Biol. Cell (1981), **40**, 119-128

Sperm Chemotaxis in Siphonophores

Danièle CARRÉ, Christian SARDET

Station Zoologique et Groupe de Biologie Marine du CEA, Station Marine de Villefranche sur Mer 06230, France.

In all siphonophores, planktonic Cnidarians with external fertilization, spermatozoa are attracted by eggs in a species-specific manner. The attractant is located in an extracellular organelle called a cupule which is secreted by the egg at its pole of maturation during oogenesis. Isolated cupules attract spermatozoa whereas eggs deprived of the cupule do not. In all species examined, the cupule is made of large globular elements arranged in regularly packed filaments that dissolve slowly in sea water. The cupule is composed of 2-3 very high molecular weight proteins (probably constituting a matrix) and a diffusing substance of lower molecular weight: the chemoattractant. The chemotactic agent from *Muggiaea kochi* migrates on non denaturing polyacrylamide gradient microgels with a mobility similar to that of bovine serum albumin, as demonstrated by sperm attraction towards this zone of the microgels. The attractant is remarkably heat stable and resists protease treatment and alcohol extraction. The chemotactic agent alters the trajectories of spermatozoa but not their speed. The trajectories which are normally slightly curved become small circles in the vicinity of the cupule. This ability to describe small circles is abolished by removal of calcium from the medium. However, the chemotactic behavior is restored, when 5-10 mM calcium is added. The ultrastructure of the siphonophore spermatozoa which is characterized by a striated rod, probably made of actin, that extends from the centriole to the tip of the sperm head, is not altered during chemotaxis. Independently of the attraction, spermatozoa undergo an acrosomal reaction at the surface of the egg. The biological role of attraction is probably to concentrate the dilute sperm to the vicinity of a specialized area at the maturation pole of the egg where fertilization always takes place.

Key-words: *Sperm - Chemotaxis - Cnidaria - Siphonophora.*

INTRODUCTION

Although the idea that animal spermatozoa are attracted by eggs is intuitive, clear examples of such an attraction have been very few. In the plant kingdom, the vectorial movement of male gametes along a chemical gradient was observed long ago (23), but until recently animal spermatozoa were considered not to have any chemotactic activity. Examples of attraction were first described in Cnidarians (4, 10, 11) and later in tunicates (15) and molluscs (18). Because the attraction of spermatozoa by eggs or gonads has been demonstrated among these widely different phyla it presumably has an important role in invertebrate reproduction (9, 17).

In the siphonophores, which are planktonic Cnidarians with external fertilization, the meeting of spermatozoon and egg can be particularly well studied. The chemical attractant is not liberated by the gonads (11, 12, 13) or the egg cortex (4, 14, 19) but, as we show here, by an extracellular structure: the cupule, overlying the polar bodies at the pole of maturation where fertilization always takes place[1]. This structure containing the chemoattractant can be isolated from the eggs. In this report we describe its properties.

MATERIAL AND METHODS

Siphonophores were collected in the bay of Villefranche sur Mer. *Muggiaea kochi* (Will, 1884), a calycophoran siphonophore, was chosen for most of the studies described here, as it was abundant. Other species were occasion-

Correspondence and reprints: Danièle Carré, Station Zoologique et Groupe de Biologie Marine du C.E.A., Station Marine de Villefranche sur Mer 06230, France.

[1] D. Carré and C. Sardet (manuscript in preparation).

nally examined (*Lensia subtilis, Chelophyes appendiculata, Abylopsis tetragona, Lensia conoïdea, Sphaeronectes gracilis, Halistemma rubra, Forskalia edwardsii...*).

For thin section electron microscopy, eggs and spermatozoa were fixed in 3% glutaraldehyde in cacodylate buffer pH 7.4 (0.1 M) with added NaCl (0.3 M) to bring the osmolarity to that of sea water. Samples were postfixed for one hour in 1% osmium tetroxyde in cacodylate buffer, embedded in Spurr resin and post fixed with uranyl acetate and lead citrate.

Cupules could be isolated by micromanipulation with tungsten needles. They were then dried on glass slides and used directly for microelectrophoresis. They were usually stored deep frozen after drying, sometimes after fixation in absolute alcohol.

For studying heat resistance isolated cupules stuck onto glass slides by fixation with absolute ethanol were placed in an autoclave (180 °C) for different periods of time.

Protease sensitivity of the attractant was examined by exposing eggs to 1 mg/mL of Proteinase K (Boehringer) or Pronase (Sigma) before addition of spermatozoa.

For microelectrophoresis in SDS polyacrylamide gels, dried cupules (5-10) were dissolved on a hot plate with 0.5 µl of SDS + DTT containing Trisglycine pH 8.4 buffer[2]. The samples were transferred with a micropipette onto a 3-40% polyacrylamide gradient made in a 5 µl capillary (Brandt) and electrophoresed with SDS + DTT containing buffer for 45 min at 60 volts (5,21). Microgels were fixed in methanol (50%) or isopropanol (25%) acetic acid (12%) for 15 min and protein bands revealed with coomassie blue or silver stain (24).

Microelectrophoresis was also performed on gradient gels in non denaturing conditions (21). The dried cupules were dissolved in Tris sulfate buffer (pH 8.8). After electrophoresis (45 min) at 60 V, the gels were extruded in sea water. Spermatozoa were added and the position of the sperm attracted area was recorded. The gels were subsequently washed, fixed and stained as above.

