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REPORT

Formation and structural organization of the egg–sperm bundle of the scleractinian coral *Montipora capitata*

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Abstract The majority of scleractinian corals are hermaphrodites that broadcast spawn their gametes separately or packaged as egg–sperm bundles during spawning events that are timed to the lunar cycle. The egg–sperm bundle is an efficient way of transporting gametes to the ocean surface where fertilization takes place, while minimizing sperm dilution and maximizing the opportunity for gamete encounters during a spawning event. To date, there are few studies that focus on the formation and structure of egg–sperm bundle. This study explores formation, ultrastructure, and longevity of the egg–sperm bundle in *Montipora capitata*, a major reef building coral in Hawai'i. Our results show that the egg–sperm bundle is formed by a mucus layer secreted by the oocytes. The sperm package is located at the center of each bundle, possibly reflecting the development of male and female gametes in different mesenteries. Once the egg–sperm bundle has reached the ocean surface, it breaks open within 10–35 min, depending on the environmental conditions (i.e., wind, water turbulence). Although the bundle has an ephemeral life span, the formation of an egg–sperm bundle is a fundamental part of the reproductive process that could be strongly influenced by climate change and deterioration of water quality (due to anthropogenic effects) and thus requires further investigation.

Keywords Gametes · Ultrastructure · Dispersal · Hermaphrodite · Spawning · Gamete packaging

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Introduction

Coral reefs are productive and diverse habitats that provide shelter for an extraordinary biodiversity and services that support the economies of many island and coastal communities (Connell 1978; Moberg and Folke 1999). Coral ecosystems worldwide are severely threatened by climate change, pollution, and overexploitation (Hughes et al. 2003; Lough 2008). Both the persistence of healthy reefs and the recovery of coral populations impacted by severe environmental disturbance are dependent on gamete production, successful fertilization, development of viable offspring, and survival of new recruits (Richmond 1997). All of these processes are variable and are influenced by interactions between coral biology and spatial and temporal fluctuations in the environment (Tomascik and Sander 1987; Harrison and Wallace 1990; Richmond and Hunter 1990; Szmant and Gassman 1990; Hughes and Tanner 2000; Baird et al. 2009).

Broadcast spawning is the dominant form of sexual reproduction in scleractinian corals (Harrison and Wallace 1990; Baird et al. 2009). Broadcast spawners can be either gonochoric or hermaphroditic and can release their gametes independently or simultaneously. Approximately 65% of scleractinian coral species studied thus far are

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hermaphroditic broadcast spawners (Richmond and Hunter 1990; Guest et al. 2008; Baird et al. 2009), and of these, the majority package and release their gametes as positively buoyant egg–sperm bundles (Arai et al. 1993; Kinzie 1996). This is in contrast to brooding corals (which can also be gonochoric or hermaphroditic), where oocytes are fertilized inside the coral polyp and well-developed larvae are released (Harrison and Wallace 1990).

Gametogenesis and gamete structure have been examined in a number of coral species representing a range of reproductive modes (Harrison and Wallace 1990; Richmond and Hunter 1990); however, relatively few studies have focused on egg–sperm bundles. Those that have, reveal that each bundle contains anywhere from 6 to 180 oocytes depending on the species (Wallace 1985; Richmond 1997). For example, in the genus *Acropora* (clade Complexa), which includes mostly hermaphroditic spawners (Baird et al. 2009), bundles contain anywhere from 6 to 13 oocytes (Wallace 1985). These are arranged peripherally around a centrally located sperm mass (Wallace 1985; Vargas-Angel et al. 2006) and the gametes develop on different, but specific mesenteries (Wallace 1985). In contrast, in bundles released by *Favites abdita* (clade Robusta), oocytes and spermatocytes are intermingled and the gametes develop on the same mesenteries (Kojis and Quinn 1982).

Egg–sperm bundles disintegrate 10–40 min after reaching the surface of the water, releasing the gametes, and making them available for fertilization (Richmond 1997). To date, no studies have examined the ultrastructure or formation of the egg–sperm bundle. The structure and organization of the egg–sperm bundle is likely to influence the time required to break open, which has implications for fertilization success and opportunities for hybridization (Wolstenholme 2004).

Here, we use electron microscopy to address this knowledge gap in *Montipora capitata* (Dana 1846; family *Acroporidae*), a major reef building coral in Hawai'i (Jokiel et al. 2004). This species belongs to the family *Acroporidae* and, like most members of this family, is a simultaneous hermaphrodite that broadcast-spawns egg–sperm bundles (Wallace and Willis 1994). This family dominates coral reef assemblages throughout the Indo-Pacific region and the Caribbean Sea and is extremely sensitive to environmental (e.g., thermal anomalies Hoegh-Guldberg 1999) and biological disturbances (e.g., crown of thorns predation, Pratchett et al. 2009). As such, the analysis of the ultrastructure and formation of egg–sperm bundles in this family contributes to our basic understanding of reproductive processes in an ecologically important group of corals and one that is increasingly threatened by climate change.

