The Journal of Experimental Biology 209, 4574-4579
Published by The Company of Biologists 2006
doi:10.1242/jeb.02500

Eggs regulate sperm flagellar motility initiation, chemotaxis and inhibition in the coral *Acropora digitifera*, *A. gemmifera* and *A. tenuis*

Masaya Morita[1,2,*], Akira Nishikawa[2], Ayako Nakajima[3], Akira Iguchi[4], Kazuhiko Sakai[2], Akihiro Takemura[2] and Makoto Okuno[3]

[1]*Department of Chemistry, Biology and Marine Sciences, University of the Ryukyus, 1 Senbaru, Nishihara, Okinawa 903-0213, Japan,* [2]*Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, 3422 Sesoko, Motobu, Okinawa 907-0227, Japan,* [3]*Department of Life Sciences, Graduate School of Arts and Sciences, University of Tokyo, Meguro-ku, Tokyo 153-8902, Japan and* [4]*Comparative Genomics Centre, Molecular Science Building, James Cook University, Townsville, Queensland 4811, Australia*

*Author for correspondence (e-mail: h063107@sci.u-ryukyu.ac.jp)

Accepted 17 August 2006

Summary

Corals perform simultaneous mass spawning around the full moon. Most *Acropora* species release gamete bundles, which are a complex of eggs and sperm, into the seawater. Then, gamete bundles are separated into eggs and sperm. Eggs are fertilized when sperm and eggs come in contact with each other. However, it is still unclear how sperm meet the eggs of the same species in the presence of many eggs of different species and how eggs guard against the fertilization attempts by sperm of different species. In this study, we observed that *A. digitifera*, *A. gemmifera* and *A. tenuis* sperm showed motility initiation/attraction close to eggs. Sperm were completely immotile in seawater, but they began to swim in circular motion when they came in close proximity to eggs, and then approached the eggs in straightforward paths. Sperm flagellar motility was not activated by an egg from different species, suggesting that motility initiation by the egg is species specific. In addition, hybridization among these species did not occur under observed conditions. Furthermore, motility-activated sperm became quiescent when many sperm approached the eggs. This study is the first report to show that the egg secretes immobilization factor(s). Our results suggest that the flagellar motility regulation has evolved to avoid hybridization among different species during the mass spawning.

Key words: coral, *Acropora*, mass spawning, sperm, chemotaxis, flagellar motility.

Introduction

Sperm motility is important for fertilization. Sperm flagellar motility is regulated by several mechanisms through a motility apparatus, the axoneme, which is highly conserved among eukaryotic flagella. Sperm flagellar motility of several animals such as ascidians and clupeids is not initiated before they come in close contact to eggs (Morisawa, 1994). The importance of the mechanism could be an increase of fertilization efficiency. Corals spawn simultaneously around the night of the full moon in a phenomenon known as 'simultaneous mass spawning' (Harrison et al., 1984; Babcock et al., 1986; Hayashibara et al., 1993). Most species release gamete bundles, which is a complex of sperm and eggs, into the seawater (Harrison and Wallece, 1990). The bundles dissipate sperm and eggs to float separately on the surface of the water where fertilization occurs. There are sperm and eggs from many species in seawater during mass spawning. Thus, sperm and eggs from the same species must find each other for efficient fertilization in the presence of sperm and eggs from many species. We hypothesized that chemotaxis might be involved. It is possible that sperm/egg have a mechanism(s), which increases fertilization through regulation of sperm flagellar motility in order to reach the egg. Within synchronously spawning species of *Acropora*, rates of intraspecific fertilization in experimental crosses are high (often >90%) compared with the rates of fertilization in many interspecific crosses (Willis et al., 1997; Hatta et al., 1999). It has been suggested in *Montipora digitata* that interspecific fertilization is reduced by a lack of the sperm attractant in eggs (Coll et al., 1994). *Acropora* species may also have regulatory mechanisms of sperm flagellar motility to increase fertilization within the same species.

In ascidians, sea urchins and clupeids, eggs secrete a sperm-activating and -attracting factor to initiate sperm flagellar motility and attract sperm. The substances, which initiate and attract sperm motility, can range from steroids to peptides (Coll et al., 1994; Oda et al., 1998; Yoshida et al., 2002). In ascidians, asymmetric flagellar beating that results in a circular trajectory increases with sperm-activating and attracting factor (SAAF).

Sperm swim towards the eggs, responding to chemoattractant (Yoshida et al., 1993). In addition, chemotaxis by SAAF is induced by an increase in $[Ca^{2+}]_i$, and this involves regulation of the symmetrical beating of flagella (Yoshida et al., 2003). Ca^{2+}/calmodulin is associated with the symmetrical flagellar beating as a result of an increase in $[Ca^{2+}]_i$ in sea urchin sperm (Brokaw and Nagayama, 1985). Navigation mechanisms may play an important role in increasing fertilization success.