Sperm motility and flagellar movements were examined with dark field optics (×16 and ×25 objectives) under stroboscopic illumination (Chadwik Helmuth Strobex). Sperm chemotaxis was observed around a cupule stuck on a glass slide which is covered with bovine serum albumin. Trajectories of spermatozoa were recorded using 1-10 seconds exposure time with flash frequencies of 20 Hz or 40 Hz. Flagellar movements were recorded with 1/2 second or monoflash exposure. Beat frequencies were measured with reference to calibrated frequencies of the flash illuminator and values were averaged on 20 spermatozoa from 5 different fields. The curvature of the trajectory was measured on printed enlargements of these micrographs.

[2] P. Chang and C. Sardet (in preparation).

RESULTS

The gametes of siphonophores

Siphonophore gametes differentiate from the ectodermal layer of the manubrium of small astomate medusae (gonophores) attached along the stem of the colony (Fig. 1*a*). Colonies are monoecious, each gonophore being either male or female. Eggs of each gonophore (1-20, depending on the species) are shed simultaneously after emission of the second polar body.

In common with other Cnidarians the eggs of siphonophores (300 to 500 µm in diameter depending on the species) have a cortical layer containing most cellular organelles surrounding large vesicles of yolk, packed against each other (Fig. 1*b*, 1*c*, 1*d*). In addition, the eggs of all siphonophore species examined have 2 special properties.

— An extracellular structure, the cupule, which is about 30-50 µ in diameter, covers the polar bodies and adhers to the egg by a mucus layer (Fig. 1*b* and 1*c*).

— A specialized zone of the egg surface situated beneath the cupule directly above the female nucleus where fertilization exclusively takes place (Fig. 1*c* and 1*d*).

Siphonophore spermatozoa are of the primitive type defined by Franzen (6), with a conical nucleus, 4-6 mitochondria forming a ring and a flagellum (9+2 type). As in most Cnidarians (1, 7), the centriolar apparatus is branched (2). In addition, siphonophore spermatozoa exhibit a striated rod extending from the centriolar apparatus to the tip of the nucleus (Fig. 1*e*).

It is associated with a large vacuole of Golgi origin (2) and, upon rupture, becomes part of the acrosomal process[1] (Fig. 1*f*).

FIGURE 1. — Fertilization in *Muggiaea kochi*.

a. — Monogastric phase showing Bract (B) and gonophore (Go) carrying eggs; × 50.

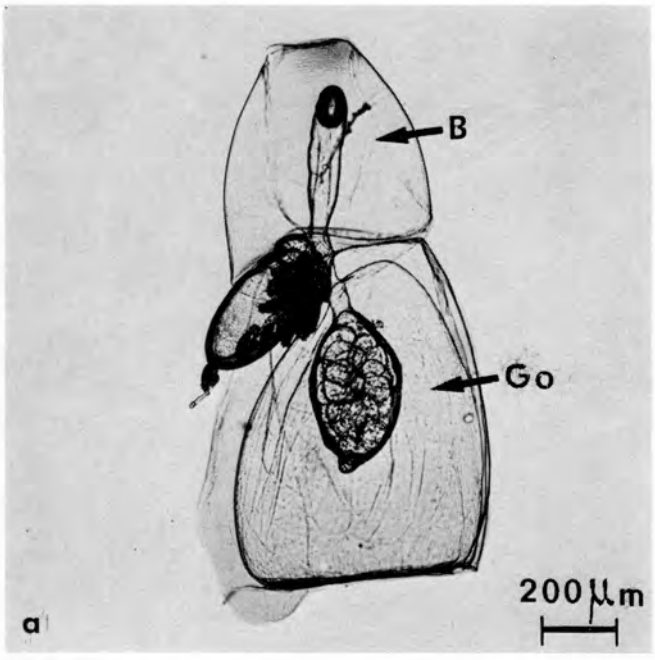
b. — Unfertilized egg with polar bodies (Pb) and cupule (C). Interference contrast; × 500.

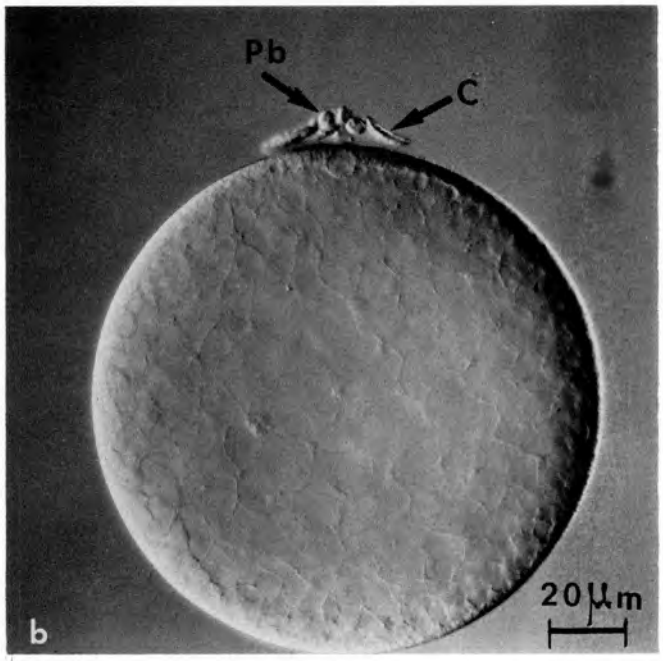
c. — The pole of maturation of the egg. The arrow shows the predetermined site of sperm entry situated just above the nucleus (N). Interference contrast; × 800.

d. — Thick section of the same area as in figure *c*. The fertilizing spermatozoon (SP) is seen in a zone devoid of cortical granules. Interference contrast; × 900.