Materials and methods

Collections and preliminary analysis

Montipora capitata releases egg–sperm bundles during the new moon from late spring through summer in Hawai'i (Hunter 1988). Egg–sperm bundles were collected from coral colonies on reefs adjacent to Moku O Lo'e Island in Kane'ohe Bay, Hawai'i 1–2 days after the new moon during spawning events in June through August in 2007 and 2008. Coral fragments were collected and dissected every 5 days for 1 month prior to spawning in order to evaluate gamete maturity and symbiont acquisition by the oocytes. Pictures of coral fragments were taken using a dissecting microscope (Olympus, SZX7) equipped with an Olympus camera (MagnaFire SP S 99810). A subset of the collected gamete samples were observed under the dissecting microscope and compound microscope (Olympus, BX51), photographed and measured using Image J digital analysis software (NIH). The remaining collections were fixed for scanning and transmission electron microscopy as described below.

Transmission Electron Microscopy (TEM)

For transmission electron microscopy, specimens were fixed with 4% glutaraldehyde in 0.1 M sodium cacodylate buffer (with 0.1 M calcium chloride, 0.35 M sucrose, buffered to pH 7.4) for 48 h, washed in 0.1 M sodium cacodylate (with 0.4 M sucrose) for 3 times 30 min each, followed by postfixation with 1% OsO_4 in 0.1 M sodium cacodylate buffer for 1 h. Tissue was dehydrated in a graded ethanol series (30, 50, 70, 85, 95, 100%), substituted with propylene oxide, and embedded in LX112 epoxy resin. Ultrathin (60–80 nm) sections were cut with a Reichert Ultracut E ultramicrotome, double stained with uranyl acetate and lead citrate, viewed on a LEO 912 EFTEM at 100 kV, and photographed with a Proscan frame-transfer CCD.

Field Emission Scanning Electron Microscopy (FESEM)

For scanning electron microscopy, samples were fixed, postfixed, and dehydrated in the same way as TEM samples. After ethanol dehydration, samples were critical point dried (Tousimis Samdri-795), mounted on aluminum stubs, sputter coated with gold/palladium to 5–8 nm thickness (Hummer 6.2), and viewed with Hitachi S-800 and Hitachi S-48000 field emission scanning electron microscopes.

Results

Development of gametes

Observations of coral tissue before the spawning event revealed that oocytes and spermatocytes developed on separate pairs of mesenteries growing deep in the skeleton (Fig. 1a). Reproductive tissue was found up to 1 cm below the surface tissue intercalated in the skeleton. As the spawning time approached, oocytes and spermatids developed and moved toward the anterior of the polyp. Oocytes were infected by endosymbiotic dinoflagellates (referred to as *Symbioidinium* throughout) ~2–3 weeks before spawning occurred.

Spawning

Spawning of *Montipora capitata* occurred between 2,145 and 2,200 h during the first quarter of the new moon in June, July, and August in 2007 and 2008. Approximately 2 h before spawning, the polyps relaxed (Fig. 1b) and expanded and were observed to produce mucus. Approximately 10–15 min before spawning, the egg–sperm bundles became visible beneath the oral disk. During spawning, the oral disk of each polyp became greatly extended, and the tentacles contracted (Fig. 1c). The egg–sperm bundles were squeezed through the polyp mouth and released into the water column. The release of bundles of the coral population in the field lasted 25–30 min.

Positively buoyant egg–sperm bundles (brownish-pink color) floated to the water surface and broke apart releasing spermatozoa and oocytes for external fertilization (Fig. 2). The breakage of the bundle was signaled by the release of spermatozoa, which was observed as a white stream generally emanating from one or more small openings between oocytes (Fig. 2b). The oocytes subsequently separated from one another, and the egg–sperm bundle dissociated completely within 5–25 min of release (Fig. 2c). When initially released, the oocytes were irregularly shaped and became ovoid over a period of 25–30 min (Fig. 2d). At the end of the reproductive season (August 2007), some oocytes remained irregularly shaped (Fig. 3). Both normal and deformed oocytes were light brown in color and reflecting the presence of *Symbiodinium* (Fig. 3b).

Ultrastructure of the egg–sperm bundle

The egg–sperm bundles released by *M. capitata* measured approximately 1 mm and contained around 15 ± 5.1 oocytes (mean ± SD, $n = 214$, from 26 colonies), surrounding a central mass of spermatozoa (Fig. 4). After rounding had occurred, oocytes measured approximately 461 ± 75 μm (mean ± SD, $n = 214$, from 26 colonies) in diameter (Fig. 4).

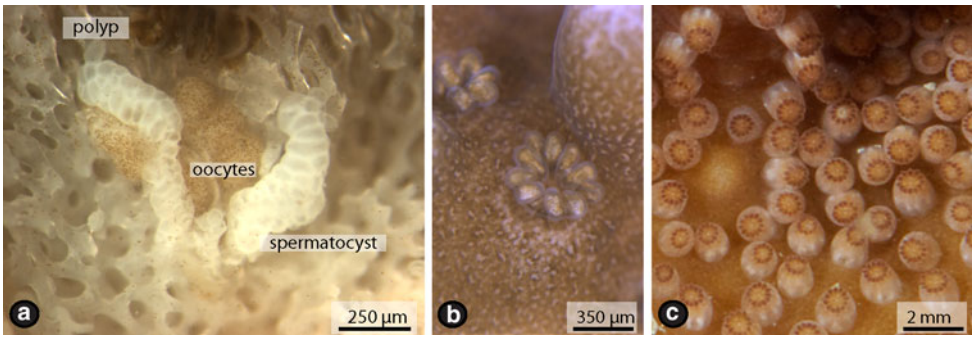

Fig. 1 *Montipora capitata* prior to spawning, **a** Oocytes and spermatozoa developing along the mesenteries deep in the skeleton. **b** Polyp starting to relax. **c** Polyps ready to release the bundle ~1 min before spawning, with bundle visible beneath the oral disc