Sperm from many species of corals are present during mass spawning. If chemoattractant(s) is not species-specific, sperm from different species approach the egg. In addition, polyspermy, which inhibits embryo development (Oliver and Babcock, 1992), might occur if eggs do not stop attracting sperm after fertilization or in response to attachment of many sperm. Therefore, eggs should be able to regulate sperm motility in a species–specific manner and have a mechanism to prevent polyspermy. In this study, we identified that sperm of *Acropora* have navigation mechanisms and motility inhibition mechanisms to prevent polyspermy. It is probable that sperm motility regulatory mechanisms are suited to the spawning phenomenon of *Acropora*.

Materials and methods

Study species: Acropora digitifera, A. tenuis and A. gemmifera

Acropora digitifera L., *A. tenuis* L. and *A. gemmifera* L. are widely distributed around Okinawa Island, Japan (26°50′46″N, 128°17′25″E). These three species perform mass spawning and do not hybridize among themselves. Colonies of *Acropora digitifera*, *A. tenuis* and *A. gemmifera* were collected at Oku fishery port in Okinawa Island. These colonies were kept in a running seawater tank under natural light conditions. These colonies were returned to their natural habitat and glued to the substratum.

Collection of sperm and eggs

Coral spawning took place in June [3rd, 7th, 8th, 10th, 14th, 22nd (full moon), 25th and 27th]. Colonies were transferred into individual buckets until gamete bundles in the polyps were confirmed. Gamete bundles from the colony were collected after they were released. The following experiments were conducted at about 28°C. Gamete bundles were washed with filtered seawater and split into eggs and sperm using a plankton net (diameter 100 μm). Eggs were washed 10 times with filtered seawater in order to wash sperm away from eggs. Detachment of sperm from washed eggs was confirmed by microscopic observation. Sperm concentration was determined using a Thoma counting chamber (Kayagaki Iruka Kogyo, Tokyo, Japan). Sperm were stored at room temperature at $1.0\times10^{7-9}$ cells ml^{-1}. Sperm and eggs were used for experiments within 5 h after collecting.

Solutions

Solutions used to test sperm motility were as follows: artificial seawater (ASW: 430 mmol l^{-1} NaCl, 10 mmol l^{-1} $CaCl_2$, 9 mmol l^{-1} KCl, 23 mmol l^{-1} $MgCl_2$, 25 mmol l^{-1} $MgSO_4$ and 10 mmol l^{-1} Hepes-NaOH, pH 8.2), and Na-free ASW (430 mmol l^{-1} choline chloride, 9 mmol l^{-1} KCl, 23 mmol l^{-1} $MgCl_2$, 25 mmol l^{-1} $MgSO_4$, 10 mmol l^{-1} $CaCl_2$ and 10 mmol l^{-1} Hepes-KOH, pH 8.2), Na-free ASW containing 10 μmol l^{-1} Ca^{2+} ionophore A23187, or Ca^{2+}-chelated ASW (430 mmol l^{-1} choline chloride, 9 mmol l^{-1} KCl, 23 mmol l^{-1} $MgCl_2$, 25 mmol l^{-1} $MgSO_4$, 5 mmol l^{-1} EGTA and 10 mmol l^{-1} Hepes-KOH, pH 8.2) containing 10 μmol l^{-1} Ca^{2+} ionophore A23187. NH_4Cl (20 mmol l^{-1}) was added to each solution to increase intracellular pH.

Motility assessment

Sperm flagellar motility was recorded using a video recorder (SLV-LF1; Victor, Tokyo, Japan/DCR-TRV900; Sony, Tokyo, Japan) and a CCD camera (63W1N, MINTRON, Taiwan) mounted on a microscope equipped with a phase-contrast or dark-field condenser (Optiphoto; Nikon, Tokyo, Japan). Motility percentage was calculated from the video recordings. Sperm were counted as motile if they either exhibited progressive movement or spontaneous flagellar beating if the sperm head was attached to the glass slide. The trajectory of sperm motility was either recorded under phase-contrast illumination and movement of sperm heads traced by macros constructed in NIH Image as described by Kihara (Kihara, 1997), or under dark-field illumination by modulating the shutter speed of a CCD camera. The swimming speed was then calculated from the length of trajectory using NIH image.