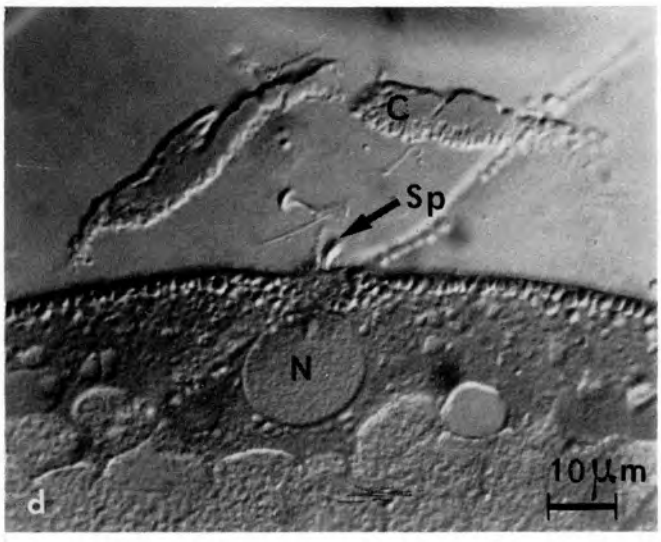
e. — Thin section of spermatozoon. Arrows show the striated rootlet of the centriolar apparatus and its prolongation, the striated root up to the tip of the nucleus. A large acrosomal vacuole is visible (Av); × 24,000.

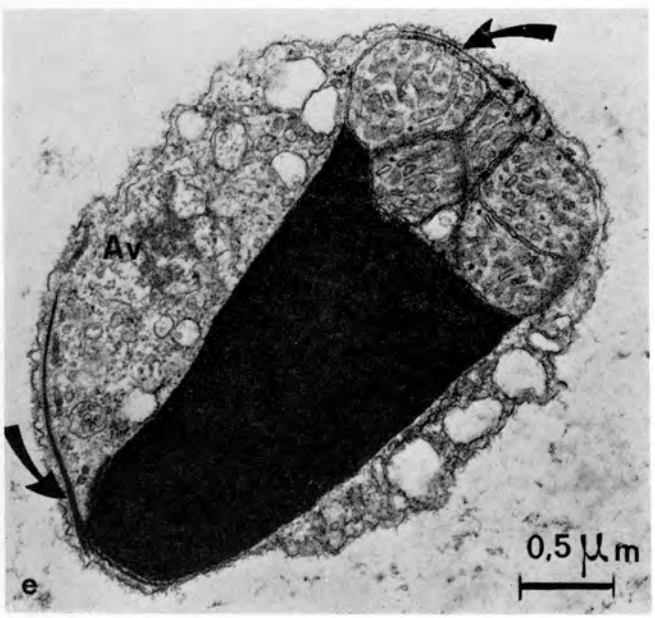
f. — The junction between egg and spermatozoon, arrow shows the participation of the striated rod in the acrosomal process; × 17,000.