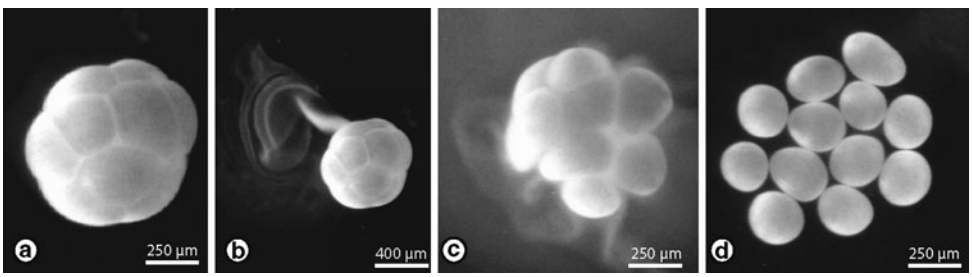

Fig. 2 Egg–sperm bundle after release, **a** Egg–sperm bundle. **b** Sudden release of spermatozoa. **c** Bundle breaking apart, oocytes with *irregular shape* and spermatozoa still present in high concentrations. **d** 30 min later, oocytes *spherically* shaped and spermatozoa diluted and no longer visible

Light microscopy and SEM observations suggest that the surface of the bundles and the spaces between the oocytes were covered with a mucus layer (Fig. 5a, b). This layer was amorphous and has no structural organization, and TEM observations revealed the material had a similar electron density to granules within the oocytes (Fig. 5c, d).

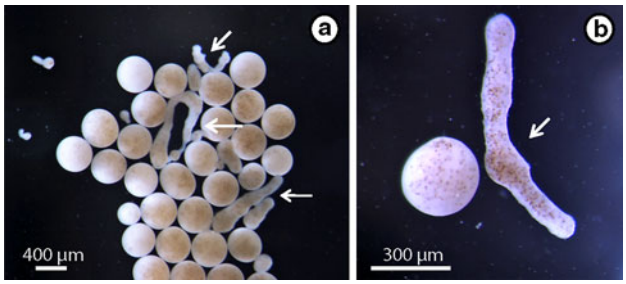

Fig. 3 Deformed oocytes released at the end of the spawning season (*arrows pointing* to the deformed eggs)

The interface between the oocytes and the mass of spermatozoa within the bundle was variable; sometimes oocytes were observed in close proximity with no bounding material between them (Fig. 5e), and sometimes a large amount of mucus separated the oocytes and spermatozoa (Fig. 5f, g). Entry of spermatozoa nuclei into the oocyte or fusion of plasma membranes was never observed.

The oocytes contained numerous lipid droplets, several types of cortical and yolk granules varying in structure, shape, and electron density, and *Symbiodinium* cells (Fig. 6). The cortical granules had, for the most part, an ovoid shape and were homogeneous and membrane bound (Fig. 6a–b). Yolk granules were larger than cortical granules and had higher irregularity in their shapes (Fig. 6c–f). Mitochondria, lipid-like inclusions, Golgi elements, and both membrane- and non-membrane-bound fibrous bodies were commonly present in the oocytes. Mitochondria were dispersed throughout the oocyte but were most common

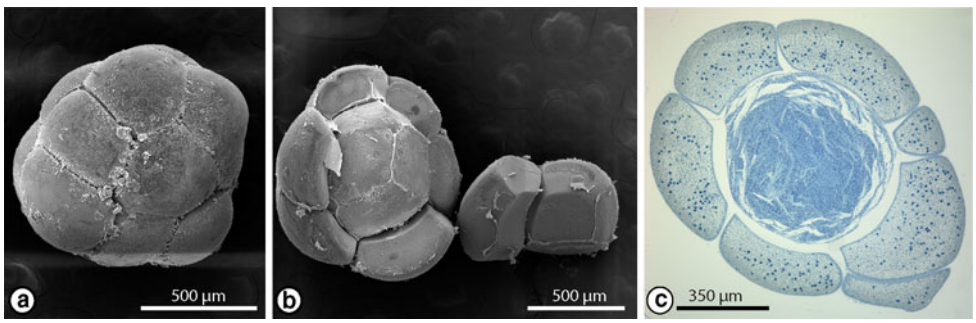

Fig. 4 Microstructure in *M. capitata* egg–sperm bundles, **a** Scanning electron micrograph of egg–sperm bundle, **b** SEM of broken egg–sperm bundle, "*sperm cluster*" visible at the *center* **c** Light micrograph of cross section of egg–sperm bundle, *darker dots* inside the oocytes are *Symbiodinium* cells

