Cah. Biol. Mar. (2002) 43 : 1-7

Ultrastructural study of the mature oocyte of *Tethya aurantium* (Porifera: Demospongiae)

Margherita SCISCIOLI[1], Elena LEPORE[1], Maria MASTRODONATO[1], Lidia SCALERA LIACI[1] and Elda GAINO[2]
[1] *Dipartimento di Zoologia, Università di Bari, Via Orabona, 4 I-70125 Bari*
Fax: (39)0805443358, E-mail: m.sciscioli@biologia.uniba.
[2] *Dipartimento di Biologia Animale ed Ecologia, Università di Perugia, Via Elce di Sotto, I-06123 Perugia*
Fax: (39)0755855733, E-mail: gaino@unipg.it

Abstract: This ultrastructural study of the oocyte of *Tethya aurantium* adds new data to the knowledge of the germinal cells in the sponges of this genus. The ooplasm contains lipid droplets, electron-dense globules, phagosomes and rod-like inclusions. The most interesting feature of the oocytes is the high number of bacteria which are present in phagocytotic vacuoles in the oocyte cytoplasm and which derive from the mesohyl. While in other sponges the vacuoles usually contain a single bacterium, in *T. aurantium* the vacuoles are large and contain groups of bacteria. The bacteria engulfed by the oocytes of *T. aurantium* may perform a trophic role but their presence may also be interpreted as a means of transfer to the embryos. The lack of nurse cells around the oocytes suggests that yolk production in *T. aurantium* occurs by active uptake of nutrients from the mesohyl.

Résumé : *Etude ultrastructurale de l'ovocyte mûr de* Tethya aurantium *(Porifera: Demonspongiae).* L'étude ultrastructurale de l'ovocyte de *Tethya aurantium* fournit de nouvelles données sur les cellules germinales des éponges du genre *Tethya*. L'ooplasme contient des globules lipidiques, des globules denses aux électrons, des phagosomes et des inclusions à bâtonnet. L'aspect le plus intéressant concerne le nombre élevé de bactéries, présentes dans des vacuoles de phagocytose du cytoplasme ovulaire, et provenant du mésohyle. Tandis que dans les autres espèces d'éponges les vacuoles contiennent généralement une seule bactérie, chez *T. aurantium* les vacuoles sont plus grandes et contiennent des groupes de bactéries. On peut supposer que les bactéries phagocytées par les ovocytes de *T. aurantium* ont une fonction trophique mais leur présence peut aussi être interprétée comme un mode de transfert aux embryons. L'absence de cellules nourricières autour des ovocytes laisse supposer que dans l'espèce *T. aurantium* la production du vitellus dérive de la capture active de nutriments présents dans le mésohyle.

Keywords : Porifera, *Tethya aurantium*, reproduction, oocyte ultrastructure, bacterial symbionts

Introduction

Two sympatric species belonging to the genus *Tethya,* namely *T. aurantium* (Pallas, 1766) and *T. citrina* Sarà & Melone, 1965, long confused under the name of *T. aurantium*, are present in the Mediterranean Sea. The distinction between the two species is mainly based on the skeletal architecture (Sarà & Melone, 1965; Bavestrello et al., 2000) and complemented by anatomical characteristics (Sarà & Gaino, 1987), electrophoretic data (Sarà et al., 1989) and ecological aspects (Corriero et al., 1989).

Reçu le 05 janvier 2001 ; accepté après révision le 20 septembre 2001.
Received 05 January 2001; accepted in revised form 20 September 2001.

A previous investigation carried out by Scalera Liaci et al. (1971) on the sexual reproduction of *T. aurantium* and *T. citrina* collected along the coasts of Southern Italy showed that in these oviparous and gonochoristic species: (a) gamete differentiation overlaps from July to October; (b) *T. citrina* oogenesis lasts longer (until November) and starts earlier (May) than that of *T. aurantium* and moreover, the sexual reproduction occurs in conjunction with the asexual production of buds in this species.

A comparison between sexual and asexual reproduction in two populations of *T. citrina* and *T. aurantium* carried out in a Mediterranean coastal lagoon (Stagnone di Marsala) by Corriero et al., (1996) revealed different reproductive strategies in bud and oocyte production between the two species.