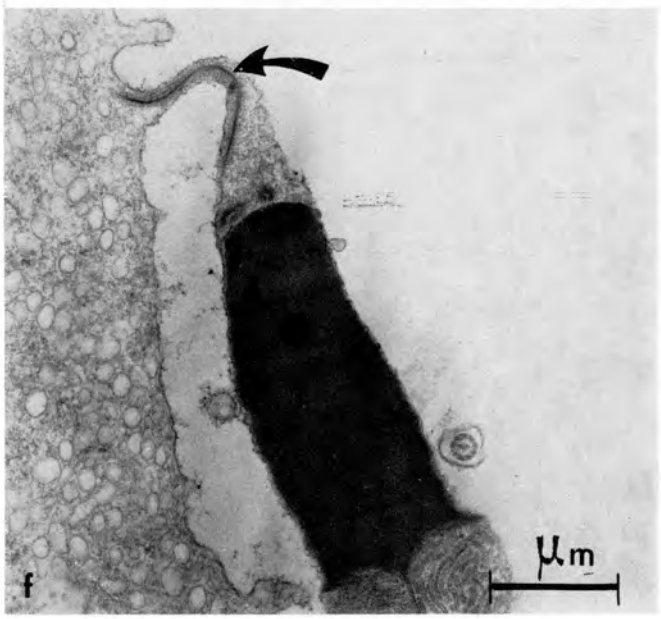

B
Go
200 µm
a


Pb
C
20 µm
b


N
10 µm
c


C
Sp
N
10 µm
d


Av
0,5 µm
e


µm
f

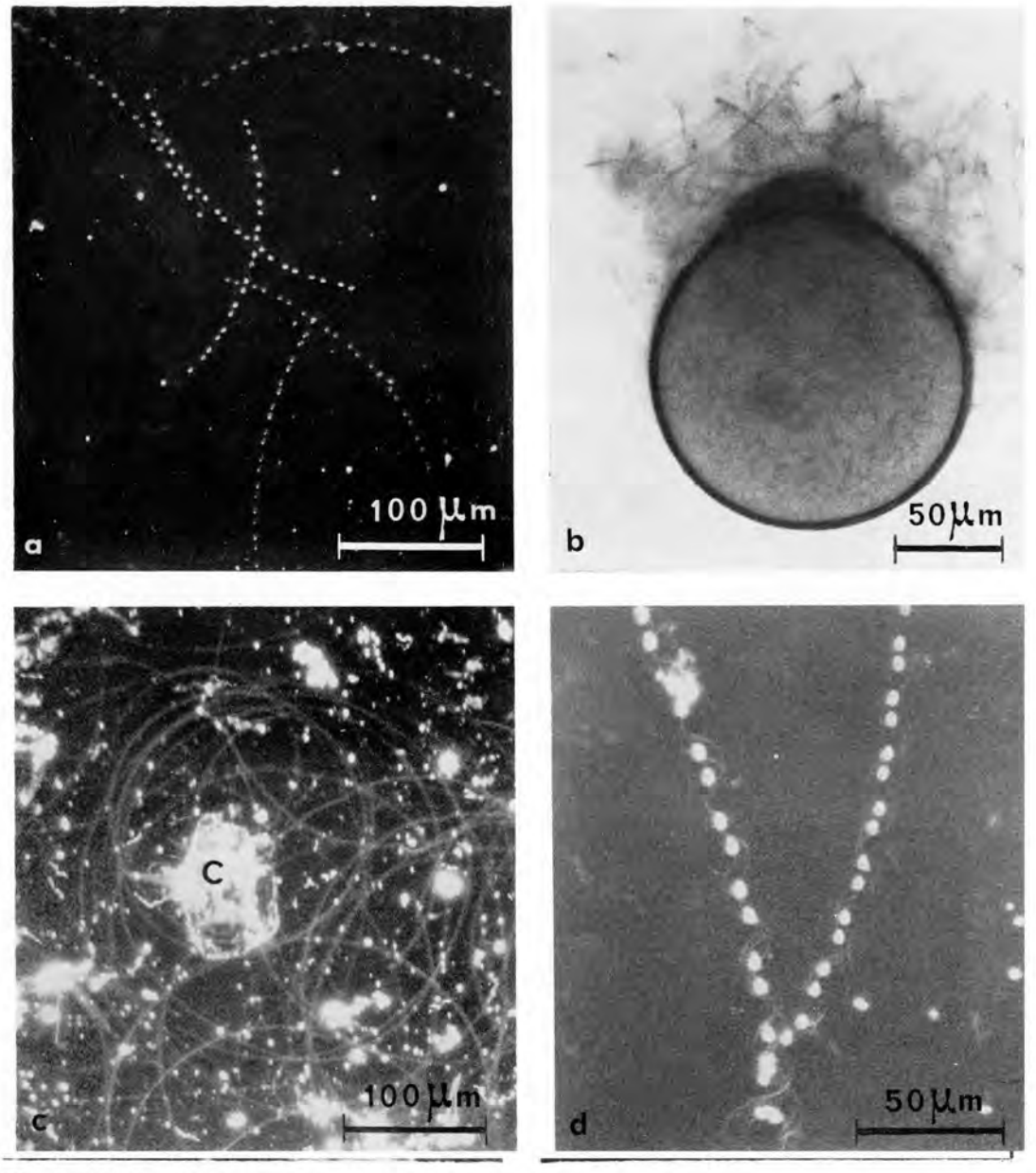


FIGURE 2. — The behavior of *Muggiaea kochi* spermatozoa.

a. — Normal trajectory of spermatozoa taken with a flash frequency of 40 Hz during 1 second; × 200.

b. — Accumulation of spermatozoa around the pole of maturation of the egg; × 300.

c. — Spermatozoa circling around a cupule (C). Exposure time: 10 seconds; × 200.

d. — Normal swimming behavior of spermatozoa. Exposure time: 1 second. Flash frequency: 20 Hz; × 400.

Evidence for the chemotaxis in siphonophores

In the absence of eggs, spermatozoa swim with slightly curved trajectories characterized by an angular deviation of 0.52±0.08 radian/second. As their flagella propagate waves at a beat frequency of 38±2 Hz, the angular rotation of the path can be described in terms of turning rate of 0.01 radian/beat (Fig. 2*a*). When eggs are introduced, a cloud of spermatozoa quickly forms above the maturation pole covered by the cupule (Fig. 2*b*). When cupule, polar bodies and the unfertilized egg are topologically separated with the help of fine tungsten needles, spermatozoa are attracted only by the isolated cupule (Fig. 2*c*). Their accumulation is the result of a change in swimming behavior. They swim in small circles around the zone of attraction (angular deviation of their paths = 2.05 ± 0.54 radians/second). We measured a beat frequency of 41 ± 0.45 Hz for the flagella, a value not significantly different from those we measured on sperm swimming in absence of chemotaxis (Fig. 2*d*). The turning rate of attracted spermatozoa is 0.05 radian/beat. Their circular swimming is reversible since some of the sperm-cells are seen escaping from the chemoattracting region.

Lack of calcium in the sea water completely inhibits chemotaxis but does not alter the swimming of the spermatozoa. Addition of calcium at 5 or 10 mM (the normal level in sea water) restores the chemotactic behavior.

We did not detect any difference in the ultrastructure of spermatozoa around isolated cupules (Fig. 3*f*), although we have not carried out detailed measurements of the periodicity of the striated rod. However, in close vicinity of eggs devoid of cupule, we have observed acrosomal processes in a few spermatozoa even with the light microscope.

Specificity of attraction

Cupules are present on eggs of all species of siphonophores examined. We had the opportunity to test the species specificity of the chemoattractant in more than 10 species. We never observed the attraction of spermatozoa by a cupule of a different species.