Hybridization experiments

Thirty eggs were put into 100 ml of filtered seawater. Sperm were diluted with seawater to 1.1×10^{5}–1.2×10^{8} cells ml^{-1} to prevent polyspermy, according to Oliver and Babcock (Oliver and Babcock, 1992). Survival rates of eggs were calculated. Unfertilized eggs broke up the next day. During these experiments, room temperature was kept at 28°C.

Statistical analysis

Data were subjected to one-way ANOVA followed by Fisher's PLSD for multiple-group comparisons of motility within different solutions. Bonferroni's *post-hoc* test was used whenever any significant differences were observed between treatments.

Results and discussion

In this study, we observed sperm motility of the coral, *A. digitifera*, *A. gemmifera* and *A. tenuis*. In these species, spawning time is different among the species (Fukami et al., 2003). As described elsewhere (Fukami et al., 2003), *A. digitifera* and *A. gemmifera* spawned from 21:00 h to 22:00 h, and *A. tenuis* spawned from 19:00 h to 20:00 h. Similar to starfish sperm, sperm of these three species were completely immotile in seawater (Fig. 1A). Sperm of most of marine invertebrates are quiescent in seawater and begin to move in response to several signals, some of which are steroids or peptides from eggs (Yoshida et al., 2002; Morisawa, 1994). In

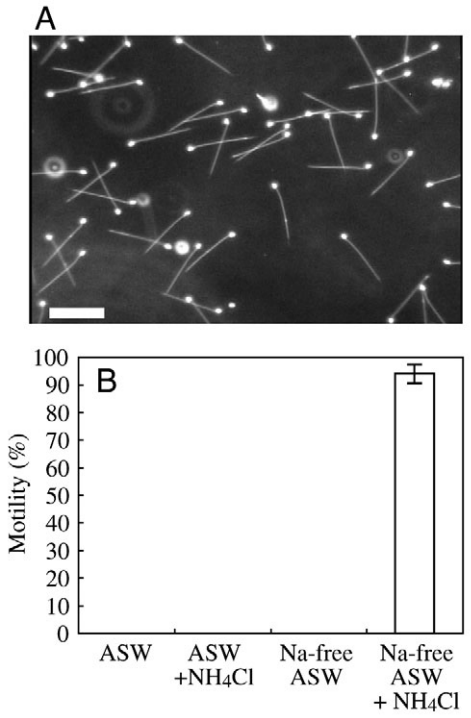

Fig. 1. Sperm motility initiation factor in *Acropora* species. (A) Sperm of *A. digitifera*, *A. gemmifera* and *A. tenuis* were completely quiescent even after sperm were split from bundles. Scale bar, 50 μm. (B) Sperm motility of *A. digitifera* in artificial seawater (ASW), ASW containing 20 mmol l^{-1} NH_4Cl, Na-free ASW, and Na-free ASW containing 20 mmol l^{-1} NH_4Cl. Other *Acropora* species also showed similar sperm motility. Values are mean ± s.d. *N*=5.

starfish sperm, an increase in intracellular pH ($[pH]_i$) induces motility activation through axonemal protein phosphorylation (Nakajima et al., 2005). When sperm were suspended in artificial seawater (ASW) containing NH_4Cl, which increases $[pH]_i$, sperm flagella bent in the proximal region but the bending motion did not propagate distally (to the tip of the flagellum), to create the cyclical sinusoidal bending motion. Instead, sperm showed vigorous motility when NaCl was replaced by choline chloride (Na-free ASW containing NH_4Cl) (Fig. 1B). Addition of a membrane-permeable cAMP, db-cAMP (50 μmol l^{-1}), did not initiate motility (data not shown), suggesting that an increase in $[pH]_i$, not an increase in $[cAMP]_i$, induces initiation of flagellar motility, and Na^+ antagonizes elevation of $[pH]_i$ even though NH_4Cl was added. It is likely that sperm flagellar motility of *Acropora* species is suppressed by Na^+ in seawater.

As shown above, sperm motility was completely suppressed in ASW containing large amount of Na^+. However, in the presence of eggs, sperm motility was initiated in ASW when sperm came to about 150–300 μm from the egg surface. At first, sperm swam in circular paths and then swam in straight paths when they approached the egg (indicated by arrows in Fig. 2A). In the absence of eggs, trajectories of sperm motility were circular (Fig. 2B). It is probable that *A. digitifera*, *A. tenuis* and *A. gemmifera* eggs secret substance(s) to initiate sperm motility *via* an increase in $[pH]_i$ even in the presence of Na^+ and modulate symmetrical flagellar beating to swim straight towards the eggs. Sea urchin sperm swim straight in the presence of sperm-activating peptide (SAP) isoform P15 (Shiba et al., 2006). These reactions were almost similar among three *Acropora* species. It is possible that each species should have species-specific mechanism(s) to initiate and attract sperm to choose appropriate eggs, which are not fertilized. *Acropora* eggs may secret species-specific substance(s) to activate and attract sperm.