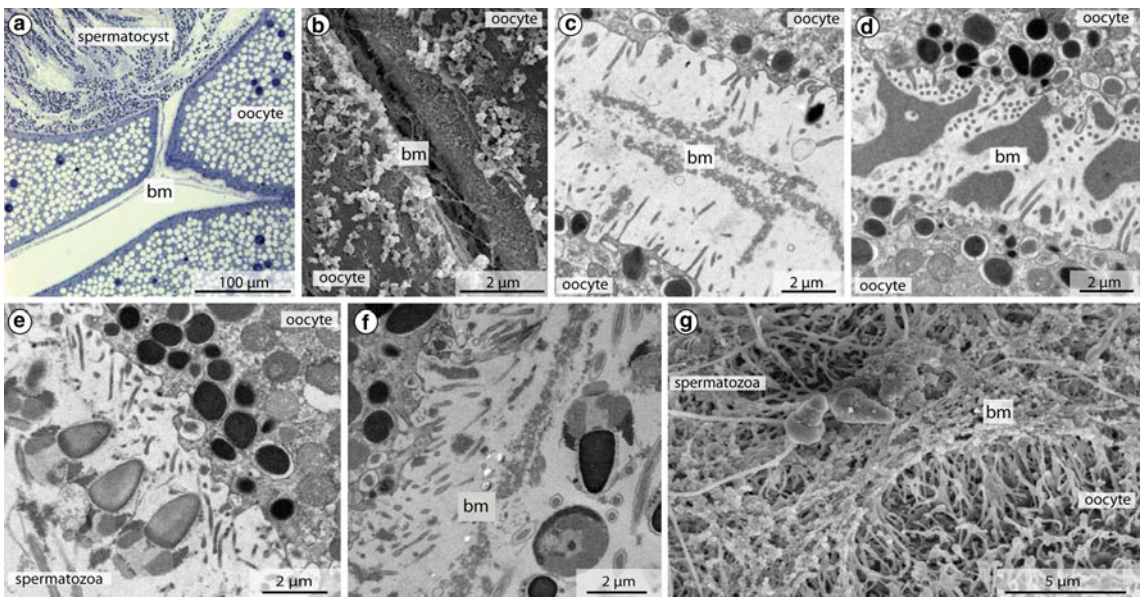

Fig. 5 Bundle material in the egg–sperm bundle, **a** Light micrograph of bundle material (*bm*) holding together the oocytes in the egg–sperm bundle, **b** SEM of bm on the eggs of the egg–sperm bundle, **c–d** TEM of bm possibly secreted by the oocytes to form the egg–sperm bundle. TEM of egg–sperm interface, **e** no mucus between gametes, **f** large amount of mucus separating oocytes and spermatozoa, **g** SEM of egg–sperm interface separated by bundle material

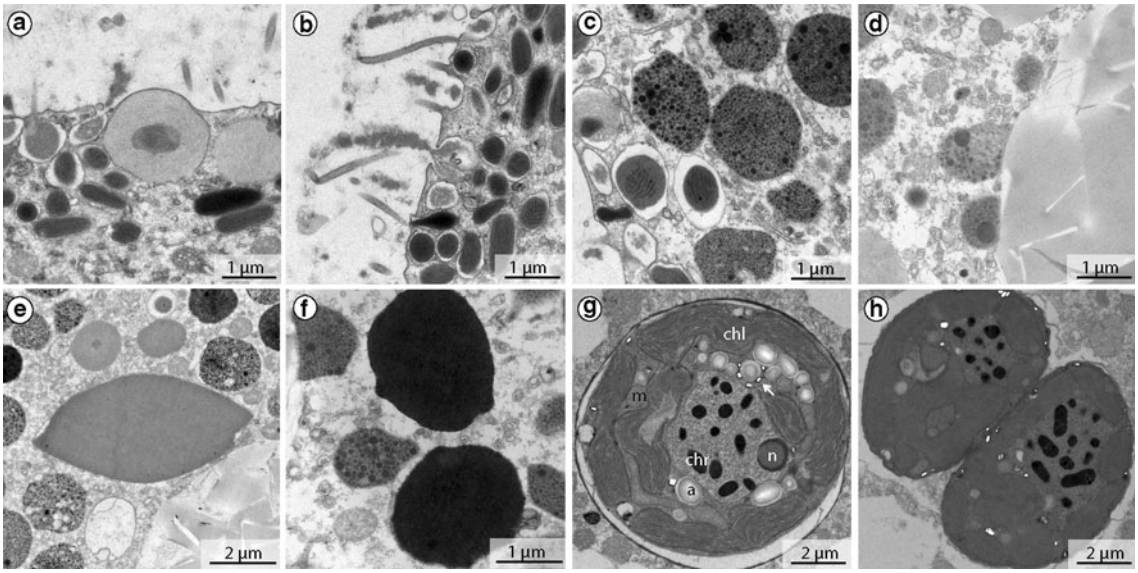

Fig. 6 TEM of components present inside the oocytes. **a–b** cortical granules with *ovoid* shape, membrane bound, and homogeneous, **c–f** several types of cortical granules varying in structure shape and electron density, membrane-, and non-membrane-bound fibrous bodies were regularly present, **g** *Symbiodinium* cell in the oocyte, *a* accumulation bodies, *chl* chloroplast, *chr* chromosomes, *m* mitochondria, *n* nucleolus, *arrow pointing* at the uric acids, **h** Some *Symbiodinium* cells were dividing

near the *Symbiodinium* cells. Germinal vesicles were not observed in the oocytes before or after the bundle broke apart. *Symbiodinium* cells were surrounded by the symbiosome membrane complex (Fig. 6g) and distributed throughout the oocyte (Fig. 4c), with some *Symbiodinium* cells dividing (Fig. 6h). Our observations of the *Symbiodinium* cells in the oocytes showed a good correspondence with previously published descriptions of this symbiont in the adult coral stage (Blank 1987); the multi-lobed chloroplasts were peripherally located, and their lamellae appeared as bands traversing their entire length (Fig. 6g–h). The nucleus contained a nucleolus, and chromosomes were widely distributed in the nucleoplasm (Fig. 6g). The *Symbiodinium* also contained mitochondria, accumulation bodies and randomly distributed uric acid deposits (Yamashita et al. 2009).