Structure and genesis of the cupule

In all species examined, thin sections show that the cupules are constituted of regular arrangement of fibers (25 nm thick), associated into loosely packed hexagonal lattices (Figs. 3*a* and 3*b*). The fibers appear to be made of globular subunits seen more clearly at the edge of the cupule dissolving slowly after fertilization (Fig. 3*d*). The cupule appears early in oogenesis between the egg and the ectodermal layer (Fig. 3*d*). It gradually spreads between the egg and the mesoglea. In thin sections we could not identify obvious precursors of the cupule in the egg. However, several observations indicate that the cupule originates from the oocyte:

— the plasma membrane of the egg immediately beneath the cupule is not clearly defined as if exocytosis was taking place (Fig. 3*c*);

— the part of the cupule closest to the surface of the egg is loosely packed in contrast to the part in contact with the envelope of the manubrium (Fig. 3*a* and 3*c*);

— the ectodermal and endodermal cells closest to the cupule do not show the features of the secretory cells.

The chemical nature of the cupule

Cupules dissected from the gonophore before maturation of the eggs already attract spermatozoa. Soon after maturation the eggs are shed. They are fertilizable for a short time. Cupules quickly detach from fertilized eggs, but remain attractive until complete dissolution, which takes more than a day. The slow dissolution in sea water is accompanied by a concomitant decrease in the attracting capabilities. In contrast, the dissolution of the cupule is quick in medium of low ionic strength. We noticed that the cupules could be fixed with absolute ethanol thus preventing their dissolution in sea water. The fixed cupules could still attract spermatozoa for more than 24 hours although they did not dissolve. This property suggests that the cupule is made of a precipitable macromolecular matrix from which an attractant could still diffuse. Treatment with proteases also points to the dual nature of the cupule. Treatment with pronase or proteinase K dissolves the cupule but does not modify the attraction of the solubilized cupule. The attractant is also remarkably heat stable since we could heat at 180 °C for 4 hours without noticing any loss of the biological activity.

The analysis of the components of dissected cupules from several species, dissolved in SDS + DTT was carried out by microelectrophoresis in polyacrylamide gradient gels. Staining with coomassie blue reveals that 2 or 3 proteins of very high molecular weight (300,000-400,000 MW) are the only constituents (Fig. 4*a*). However, in *Muggiaea kochi,* more sensitive detection of proteins with a silver stain, which allows us to detect picogram amounts of proteins[2], and fixation of the gel in isopropanol to retain small molecular weight proteins (5), show that besides the high molecular weight proteins, the cupule also contains low molecular weight material (10,000 - 20,000 MW) Fig. 4*b*).

Microelectrophoresis on polyacrylamide gradient gels of 10 whole cupules of *Muggiaea kochi* dissolved in low ionic strength buffer without SDS shows that the attractant migrates in the gradient gel in a position comparable to that of bovine serum albumin. Spermatozoa added to the gel immediately after electrophoresis concentrate around this single region of the gel (Fig. 4*c*). After washing the spermatozoa and staining with

FIGURE 3. — Structure and genesis of the cupule of *Muggiaea kochi.*

a. — Thin section through the cupule (C) before shedding of the egg. The cupule is situated between the ectoderm (EC) and the oocyte (O) with its nucleus (N) near the pole of maturation; × 8,000.

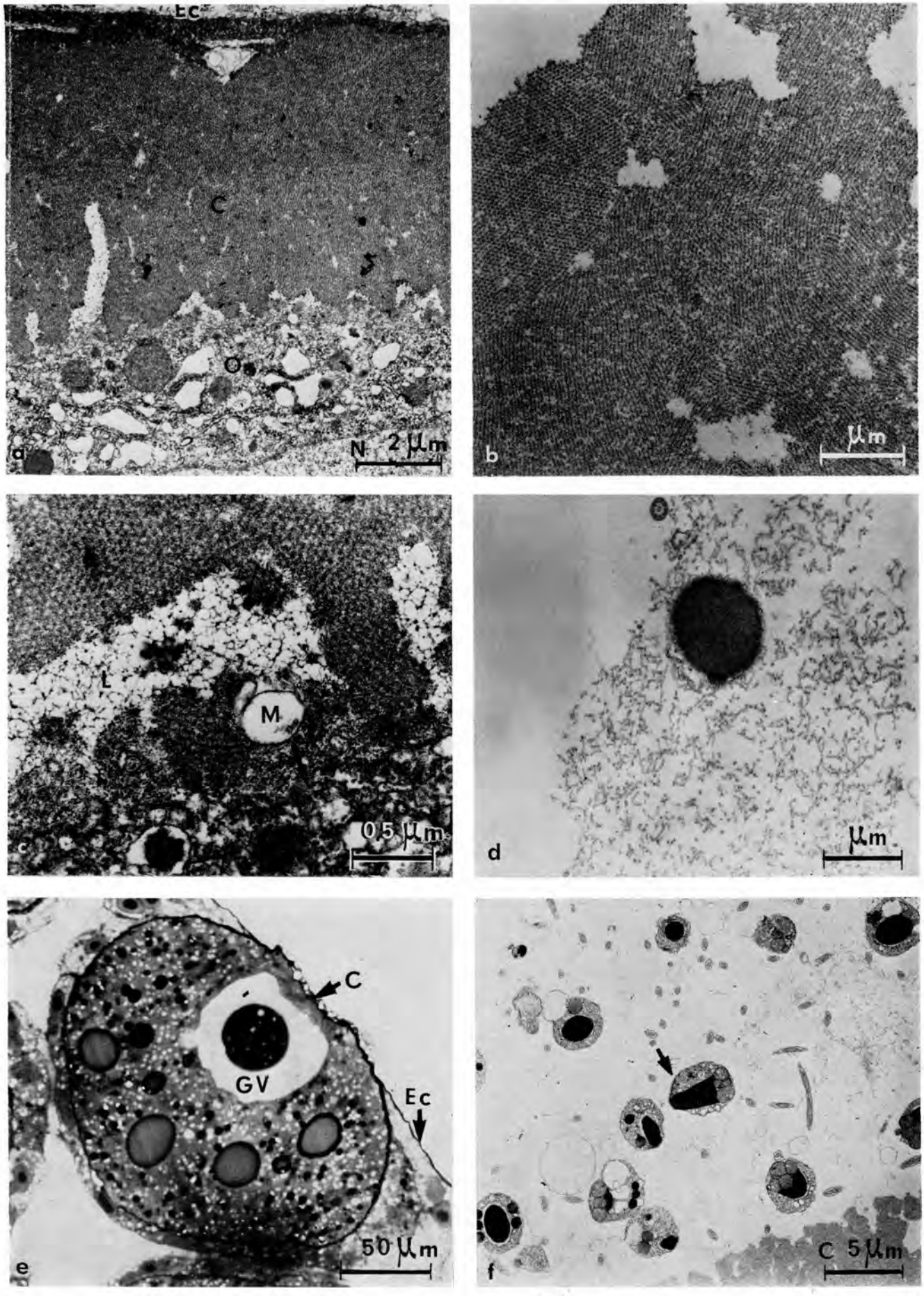
b. — Ordered structure of the cupule; × 16,000.