Sperm flagellar motility was initiated by NH_4Cl treatment and sperm became quiescent within 1 min when they came close to eggs after many sperm had attached to the egg surface (Fig. 3A,B). In the absence of eggs, sperm showed motility for a longer period (Fig. 3B), suggesting that a secretion of motility inhibition substance(s) from eggs occurs when many sperm attach to the egg. In addition, quiescent sperm reinitiate their flagellar motility by a re-addition of NH_4Cl, suggesting that a decrease in $[pH]_i$ causes a suppression of sperm motility. It is possible that an inhibition of flagellar motility by eggs is reversible. Sperm motility and swimming velocity decreased quickly unlike those in the absence of an egg (Fig. 3B,C). However, swimming speed was faster in the first 10 s after dilution (Fig. 3C), implying that fertilization occurs within 10 s and the egg begins to secret motility suppressor(s). In ascidians,

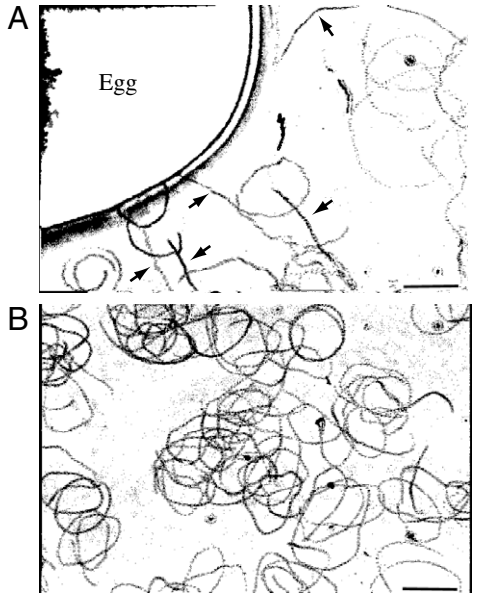

Fig. 2. Trajectory of swimming sperm with or without eggs. Sperm movements in *Acropora digitifera* were recorded. The movement of sperm heads was traced by NIH images and the trajectories drawn. (A) The trajectories of sperm heads in the presence of an egg in ASW. Arrows indicate straight direct paths, a chemotactic behaviour observed close to eggs. (B) The trajectories of sperm heads when no egg was present in Na-free ASW containing 20 mmol l^{-1} NH_4Cl. Other *Acropora* species, *A. gemmifera* and *A. tenuis* showed similar movement (data not shown). Scale bars, 50 μm.