The microvilli and cortical layer of the oocytes were different before and after the egg–sperm bundles broke (Fig. 7). When contained in the egg–sperm bundle, the microvilli were thick and close together (Fig. 7a) and the cortical granular layer contained smaller granules (approximately 860 nm) at the periphery, and larger granules toward the center of the oocyte (Fig. 7b). Once released from the bundle and rounded, the microvilli were thinner and more spaced out (Fig. 7c), and the granules at the periphery of the oocyte were larger and had a different shape and electron density (Fig. 7d).

The spermatozoa mass occupied approximately 20% of the total egg–sperm bundle volume and was held together in spherical shape with a mucous coating similar to the one found

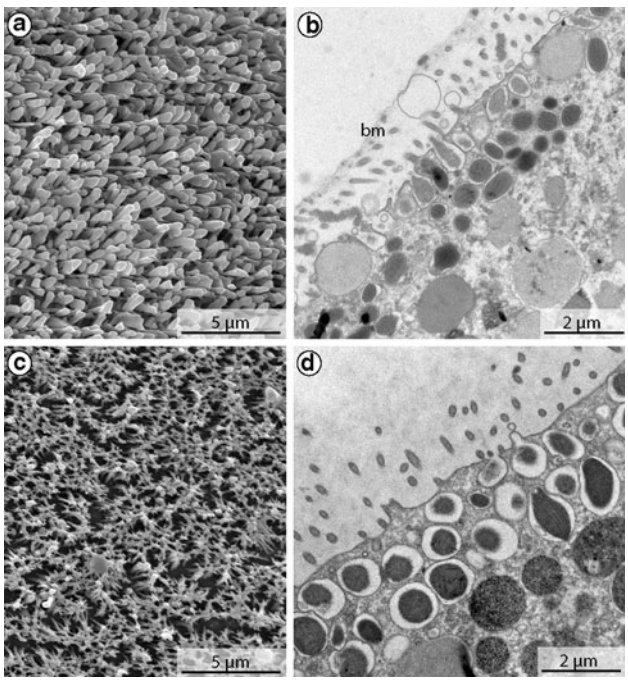

Fig. 7 Microvilli and cortical layer of the oocytes before and after egg–sperm bundle breakage. **a** SEM of microvilli and **b** TEM of cortical layer before bundle breakage when oocytes still have an *irregular shape* and some bundle material (*bm*) is still present, **c** SEM of microvilli and **d** TEM cortical layer after oocytes have been released from the bundle and became *rounded*

on the surface of the bundle and between oocytes (Fig. 8a–c). The spermatozoa had no particular orientation (Fig. 8d–e), and a mucous web was present within the mass (Fig. 8f–h).