Ultrastructural analysis on the eggs of *T. citrina* (Gaino et al., 1987) and of two tropical species, *T. seychellensis* and *T. tenuisclera* (Gaino & Sarà, 1994) showed organizational differences from species to species along with structural changes in the stored material in relation to the egg metabolic activity.

Our aim was to widen the knowledge on the fine structure of the oocytes of the species belonging to the genus *Tethya*, in order to highlight species-specific differences in sponge oocytes.

Materials and methods

Tethya aurantium (Pallas) occurs commonly along the Adriatic coast close to Bari (Italy). On the basis of previous data on the sexual cycle of this species (Scalera Liaci et al., 1971), 150 specimens were collected from July to September 1999. Only 21 specimens had female germinal cells, a feature showing a very low reproductive activity. Fragments of 150 specimens were fixed and embedded either for histology or for transmission electron microscope (TEM) studies.

For histological observations samples were fixed in a solution of 10% formalin in filtered sea water, buffered with 0.1 N NaOH to a final pH of 7.6 - 7.8. Then, mesohyl fragments approximately 1 cm long were cut from each specimen, rinsed in filtered sea water, desilicified by immersion in 5% hydrofluoric acid in filtered sea water for one hour and a half, dehydrated in an ethanol series and embedded in paraffin. The 5-7 μm thick histological sections were stained with haematoxylin-eosin and observed under a Leica microscope.

For TEM investigations 2-3 mm mesohyl sponge fragments were cut from the mother sponges and fixed in a mixture of 25% glutaraldehyde (1 volume) in cacodylate buffer (0.4 M) (4 volumes) and filtered natural sea water (pH 7.4) (5 volumes). Subsequently, the specimens were postfixed for one hour in a mixture of 1% OsO_4 in sea water at 4°C and desilicified by immersion in a mixture of 5% hydrofluoric acid in filtered sea water for 50 minutes. The specimens were then dehydrated in a graded series of acetone and embedded in araldite. Ultrathin sections were contrasted with uranyl acetate and lead citrate, and examined with a Zeiss EM 109 microscope.

Figure 1. Histological section of *Tethya aurantium* showing a clump of young oocytes (*arrows*). Haematoxylin-eosin. Scale bar: 20 μm.

Figure 2. Transmission electron micrographs (TEM). Young oocyte. (*b*) bacteria in the mesohyl; (*n*) nucleus; (*nu*) nucleolus; (*ph*) phagosome; Scale bar: 1 μm.

Figure 3. Histological section of two mature oocytes (*o*) within the sponge choanosome. (*chc*) choanocyte chambers. Haematoxylin-eosin. Scale bar: 20 μm.

Figures 4-6. TEM Micrographs. **Figure 4.** Section showing a sector of a mature oocyte. The cytoplasm comprises various inclusions. Note the phagocytosis of the bacteria (*b*) occurring at the oocyte surface. (*n*) nucleus; (*ph*) phagosomes; (*r*) rod-like inclusions; (*y*) yolk. Scale bar: 2.5 μm.

Figure 5. Detail of oocyte cytoplasm showing various inclusions. (*l*) lipid droplets; (*r*) rod-like inclusions; (*y*) yolk. Scale bar: 1 μm.

Figure 6. Detail of ovular cytoplasm: (*l*) lipid droplets; (*ph*) phagosomes and (*r*) rod-like inclusions. Scale bar: 1 μm.

Figure 1. Coupe histologique de *Tethya aurantium* montrant de jeunes ovocytes (*flèches*). Hématoxyline-éosine. Echelle: 20 μm.

Figure 2. Microscopie électronique à transmission (MET). Jeune ovocyte. (*b*) bactéries dans le mésohyle ; (*n*) noyau ; (*nu*) nucléole ; (*ph*) phagosome. Echelle : 1μm.

Figure 3. Coupe histologique de deux ovocytes mûrs (*o*) dans le choanosome de l'éponge. (*chc*) chambres choanocytaires. Hématoxyline-éosine. Echelle : 20 μm.