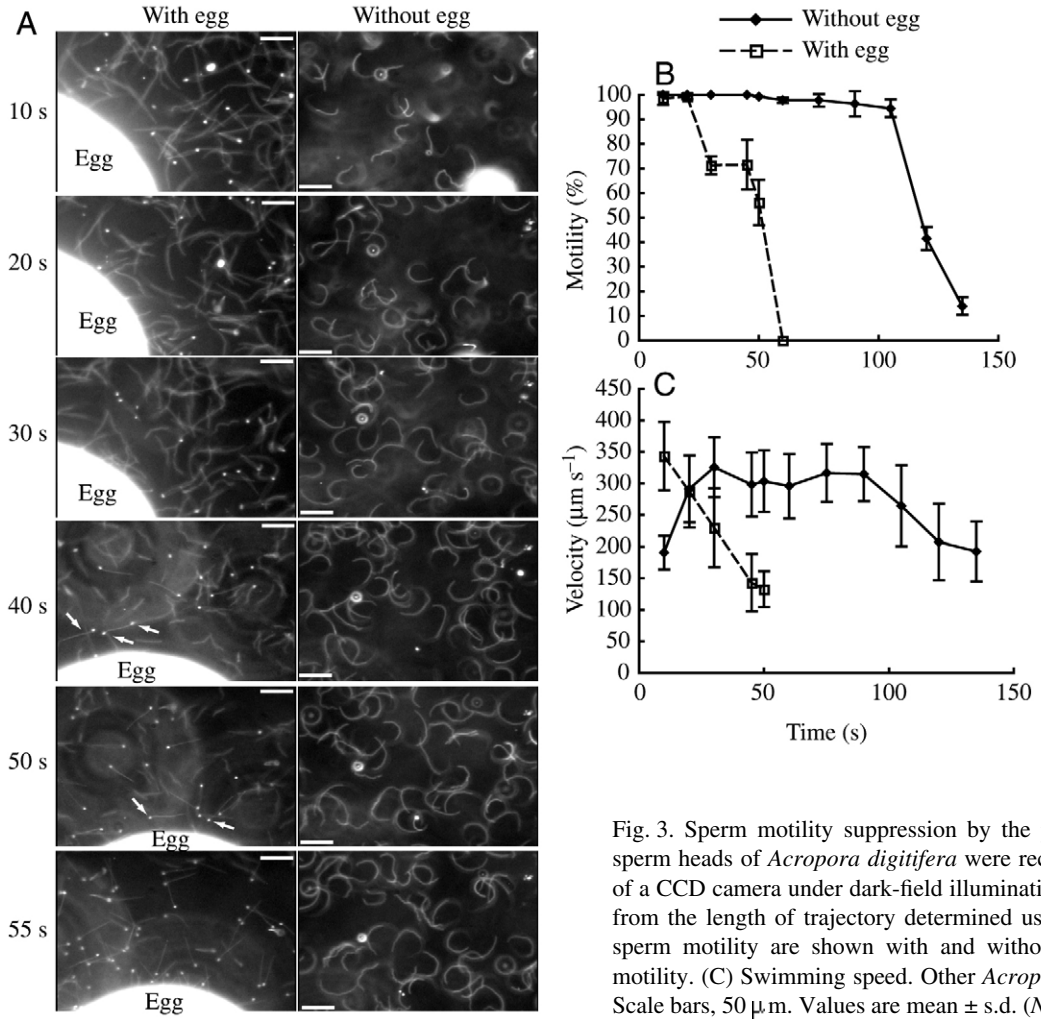

Fig. 3. Sperm motility suppression by the presence of an egg. Trajectories of sperm heads of *Acropora digitifera* were recorded by modulating the sensitivity of a CCD camera under dark-field illumination. Swimming speed was calculated from the length of trajectory determined using NIH Image. (A) Trajectories of sperm motility are shown with and without eggs. (B) Percentages of sperm motility. (C) Swimming speed. Other *Acropora* species showed similar patterns. Scale bars, 50 μm. Values are mean ± s.d. (*N*=150 sperm from three individuals).

sperm-activating and -attracting activities of the egg disappear when fertilization is completed (Yoshida et al., 1993). Therefore, swimming velocity could increase in the presence of eggs that are not fertilized. However, swimming velocity of sea urchin sperm is not changed in the presence of asterosap isoform p15 (Shiba et al., 2006). It is reasonable to assume that the egg secretes motility suppressor(s) to prevent polyspermy, which inhibits embryo development (Oliver and Babcock, 1992). One clue about the evolution of a sperm motility inhibition mechanisms is that eggs of coral do not have a fertilization membrane, which develops after fertilization to prevent sperm entry (Babcock and Heyward, 1986; Hayashibara et al., 1997). In corals, many different species spawn at once, and many eggs and sperm from other species are present during mass spawning. Mechanism(s) to restrict sperm motility may be useful to increase the fertilization success.

This study suggests that an elevation of $[pH]_i$ induces motility initiation and a decrease in $[pH]_i$ suppresses motility. However, chemotactic behaviour of sperm, swimming in a straight path, did not occur with the addition of NH_4Cl. In the ascidians *Ciona intestinalis* and *C. savignyi*, an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) induces an increase in asymmetrical flagellar beating (Yoshida et al., 1994; Yoshida et al., 2003). In contrast to ascidians, *Acropora* sperm swam straight when they approach the egg (Fig. 2A, Fig. 3A). It is possible that Ca^{2+} affects chemotactic behaviour of sperm. To elucidate the role(s) of Ca^{2+} in chemotaxis, the effect of Ca^{2+} on trajectory and swimming velocity was considered. In *A. digitifera*, sperm swam straight in a solution containing EGTA and the Ca^{2+} ionophore A23108 (which decreases $[Ca^{2+}]_i$) (Fig. 4A). By contrast, sperm swam in circles when extracellular Ca^{2+} and Ca^{2+} ionophore A23187 was present (Fig. 4B,C). Sperm swam slower in the presence of Ca^{2+} or Ca^{2+} and A23187 (Fig. 4D). Therefore, a reduction of $[Ca^{2+}]_i$ induces an increase in swimming velocity and symmetrical flagellar beating. It is likely that chemotaxis does not depend on a rise of $[pH]_i$ but regulation of $[Ca^{2+}]_i$.