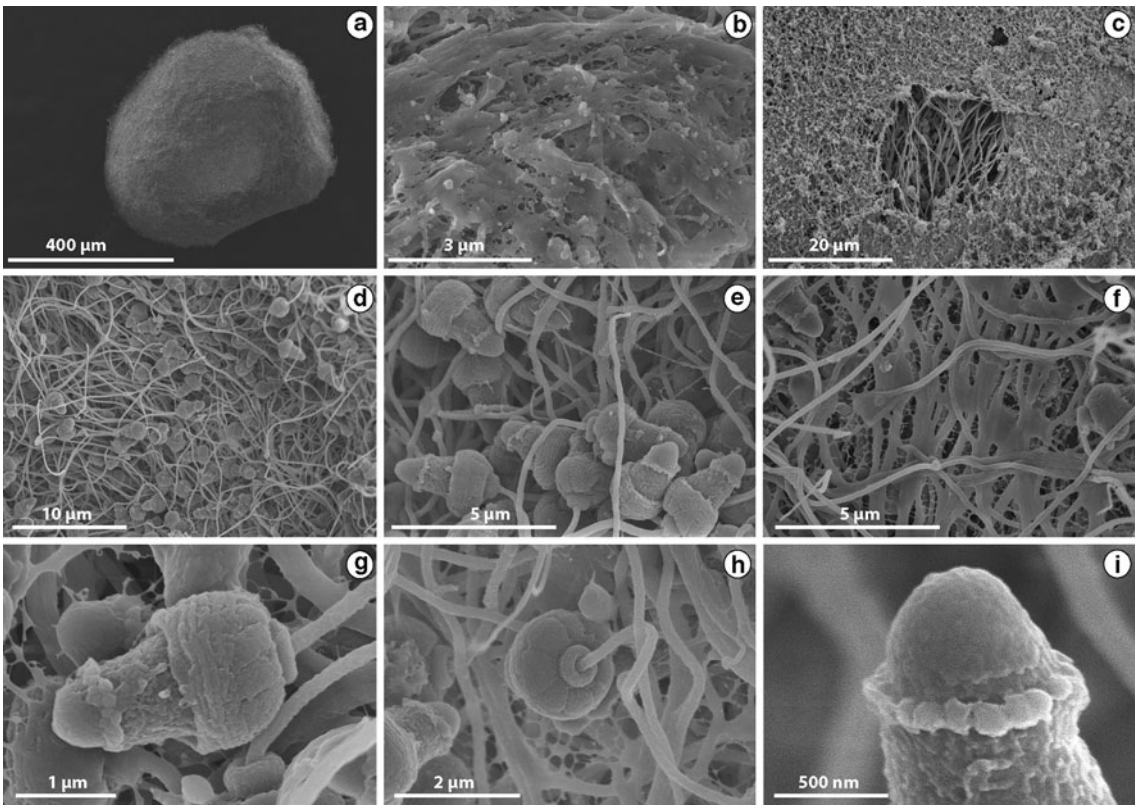

Fig. 8 SEM of sperm bundle (spermatozoa mass). **a** Sperm clump isolated from an egg–sperm bundle, **b** "*glue material*" surrounding the sperm clump, **c** hole in the sperm clump, spermatozoa are visible, **d–e** spermatozoa with no particular orientation, **f** "*sperm net*" material possibly holding together the spermatozoa inside the sperm clump, **g-i** spermatozoa *close up*

Montipora capitata had ovoid ect-aqua sperm (fertilize externally in contact with water, Rouse and Jamieson 1987) that comprised an approximately 1.6-µm head and 1.3-µm midpiece. The nucleus was bullet shaped and contained a zone of electron dense material at the top (Figs. 8, 9). The anterior part of the nucleus was surrounded by small vesicles (Figs. 8i, 9a–b), and the anterior and lateral regions of the midpiece were occupied by lamellae layers stacked in parallel arrays (Fig. 9a–c). The midpiece also contained aggregated mitochondria and a large lipid body on one side (Fig. 9a–b, d). The intracentriolar ligament was found at the center of the midpiece. The cytoplasmic collar was at the base of the spermatozoa and surrounded the anterior portion of the flagellum (Figs. 8h, 9b). The flagellum exhibited the usual 9 + 2 arrangement of microtubules, and the flagellar membrane expanded laterally (Fig. 9e–f). In some instances, spermatocytes (early stage sperm) were observed in bundles collected at the beginning of the reproductive season (June). The only detectable structures at this early stage were the nucleus, mitochondria, and lamellate bodies (Fig. 9g–h).

Discussion

The longevity of an egg–sperm bundle is limited. It is formed a few hours before spawning (Wallace 1985) and breaks apart in less than 10–40 min after it has been released from the polyp. Although it has a short life, this bundle carries out the important function of transporting gametes to the surface and maximizing the chances of encounter between gametes with very different buoyancies (i.e., sperm is denser and sinks, whereas oocytes are generally positively buoyant). This strategy increases sperm availability and facilitates outcrossing (Harrison and Wallace 1990; Richmond 1997).

This work is the first systematic study of the ultrastructure of the egg–sperm bundles in scleractinian corals and presents important new observations to understanding bundle structure and formation. A layer of mucus is present around the oocytes and within the egg–sperm interface, and this material appears to be forming and holding the egg–sperm bundle together for ejection from the polyp coral. TEM observations revealed that there is a significant

discharge of granule content from the oocytes, and this material has very similar electron density to the mucous layer, suggesting that bundle formation is achieved, at least in part, by the excretion of oocyte material.

The release of cortical material from oocytes in response to seawater has been observed in other invertebrates such as the polychaete *Sabellaria vulgaris* (Waterman 1936) and the crustacean *Penaeus aztecus* (Clark et al. 1980). In *S. vulgaris*, the newly shed eggs have a very irregular shape due to mechanical pressure in the coelomic cavities. After exposure to seawater, the egg membrane undergoes physical alteration, and the eggs become spherical. During this period, fertilization is limited. In *P. aztecus,* the contact of eggs with seawater results in a dramatic and massive release of a jelly precursor material from the cortical crypts (Clark et al. 1980). The jelly precursor material is made of 25–30% carbohydrate and 70–75% protein (Lynn and Clark 1987) and is believed to be responsible for the prevention of polyspermy and establishing a microenvironment inside the oocyte suitable for embryo development (Clark et al. 1980).

From our data, it appears that the mucous material in *M. capitata* is secreted by the oocytes in response to seawater contact (as they near the oral area prior to spawning). This secretion facilitates oocyte adhesion during the bundle formation. The hypothesized process of bundle formation of *M. capitata* is shown diagrammatically in Fig. 10I. Male and female gametes develop along the 8 mesenteries, with two male alternating with two female mesenteries (Heyward 1986). Between 3 and 5, oocytes (Heyward 1986) are lined up vertically along the mesenteries (Fig. 10a, I. In *M. capitata* (a perforate coral), most of the oocyte and spermatozoa development occurs deep in the skeleton. Hours before spawning, the polyps elongate and gather the gametes near the oral disk (Heyward 1986). During this time, the seawater flux inside the gastrodermal cavity may increase and induce the granule secretion of the cortical layer and facilitate oocyte adhesion (Fig. 10b–d, I). As the oocytes move toward the distended oral disc, the secretion continues and the bundle forms (Fig. 10e, I). Most polyps of *M. capitata* release one egg–sperm bundle per polyp in a single evening, though on rare occasions two egg–sperm bundles can be released (Babcock and Heyward 1986; Stanton 1992). Releasing two smaller bundles instead of one may be a good strategy if the gamete material is too large to fit through the polyp's mouth (Stanton 1992). For corals in which oocytes and spermatozoa develop on the same mesentery, the process of bundle formation could result in an egg–sperm bundle in which oocytes and spermatozoa are intermingled (Fig. 10II), such as the coral *Favites abdita* (Kojis and Quinn 1982).