c. — Detail of the pole of maturation during oogenesis. Note the presence of lacunae (L) between egg and cupule, and the discontinuous appearance of the egg membrane (M); × 30,000.

d. — Cupule near complete dissolution, filaments dissolve into globular subunits; × 15,000.

e. — Thick section of an egg during oogenesis; germinal vesicle stage (GV). The cupule (C) is situated between the egg and the ectodermal layer (Ec); × 350.

f. — Spermatozoa near an isolated cupule (C) have not undergone the acrosomal process (*arrow*); × 3,000.


Ec
C
O
N
2 µm
a
µm
b
L
M
0,5 µm
c
µm
d
C
GV
Ec
50 µm
e
C 5 µm
f

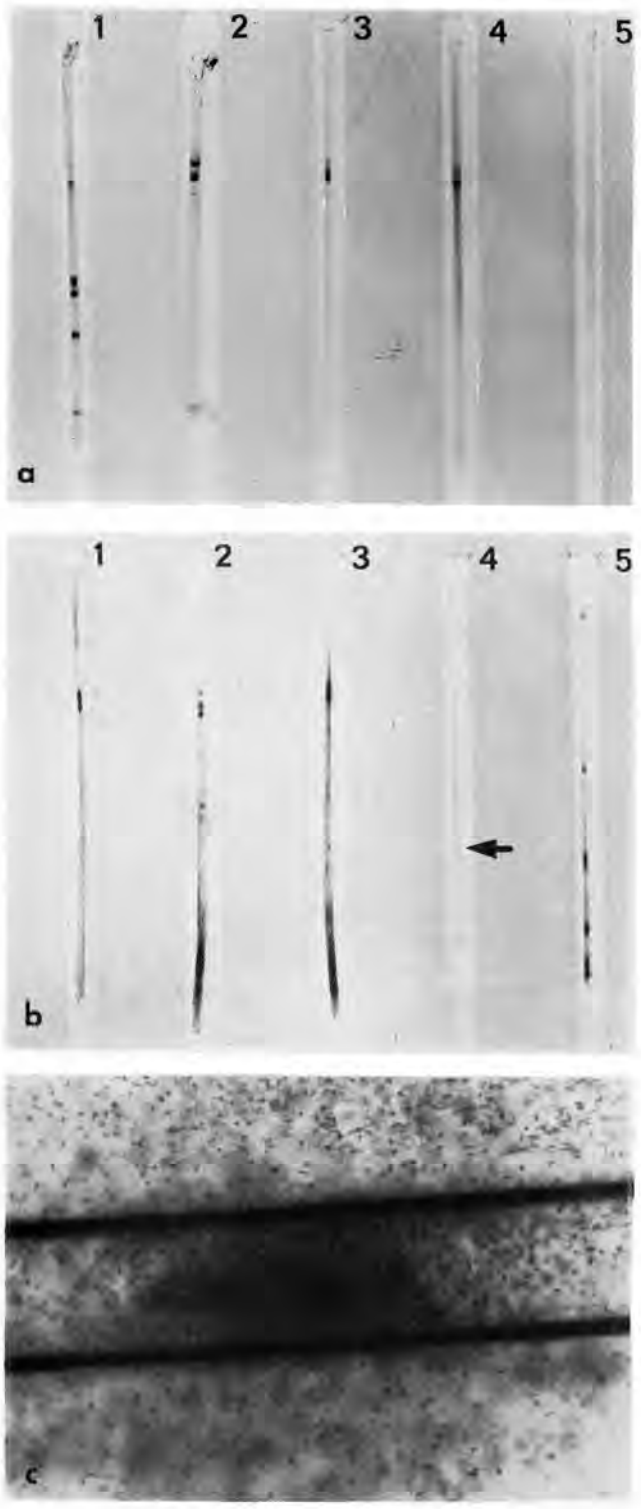


FIGURE 4. — Microelectrophoresis of cupules.

a. — Polyacrylamide electrophoresis on gradient (3-40 %) microgels of cupules dissolved in SDS + DTT. The gels were fixed and stained in Ethanol Acid Acetic + Coomassie blue R 250.