From these results, it is possible to speculate that Ca^{2+} binds to Ca^{2+}-binding protein(s) to modulate symmetry of flagellar beating and swimming velocity. Ca^{2+}-binding protein, CaM, is known to regulate the asymmetry of flagellar beating in sea urchin sperm (Brokaw and Nagayama, 1985). In higher Ca^{2+} concentrations, sperm swim circular paths as a result of an

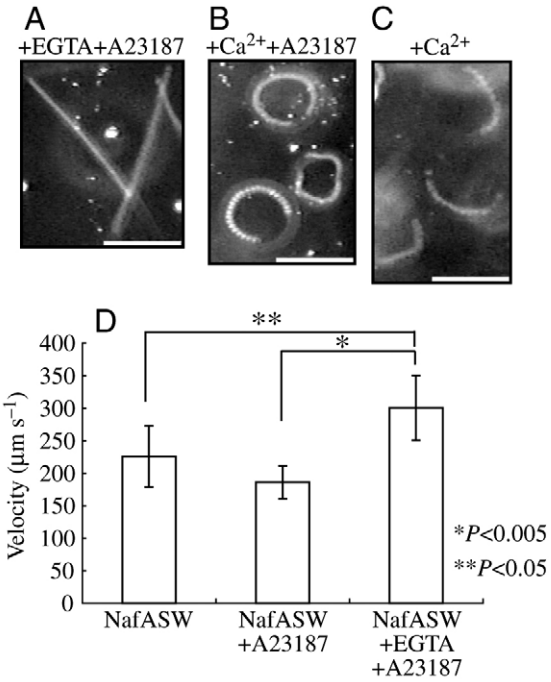

Fig. 4. Effect of Ca^{2+} on trajectories and swimming speed. Trajectories in the presence or absence of Ca^{2+} are shown. Sperm trajectories of *Acropora digitifera* in Na-free ASW containing (A) 5 mmol l^{-1} EGTA and 10 μmol l^{-1} of the Ca^{2+} ionophore A23187, (B) 10 mmol l^{-1} $CaCl_2$ and 10 μmol l^{-1} A23187, and (C) 10 mmol l^{-1} $CaCl_2$. (D) Swimming speed in each of the solutions. Other species showed similar patterns (data not shown). Values are mean ± s.d. (*N*=150 sperm from five individuals). Scale bars, 50 μm.

increase in asymmetry of flagellar beating through Ca^{2+}/CaM-dependent regulation. It is possible that this type of regulation exists in *Acropora*. However, further studies are required to identify the role of CaM on chemotactic behaviour in *Acropora* sperm.

There appears to be species specificity of initiation/chemotaxis of sperm among the three species. It is reported that the chemoattractants of *Montipora digitata* are diverse and the ratio of substances are important for chemotaxis of sperm (Coll et al., 1994). *Acropora* species may have species-specific substance(s), which induces sperm flagellar motility initiation, implying that fertilization among *A. digitifera*, *A. gemmifera* and *A. tenuis* could not occur as a result of the lack of sperm flagellar motility initiation by eggs from different species. Sperm of each *Acropora* species did not respond to eggs of the other two species, suggesting that a substance(s) from eggs that induces flagellar motility initiation is species specific. In mass spawning, eggs of different species should be present in the seawater. It is likely that sperm begin to move when they come in close to eggs of the same species. Therefore, hybridization among different species could be avoided and sperm and eggs of same species could undergo fertilization.

As shown above, sperm did not respond to eggs from other species in the present study, because motility initiation occurred in a species-specific manner. However, hybridization does occur among some *Acropora* species (Hatta et al., 1999). Hatta et al. (Hatta et al., 1999) described that 71.5±28.5% fertilization success was observed when sperm of *A. nobilis* and eggs of *A. florida* were mixed. It seems that eggs of *A. florida* activates *A. nobilis* sperm. On the other hand, fertilization did not occur when sperm of *A. digitifera* and eggs of *A. florida* were mixed (Hatta et al., 1999). Thus, it is possible that specificity of activation factor(s) from eggs is different among species. One possible reason why hybridization within the genus *Acropoa* occurs is that *Acropora* have possibly evolved in reticulate ways (Veron, 1995a). Some species could have the same activating substrate(s) and fertilization mechanism(s). Further studies are necessary to elucidate the importance of substances that initiate sperm motility and attract sperm during fertilization among different species.