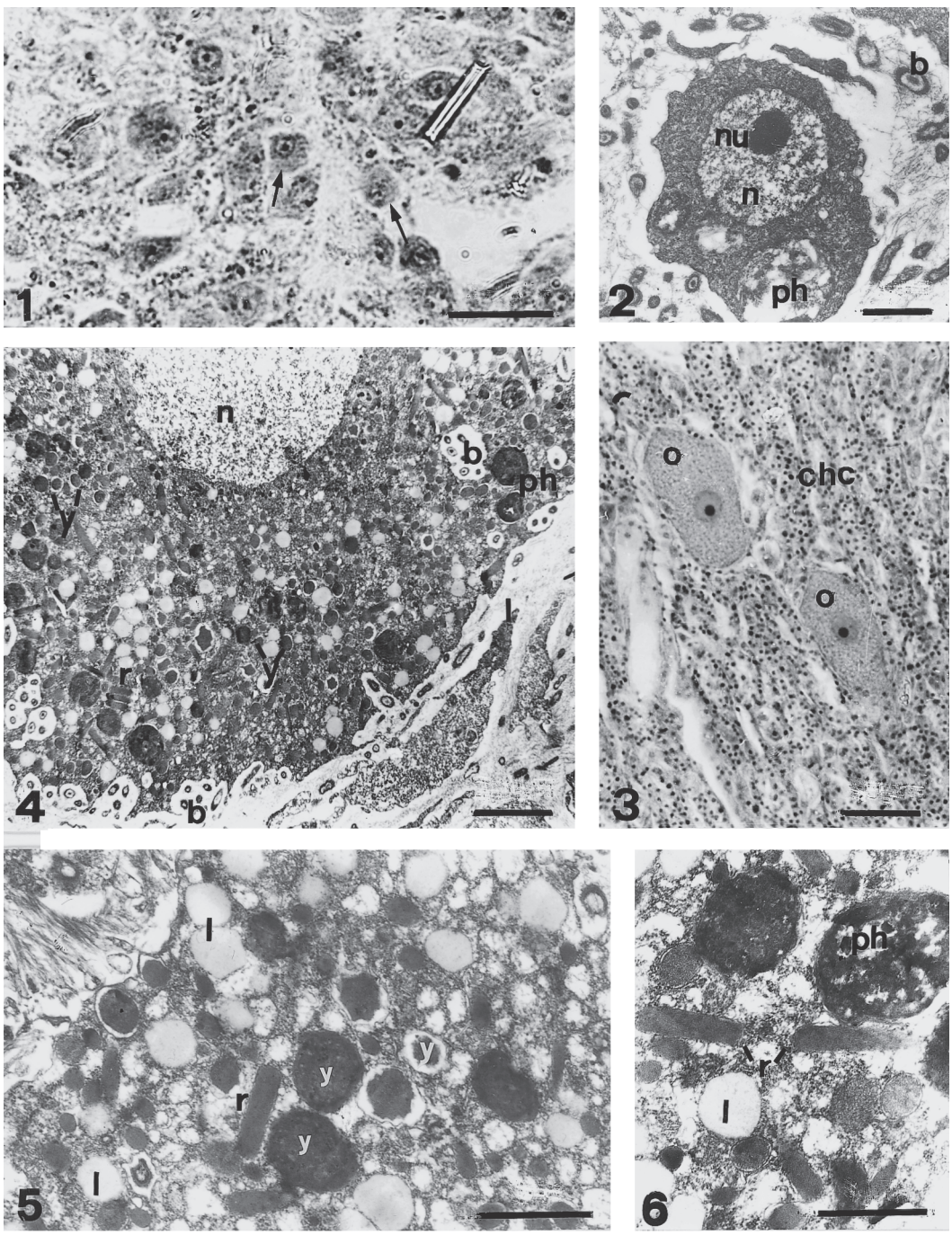
Figures 4 - 6. Micrographies MET. **Figure 4.** Coupe montrant un secteur d'ovocyte mûr. Le cytoplasme comporte diverses inclusions. On remarque la phagocytose des bactéries (*b*) se produisant à la surface de l'ovocyte. (*n*) noyau ; (*ph*) phagosomes ; (*r*) inclusions à bâtonnet ; (*y*) vitellus. Echelle : 2,5 μm.

