate with brooding *C. vaginalis*. Brittlestars may be unable to determine when a host *C. vaginalis* is brooding larvae, and they may not have long to wait until a non-brooding individual becomes reproductive, making the wait a better option than the risk of predation when moving to another sponge.

Increased probability of finding a mate could be a strong mechanism for maintaining host specificity in addition to the role of food resources in structuring the association of *Ophiothrix lineata* with *Callyspongia vaginalis*. The importance of mating success in structuring species-specific associations has received little attention in marine systems (Sotka 2005); however, it is known to be an important mechanism in insects with low mobility (Bush & Smith 1998, Hawthorne & Via 2001). Mate availability has also been suggested to drive selection for maintaining host specificity in 4 species of bat fly that have high dispersal potential to multiple hosts (Dick & Patterson 2007). Fertilization success may be important in structuring the *O. lineata*–*C. vaginalis* association given the low mobility of larger *O. lineata*. Fertilization success has been found to increase with greater densities of free-spawning sea urchins (Levitan 2005). The abundance of mature *O. lineata* increased with increasing sponge size (Fig. 4), and the tubes of *C. vaginalis* are usually connected at the base, allowing movement of brittlestars between tubes within the larger sponge. The greater density of males, and potential higher concentration of sperm during spawning, relative to females within a sponge (Fig. 3), may increase fertilization success considering that gametes are released into a high-flow environment: the osculum of *C. vaginalis* can pump $3.5 \ l \ s^{-1} \ kg^{-1}$ dry tissue (Weisz et al. 2008). In addition, the high pumping rate of *C. vaginalis* may provide a dispersal mechanism for fertilized eggs that are slightly heavier than seawater (T. P. Henkel pers. obs.). While 65.7% of *C. vaginalis* surveyed had at least a 1:1 male to female ratio, a quarter had only 1 sex present (Fig. 3). Given the limited mobility of large *O. lineata* (Henkel & Pawlik 2005), solitary individuals would likely be non-reproductive, but the mobility of smaller *O. lineata* (Henkel & Pawlik 2005) combined with their chemotactic ability to locate host sponges would enhance pairing and reproductive success.

As noted by Hendler (1984), the deposit-feeding *Ophiothrix lineata* is distinct among its congeners, all of which are suspension feeders. In laboratory assays, *O. lineata* captured swimming larvae using tube feet to pass and guide larvae into its mouth (T. P. Henkel unpubl. data). This raises some interesting evolutionary questions regarding the potential importance of both food and mating success in structuring the association. The increased probability of finding a mate may have resulted in a regular association with *Callyspongia vaginalis*, and subsequently *O. lineata* shifted towards deposit-feeding on the surface of the sponge. Conversely, *O. lineata* may have first diverged from its congeners as a deposit feeder, benefiting from the resources available on the surface of *C. vaginalis*, as well as from larval predation. Increased mating success, associated with multiple individual *O. lineata* occupying a single sponge, could have served to strengthen the association, resulting in the obligate relationship the brittlestar exhibits today. The association provides an excellent model for examining evolutionary trade-offs and factors maintaining host specialization considering *O. lineata* is unique within its genus with respect to a high level of host specificity, mode of feeding, and a direct-developing larval stage.

Host limitations on growth of *Ophiothrix lineata*

The abundance of *Ophiothrix lineata* in reefs of the northern Florida Keys is positively correlated with the abundance of *Callyspongia vaginalis* (Henkel & Pawlik 2005). Furthermore, the number of large *O. lineata* in a single *C. vaginalis* rarely exceeds 1 individual per sponge tube, which may be a response to available food resources (Henkel & Pawlik 2005). In the present study, *O. lineata* grew significantly more when living in longer tubes of *C. vaginalis* (Fig. 1). Assuming that larger *O. lineata* have increased fecundity, which has been demonstrated for other brittlestar species (McGovern 2002), brittlestars in longer tubes may also have greater reproductive output. Enhanced growth in longer tubes could be due to decreased non-lethal predation (arm grazing by fish predators) on *O. lineata* within larger sponges, a greater surface area for deposit-feeding, or increased larval output of larger sponges.

Larger brittlestars living in small refuges may sustain increased non-lethal predation of arms, resulting in less growth in disk diameter, as more energy is diverted to healing. Arm regeneration results in reduced lipid and gonad production in the brittlestar *Ophiocoma echinata* (Pomory & Lawrence 1999). Large *Ophiothrix lineata* are cryptic during the day and only extend their arms out of sponges at night to feed when predators are less abundant (Hendler 1984). While a shorter sponge tube may increase exposure of long arms to damage, brittlestars are capable of retracting their arms into small areas for protection. In the pre-

sent study, there was no observable difference in arm damage for brittlestars that had been transplanted into short or long sponge tubes. Therefore, the impact of predation is unlikely to explain the differences in growth of *O. lineata* in sponges of different sizes.

Another explanation for enhanced growth of *Ophiothrix lineata* in longer sponge tubes is more surface area for the brittlestar to deposit feed. The average arm length of *O. lineata* is about 10 times that of the disk diameter (Hendler 2005); the *O. lineata* used in this study had an initial arm length of 55 to 80 mm, surpassing the length of the short sponge tubes (60 mm). Brittlestars in long sponge tubes (120 mm) had a larger surface area for deposit-feeding, but several observations suggest that this extra surface area may not be used by *O. lineata*. First, as *O. lineata* extends its arms out of a sponge tube and over the surface to deposit-feed, the disk does not leave the inside of the sponge and only part of the arm or arms move over the sponge to deposit-feed (T. Henkel pers. obs.). Second, *O. lineata* transferred to the non-preferred sponge *Niphates digitalis* grew similarly to individual brittlestars living in *Callyspongia vaginalis*, even though the surface area of *N. digitalis* is almost twice that of *C. vaginalis* of similar height (Henkel & Pawlik 2005). This suggests that *O. lineata* may not differentially benefit from a greater surface area for deposit-feeding.

Deposit-feeding is the purported primary feeding mode of *Ophiothrix lineata* (Hendler 1984); however, the ability of *O. lineata* to consume larvae of *Callyspongia vaginalis* and *Niphates digitalis* suggests that the increased growth of *O. lineata* in longer sponge tubes may be due to the increased reproductive output of larger sponges. Longer tubes of *C. vaginalis* had significantly more brood chambers per tube than shorter sponge tubes (Fig. 1), and may provide a greater larval food source for *O. lineata*, given that 81 % of long tubes had at least 1 brood chamber compared with only 41 % of short tubes. Although enhanced growth of *O. lineata* in longer sponge tubes may be due to a combination of all 3 factors — greater shelter, more surface area for deposit-feeding, and more opportunity to feed on sponge larvae — the last of these 3 may be the most important factor.

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