To gain insight into this relationship, hybridization experiments among *A. digitifera*, *A. gemmifera* and *A. tenuis* were carried out. Motility initiation did not occur among these species. Hybridization among *A. digitifera*, *A. tenuis* and *A. gemmifera* did not completely occur. Therefore, it is possible that initiation of sperm flagellar motility in a species-specific manner plays an important role in fertilization. However, *A. nobilis* hybridizes with *A. florida* (Hatta et al., 1999), but it is unknown whether sperm of *A.*

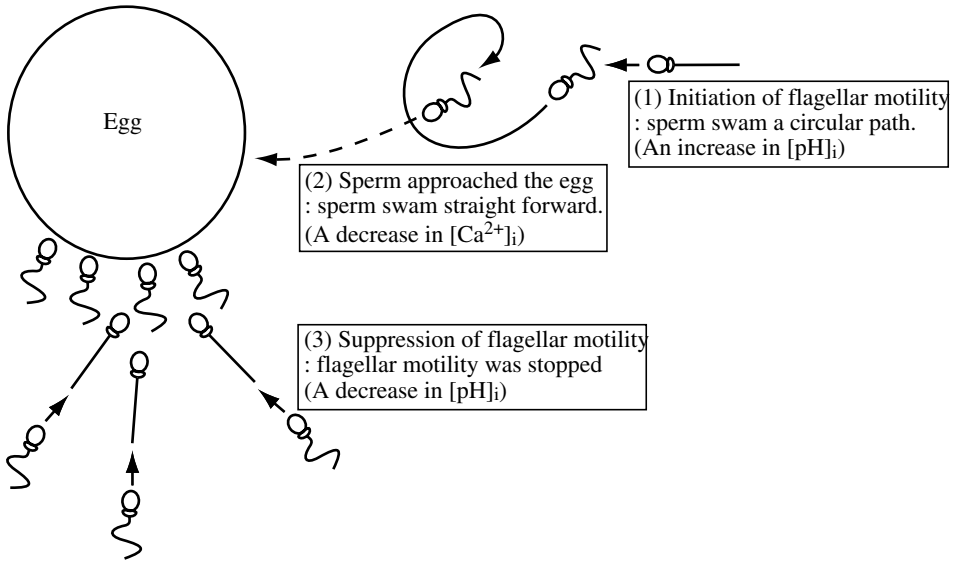

Fig. 5. Schema of flagellar motility regulation in sperm of *Acropora digitifera, A. gemmifera* and *A. tenuis.*

nobilis show initiation/chemotaxis toward eggs of *A. florida*. Further studies are necessary to identify the role of motility initiation and chemotaxis on hybridization among *Acropora* species.

In summary, in the corals, *A. digitifera*, *A. tenuis* and *A. gemmifera*, eggs suppressed sperm motility when many sperm approached (Fig. 5). This study is the first report to show that eggs inhibit sperm motility in response to sperm attachment. Eggs initiated sperm motility *via* an increase in $[pH]_i$ (Fig. 5). In addition, sperm approached eggs by modulating bending motion to swim straight, in a $[Ca^{2+}]_i$-dependent manner (Fig. 5). These phenomena could be induced by a secretion of substance(s) from eggs. Mass spawning occurs synchronously in many coral species. It has been reported that there are more than 150 *Acropora* species (Veron, 1995b). Since sperm must fertilize eggs in the presence of many different *Acropora* species, it is possible that sperm motility regulatory mechanisms could have been selected to increase fertilization success.

The authors want to express gratitude to Ms Ruth S. Pisingan, Ms S. Kai and Ms M. Nakamura (University of the Ryukyus) and Dr N. Aluru, Department of Biology, University of Waterloo for helpful advice on the manuscript. This study was partially supported by a grant for the 21st Century COE program 'The Comprehensive Analyses on Biodiversity in Coral Reef and Island Ecosystems in Asian and Pacific Regions' from the Ministry of Education, Culture, Sports, Science and Technology, Japan (Monbukagakusho) to M.M. and A.N., a Grant-in-Aid for Scientific research (B) (15405029) to A.T. and a Grant-in-Aid for Scientific research (B) (16310158) to K.S.

References

Babcock, R. C. and Heyward, A. J. (1986). Larval development of certain gamete-spawning scleractinian corals. *Coral Reefs* **5**, 111-116.

Babcock, R. C., Bull, G. D., Harrison, P. L., Heyward, A. J., Oliver, C. C., Wallace, C. C. and Willis. B. L. (1986). Synchronous spawning of 105 scleractinian coral species on the Great Barrier Reef. *Mar. Biol.* **90**, 379-394.

Brokaw, C. J. and Nagayama, S. M. (1985). Modulation of the asymmetry of sea urchin sperm flagellar bending by calmodulin. *J. Cell Biol.* **100**, 1875-1883.

Coll, J. C., Bowden, B. F., Meehan, G. V., Konig, G. M., Carroll, A. R., Tapiolas, D. M., Alin, P. M., Heaton, A., De Nys, R., Leone, P. A. et al. (1994). Chemical aspects of mass spawning in corals. I. Sperm-attractant molecules in the eggs of the scleractinian coral *Montipora digitata*. *Mar. Biol*. **118**, 177-182.