1. Reference proteins (Pharmacia HMW) from top to bottom: thyroglobulin (330000 MW), ferritin (220000 MW), catalase (600000 MW), albumin (67000 MW), lactate dehydrogenase (36000 MW), ferritin (18500 MW).

2. 3 cupules from *Abylopsis tetragona*, polar bodies removed.

3. 10 cupules from *Muggiaea kochi* with polar bodies.

4. 10 cupules from *Muggiaea kochi* with polar bodies.

5. 16 polar bodies from *Muggiaea kochi.*

b. — Polyacrylamide gel electrophoresis on gradient microgels (3-40%) of cupules.

1,2,3: cupules were dissolved in SDS + DTT, fixed and stained by a silver staining procedure (29).

4,5: cupules of reference proteins (same as above) were dissolved in Tris sulfate buffer pH 8.8 (20) and electrophoresed 45 min at 60 volts (non denaturing conditions).

1. Denaturing gel fixed in methanol acetic acid. 7 cupules from *Muggiaea kochi.*

2. Denaturing gel fixed in isopropanol acetic acid. 7 cupules from *Muggiaea kochi.*

3. Denaturing gel fixed in isopropanol acetic acid. 30 cupules from *Muggiaea kochi.*

4. Non denaturing gel fixed in isopropanol acetic acid. Arrow points to the faintly staining band corresponding to the zone where spermatozoa were attracted. 10 cupules from *Muggiaea kochi.*

5. Non denaturing gel of reference proteins (Pharmacia HMW) from top to bottom: thyroglobulin (669000 MW), ferritin (440000 MW), catalase (232000 MW), lactate de hydrogenase (140000 MW), albumin (67000 MW).

c. — The zone of attraction on a gradient microgel after 10 cupules from *Muggiaea kochi* have been electrophoresed. Sperm trajectories are visible around the gel.

silver stain a faint band could be detected at the place where spermatozoa had been attracted (Fig. 4*b*). Although the attraction by this zone of the gel could be seen with the naked eye, the amount of substance in that zone was at the limit of detection possible with silver staining ($\simeq$ 100 pg) perhaps indicating that most of the cupule material did not enter the gradient gel.

DISCUSSION

Siphonophore eggs have developed a particular structure, localized above the maturation pole, which species-specifically, attracts spermatozoa over a narrow range (about the egg's diameter). The real biological function of the attraction could be to concentrate spermatozoa in the vicinity of the pole of maturation where fertilization must take place, rather than simply around the egg. This may be true for all Cnidarian with external fertilization, since they show the same characteristics as siphonophores eggs, i.e., a depression at the maturation pole under which is situated the female pronucleus (8, 4) and a clear attraction of the spermatozoa centers around this pole (4, 19).

In contrast with what has been described by Miller (19) in the leptomedusa *Orthopyxis,* where chemotaxis exists only between maturation and first division, in siphonophores the cupule is capable of attracting the spermatozoa for more than a day. However, the 2 situations are biologically equivalent since eggs are fertilizable only for a short time after being shed and the attraction by the cupule is masked in the gonophore and is useless after fertilization.

The question of species-specificity of attraction has been examined in detail by Miller (20). Cross attraction is extremely rare among the many species of hydromedusae he observed. Siphonophore chemotactic cupules, although they share an identical ultrastructural appearance, only attract the spermatozoa of the same species. This appears as the first barrier of specificity in reproduction. As in other species, other barriers must exist at the level of the sperm activation and recognition of the egg surface, to prevent hybridization.

The regularly repeating (paracrystalline) structure of the cupule, seen in thin section electron microscopy, suggested some simplicity at the molecular level which was demonstrated by SDS polyacrylamide electrophoresis with coomassie blue staining. Cupules from different species seem to be made of only 2 or 3 very high molecular weight proteins (400 000 MW). However, in *Muggiaea kochi,* the use of silver staining, which increase the sensitivity of the technique, reveals protein material of low molecular weight, not detected with coomassie blue, near the 40% end of the gradient gel. The exact chemical nature of the attractant remains to be determined. Its migration on polyacrylamide gradient gels is similar to that of bovine serum albumin and its detection with the silver stain procedure would indicate it is a protein. However its remarkable heat resistance (180 °C for 4 hours) and insensitivity to proteases cast a doubt on the proteinaceous nature of the chemotactic agent. We do not know at present whether the attractant has sugar residues as might be expected of a molecule involved in recognition but attempts to inhibit chemotaxis with various sugars were unsuccessful.

It is difficult to compare our results with those of Miller (20) since in his case, attractants were obtained after lengthy alcoholic extraction of whole tissues or eggs and subjected to gel filtration for estimating molecular weight. The basic concept that attractants are stable low molecular weight proteins will need further examination.

In siphonophore spermatozoa, it is clear that attraction and activation (stimulation, acrosomal process) are distinct. Isolated cupules do not modify the morphology of the spermatozoa, in contrast to what has been described for *Campanularia* (22). Eggs whose cupule has been removed still induce the acrosomal process and remain fertilizable provided enough spermatozoa are present.

The main modification of the swimming behavior of spermatozoa introduced by the attractant concerns the curvature of their trajectories. Their speed and frequency of flagellar beating are not affected. The chemotatic behavior is dependent on Ca^{2+} since its removal from the seawater reversibly abolishes attraction although it does not affect the normal swimming behavior[3] (16).