With the mucus being secreted by the oocytes, the energy investment from the coral polyp is minimized.

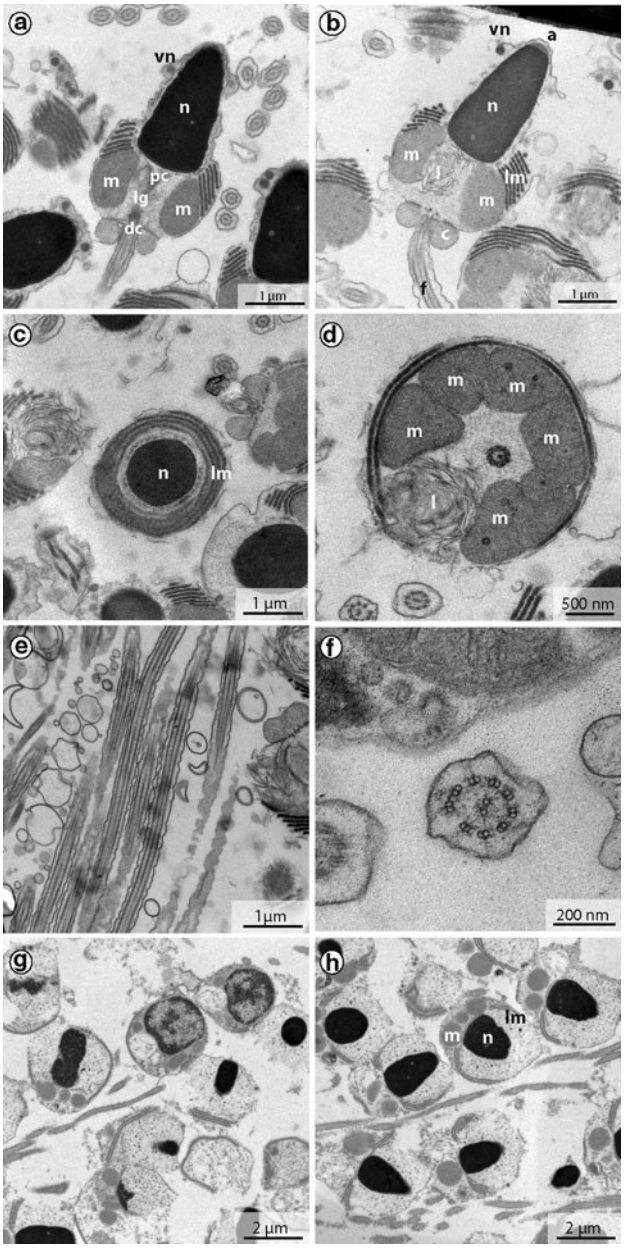

Fig. 9 TEM of *M. capitata* spermatozoa structure, **a**, **b** *a* anterior less dense nuclear zone, *c* collar, *dc* distal centriole, *l* lipid body, *lg* intercentriolar ligament, *lm* lamellae, *m* mitochondria, *n* nucleus, *pc* proximal centriole, *vn* vesicle apically of nucleus. **c** Spermatozoa cross section, frontal plane, **d** spermatozoa cross section, caudal plane, **e** spermatozoa tails with membrane oblique section, **f** Axonemes (9 + 2 arrangement of microtubules), and **g**–**h** spermatocytes (early stage sperm) released in the egg–sperm bundle during the beginning of spawning season

More complex structures such as membranes (composed of phospholipids and proteins) would be both more difficult to produce in a short period of time and more energetically costly (Vance 2002; Voelker 2002). SEM observations of the mucous layer in the bundle (Figs. 5, 8) resemble SEM observations of the mucous floc and web material produced

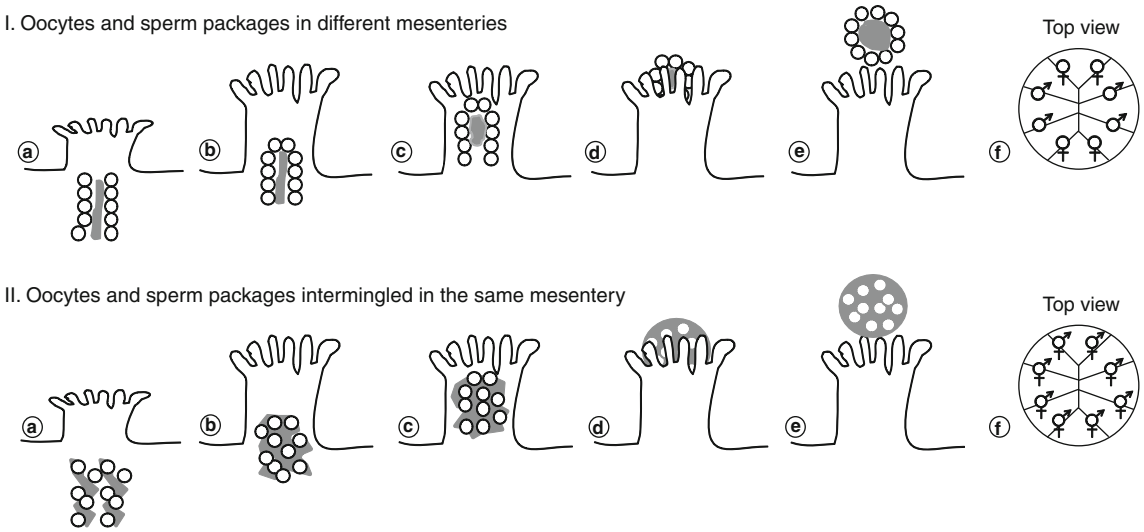

Fig. 10 Proposed models of egg–sperm bundle formation. *I* Model if oocytes and sperm packages develop in different mesenteries. *II* Model if oocytes and sperm packages develop intermingled in the same mesentery. Top views show the gametes on the mesenteries prior to initiation of bundle formation