Fukami, H., Omori, M., Shimoike, K., Hayashibara, T. and Hatta, M. (2003). Ecological and genetic aspects of reproductive isolation by different spawning times in *Acropora* corals. *Mar. Biol*. **142**, 679-684.

Harrison, P. L. and Wallace, C. C. (1990). Reproduction, dispersal and recruitment of scleractinian corals. In *Ecosystems of the World: Coral Reefs* (ed. Z. Dubinsky), pp. 133-207. Amsterdam: Elsevier Science.

Harrison, P. L., Babcock, R. C., Bull, G. D., Oliver, J. K., Wallace, C. C. and Willis, B. L. (1984). Mass spawning in tropical reef corals. *Science* **223**, 1186-1189.

Hatta, M., Fukami, H., Wang, W., Omori, M., Shimoike, K., Hayashibara, T., Ina, Y. and Sugiyama, T. (1999). Reproductive and genetic evidence for a reticulate evolutionary history of mass-spawning corals. *Mol. Biol. Evol*. **16**, 1607-1613.

Hayashibara, T., Shimoike, K., Kimura, T., Hosoya, S., Heyward, A., Harrison, P., Kudo, K. and Omori, M. (1993). Patterns of coral spawning at Akajima Island, Okinawa, Japan. *Mar. Ecol. Prog. Ser.* **101**, 253-262.

Hayashibara, T., Ohike, S. and Kakinuma, Y. (1997). Embryonic and larval development and planula metamorphosis of four gamete-spawning *Acropora* (*Anthozoa*, *scleractinia*). *Proc. 8th. Int. Coral Reef Symp.* **2**, 1231-1236.

Kihara, A. (1997). Ugoki (keiji henka) no kaiseki. In *Gazoukaiseki Text* (ed. K. Kojima and Y. Okamoto), pp. 120-142. Tokyo: Yodosya. (In Japanese.)

Morisawa, M. (1994). Cell signaling mechanism for sperm motility. *Zool. Sci.* **11**, 647-662.

Nakajima, A., Morita, M., Takemura, A., Kamimura, S. and Okuno, M. (2005). Increase in intracellular pH induces phosphorylation of axonemal proteins for flagellar motility activation in starfish sperm. *J. Exp. Biol.* **208**, 4411-4418.

Oda, S., Igarashi, Y., Manaka, K., Koibuchi, N., Sakai-Sawada, M., Sakai, M., Morisawa, M., Ohtake, H. and Shimizu, N. (1998). Sperm-activating proteins obtained from the herring eggs are homologous to trypsin inhibitors and synchronized in follicle cells. *Dev. Biol.* **204**, 55-63.

Oliver, J. K. and Babcock, R. C. (1992). Aspects of the fertilization ecology of broadcast of spawning corals: sperm dilution effects and *in situ* measurements of fertilization. *Biol. Bull.* **183**, 409-417.

Shiba, K., Tagata, T., Ohmuro, J., Mogami, Y., Matsumoto, M., Hoshi, M. and Baba, S. (2006). Peptide-induced hyperactivation-like vigorous flagellar movement in starfish sperm. *Zygote* **14**, 23-32.

Veron, J. E. N. (1995a). *Corals in Space and Time: The Biogeography and Evolution of the Scleractinia*. Ithaca, London: Comstock/Cornell.

Veron, J. E. N. (1995b). *Corals of the World*. Townsville, Australia: Australian Institute of Marine Sciences.

Willis, B. L., Babcock, R. C., Harrison, P. L. and Wallace, C. C. (1997). Experimental hybridization and breeding incompatibilities within the mating systems of mass spawning corals. *Coral Reefs* **16**, S53-S65.

Yoshida, M., Inaba, K. and Morisawa, M. (1993). Sperm chemotaxis during the process of fertilization in the ascidians *Ciona savignyi* and *Ciona intestinalis*. *Dev. Biol.* **157**, 497-506.

Yoshida, M., Inaba, K., Ishida, K. and Morisawa, M. (1994). Calcium and cyclic AMP mediate sperm activation, but Ca^{2+} alone contributes sperm chemotaxis in the ascidian, *Ciona savignyi*. *Dev. Growth Differ.* **36**, 583-595.

Yoshida, M., Murata, M., Inaba, K. and Morisawa, M. (2002). A chemoattractant for ascidian spermatozoa is a sulfated steroid *Proc. Natl. Acad. Sci. USA* **99**, 14831-14836.

Yoshida, M., Ishikawa, M., Izumi, H., Santis, R. and Morisawa, M. (2003). Store-operated calcium channel regulates the chemotactic behavior of ascidian sperm. *Proc. Natl. Acad. Sci. USA* **100**, 149-154.