It seems reasonable to relate the chemotactic behavior of Cnidarian spermatozoa to the peculiar structure of its centriolar complex (7, 2). In addition to other Cnidarians, siphonophores possess a striated rod that extends from the centriole up to the tip of the nucleus. In light of the presence of actin in the centriolar satellite apparatus of *Hydractinia spermatozoa* (7), it is tempting to speculate that the rod is contractile and plays a role in the transduction of the chemotactic signal, perhaps via a control of bend between the head and the flagella of the attracted spermatozoa. However, it must be kept in mind that the striated rod also plays a role in the acrosomal process, an event which takes place after the spermatozoon has undergone attraction.

[3] M.P. Cosson, J. Cosson, D. Carré, C. Sardet (in preparation).

Acknowledgements

We thank M.P. Cosson, J. Cosson, P. Chang who contributed enthusiastically to this work, R. Lallier for his interest and advice, A. Helenius and Q. Bone for their comments on the manuscript.

REFERENCES

1. Afzélius B.A. and Franzen A., 1971. The spermatozoon of the jellyfish Nausithöe. *J. Ultrastruct. Res.,* **37,** 186-199.
2. Carré D., 1979. An ultrastructural study of spermiogenesis and the mature sperm in the Siphonophore calycophore *Muggiaea kochi* (Cnidaria). *Zoon.,* **7,** 143-148.
3. Carré D., 1979. Chimiotactisme chez le Siphonophore *Muggiaea kochi.* The 4th Intern. Coelenterate Conference. Interlaken. Switzerland 4-8 september, 1979 (Poster).
4. Dan J.C., 1950. Fertilization in the medusan *Spirocodon saltarix. Biol. Bull.,* **99,** 412-415.
5. Fairbanks G., Steck T.L. and Wallach D.F.H., 1971. Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. *Biochem.,* **10,** 2606-2616.
6. Franzen A., 1956. On spermiogenesis, morphology of the spermatozoon, and biology of fertilization among Invertebrates. *Zool. Bird. Upps.,* **31,** 355-482.
7. Kleve M.G. and Clark W.H., 1980. Association of actin with sperm centriole: isolation of centriolar complexes and immunofluorescent localization of actin. *J. Cell Biol.,* **86,** 87-95.
8. Metschnikoff E., 1886. Embryologische studien an Medusen. Ein Beitrag Zur Genealogie der Primitiv. Organ. Alfred Hölder. Vienne.
9. Metz C., 1978. Sperm and egg receptors in fertilization. *Current Topics in Dev. Biol.,* **12,** 107-147.
10. Miller R.L. and Nelson L., 1962. Evidence for a chemotactic substance in the female gonangium of *Campanularia. Biol. Bull.,* **123,** 477.
11. Miller R.L., 1966. Chemotaxis during fertilization in the Hydroid *Campanularia. J. Exp. Zool.,* **162,** 22-45.
12. Miller R.L., 1970. Sperm migration prior to fertilization in the Hydroid *Gonothyrea loveni. J. Exp.* Zool., **175,** 493-503.
13. Miller R.L., 1973. The role of gonomedusa and gonangium in the sexual reproduction (fertilization) of the Hydrozoa. *J. Seto. Mar. Biol.,* **20,** 367-400.
14. Miller R.L., 1974. Sperm behavior close to *Hydractinia* and *Ciona* eggs. *Amer. Zool.,* **14,** 1250.

15. MILLER R.L., 1975. Chemotaxis of the spermatozoa of *Ciona intestinalis*. *Nature*, **254**, 244-245.
16. MILLER R.L., 1975. Effect of calcium on Tubularia sperm chemotaxis. *J. Cell. Biol.*, **67**, Pt2, 1*a*-483*a*.
17. MILLER R.L., 1977. Distribution of sperm chemotaxis in the animal kingdom. In: Advances in Invertebrates Reproduction. Vol. 1. K.G. Adiyodi and R.G. Adiyodi Eds. Peralam Kenoth Ed. Kerala. India.
18. MILLER R.L., 1978. Chemotactic behavior of the sperm of *Chitons* (Mollusca Polyplacophora). *J. Exp. Zool.*, **202**, 203-212.
19. MILLER R.L., 1978. Site specific sperm agglutination and the time release of a sperm attractant by the egg of the Leptomedusan *Orthopyxis caliculata*. *J. Exp. Zool.*, **205**, 385-391.
20. MILLER R.L., 1979. Sperm chemotaxis in the Hydromedusae. I. Species-specificity and sperm behavior. II. Properties of Medusa sperm attractant. *Mar. Biol.*, **53**, 99-124.
21. NEUHOFF V., 1973. Micromethods in molecular biology. Springer Verlag. Berlin.
22. O'RAND M.G. and MILLER R.L., 1974. Spermatozoon vesicle loss during penetration of the female gonangium in the Hydroid *Campanularia flexuosa*. *J. Exp. Zool.*, **188**, 179-194.
23. PFEFFER W., 1884. Locomotorische Richtungsbewegung durch chemische Reize. Untersuch. a.d. Botanische Institut Zu Tübingen. **1**, 363-481.
24. SWITZER R.C., MERRIL C.R. and SHIFRIN S., 1979. A highly sensitive silver stain for detecting proteins and peptides in polyacrylamide gels. *Anal. Biochem.*, **98**, 231-237.

Manuscrit reçu le 18 novembre 1980.

Accepté pour publication le 21 décembre 1980.