by the coral *Porites astreoides* (Ducklow and Mitchell 1979). Coral mucus is mostly composed of carbohydrates (Coffroth 1990) and to a lesser degree glycoproteins (Krupp 1985; Vacelet and Thomassin 1991) and lipids (Benson and Muscatine 1974; Crossland et al. 1980). Further studies of the structure and macromolecular composition in the bundle mucus layer are necessary.

TEM observations of the egg–sperm interface showed areas where mucus separated the oocytes and spermatozoa and other areas where this layer was minimal or otherwise not visualized. Regardless of the presence of mucus, no fusion of gametes was observed within the bundles. This is consistent with previous research that has found that self-fertilization is very uncommon in *M. capitata* (Hodgson 1988; Maté et al. 1997). It is also possible that, while inside the egg–sperm bundle, the oocytes possess biochemical blocks to self- or total fertilization or that cortical rearrangement and acquisition of spherical shape of the oocyte must occur before fertilization can take place (by which time spermatozoa have been released and dispersed through water currents to other colonies). Hodgson (1988) found evidence that oocytes of *M. capitata* do not self- or cross- fertilize for at least 1 min after the bundle has broken open, and self-fertilization blocks have been reported to last 3 h or more in *Favia pallida, Platygyra pini*, *and P. ryukyensis* (Heyward and Babcock 1986).

Most egg–sperm bundles broke open within 30 min after release, which is approximately the time that the spawning events lasted. Agitation of water or exposure to higher temperatures accelerated the bundle breakage. However, faster breakage does not necessarily mean more or faster fertilization. On the contrary, when the egg–sperm bundles broke quickly (i.e., by higher wave turbulence), the spermatozoa mass separated from the oocytes and sank before the spermatozoa were released. Sinking of the sperm mass thus likely reduced the likelihood of these sperm encountering oocytes because oocytes are positively buoyant and remain on the surface, while the spermatozoa sink in the water column and became diluted at depth. Thus, environmental conditions during spawning events have the potential to significantly influence egg–sperm bundle breakage and fertilization rates and may diminish or promote local reproductive success.

The release of deformed oocytes toward the end of the spawning season (August) did not coincide with unusual environmental conditions, and colonies releasing deformed oocytes were found adjacent to colonies that produced regular egg–sperm bundles. These observations raise a number of interesting questions regarding the abiotic and biotic conditions leading to the production of deformed oocytes, whether or not the deformed oocytes are viable, and the selective value of releasing deformed oocytes rather than reabsorbing them. Reabsorption of unspawned oocytes has been described for brooding (Rinkevich and Loya 1979) and spawning (Neves and Pires 2002) corals and could be an important means of conserving nutrients. If the deformed oocytes result from stress, releasing deformed oocytes could be either a response with beneficial value (i.e., release of a toxin via the gametes), or an indication of impaired reproductive capacity. Failure of bundle formation and release of prematurely aborted eggs were observed in *Leptoria phrygia* in response to stress

(Kojis and Quinn 1982). Elucidating the factors leading to the failure of bundle formation and production of deformed eggs and their occurrence in nature could prove to be an important component of understanding the dynamics of coral populations.

The formation of an egg–sperm bundle and synchronicity of spawning are critical aspects for the reproductive success of broadcast spawners, which represent the majority of coral species (Fadlallah 1983; Harrison and Wallace 1990; Baird et al. 2009). These two aspects increase the abundance of female and male gametes in seawater and increase the chances of outcrossing. The selective advantage of the egg–sperm bundle in corals is unequivocal and is exemplified by its occurrence in many species of corals from both the Robust and Complex clades (Kerr 2005). However, it is unclear if the egg–sperm bundle has been acquired or lost several times throughout evolution. Bundles from the two coral clades show differences in structure, with bundles of Acroporids (clade complexa) having the oocytes around the sperm package, whereas bundles of *Favites* sp. (clade robusta) have the oocytes embedded within the sperm cluster (Kojis and Quinn 1982; Wallace 1985; Harrison and Wallace 1990; Richmond 1997). Interestingly, in both clades, the oocytes released in the bundle do not have a visible germinal vesicle (Kojis and Quinn 1982, this study), and it is unknown if this observation is associated with the process of bundle formation and/or the release of cortical material in response to seawater. Future studies on bundle structure of both clades may provide insights into different mechanisms of bundle formation and evolution of reproductive strategies in corals.

Little is published on the egg–sperm bundle due to its ephemeral lifespan. However, the failure of bundle formation could have detrimental effects on the reproduction and success of coral populations. As climate change and water quality deterioration (due to anthropogenic effects) continue, environmental cues for spawning may change and impact the different steps of the reproductive cycle. Bundle formation and breakage are critical components that can be strongly influenced by the environment and thus require further investigation.

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