Figure 5. Détail du cytoplasme ovocytaire montrant diverses inclusions. (*l*) globules lipidiques ; (*r*) inclusions à bâtonnet ; (*y*) vitellus. Echelle : 1 μm.

Figure 6. Détail du cytoplasme ovulaire : (*l*) globules lipidiques ; (*ph*) phagosomes et (*r*) inclusions à bâtonnet. Echelle : 1 μm.

Results

During the reproductive season of *T. aurantium*, previtellogenic oocytes (ca. 8-10 µm in diameter) of specimens collected in July accumulate in the mesohyl of the mother sponge (Fig. 1). Their morphology is consistent with their origin from archeocytes. Indeed, at the ultrastructural level, the young oocytes show a vesiculated nucleus (1-2 µm in diameter) with a diffuse chromatin and an electron-dense nucleolus (Fig. 2). The nucleoplasmatic index is high, since the cytoplasm is reduced to a thin strip, devoid of yolk inclusions but showing large phagosomes. These young oocytes are neither surrounded by lophocytes nor enclosed with a collagen envelope.



In August, most of the examined specimens have mature oocytes, measuring 40-50 µm with a nucleolated nucleus measuring 5-6 µm in diameter (Fig. 3). This feature is probably due to a fast vitellogenesis which assures a rapid growth of the oocytes. Under TEM, oocytes are characterized by a cytoplasm filled with inclusions, namely electron-dense globules, lipid droplets, phagosomes and rod-like inclusions (Figs 4, 5, 6).

The size and morphology of the electron-dense globules vary: they are 0.3-0.6 µm in diameter and can be either uniformly electron-dense or with an electron-dense core surrounded by a translucent border (Figs 4, 5). Dispersed lipid droplets occasionally merge with each other giving rise to larger inclusions (0.2-0.4 µm in diameter) (Figs 5, 6).

The phagosomes, already evident in the young oocyte, are abundant in the mature oocyte. Likewise observed in the initial phase of gamete differentiation, these inclusions have a fairly round shape and an heterogeneous content (Fig. 6).

The rod-like inclusions appear as electron-dense bodies often arranged in parallel rows (Figs 4, 5, 6, 10).

In the perinuclear region, the involvement of the Golgi apparatus in the synthesis of small electron-dense vesicles is evident (Figs 7, 8) along with the accumulation of mitochondria with a dense matrix (Fig. 8). The passage of a granular material can be observed within the pores of the nuclear membrane (Fig. 8).

The mesohyl of *T. aurantium* harbours a remarkable quantity of bacteria (Fig. 9). As the gamete maturation proceeds, they tend to accumulate around the oocytes. Bacteria are then included in deep depression of the oocyte cell surface, thereby forming large phagocytotic vacuoles (Figs 4, 10). These vacuoles do not reach the perinuclear region (Figs 4, 7, 8), being first located close to the oocyte surface (Fig. 10) and then in the middle part of the cytoplasm (Fig. 11). The interposition of lophocyte-like cells, tends to isolate mature oocytes from the rest of the sponge tissue (Fig. 12).

Discussion

The ultrastructural observations carried out on the oogenesis of *T. aurantium* show that oocyte maturation requires the accumulation of different kinds of inclusions among which the phagosomes are an important component. The species of the genus *Tethya* studied so far lack nurse cells around the oocyte, thereby suggesting that also in *T. aurantium* yolk production can occur by active uptake of nutrients from the mesohyl.

Sponge oocytes filled with inclusions are typical of oviparous species (Gallissian & Vacelet, 1976; Watanabe, 1978; Gaino, 1980; Gaino et al., 1987; Sciscioli et al., 1991, 1994; Lepore et al., 1995, 2000). Reserves of energy are necessary in these oviparous species, since the further development of embryos cannot be insured by a direct uptake of nutrients from the mother sponge.

As far as the genus *Tethya* is concerned, there is a rich amount of inclusions in eggs of *T. citrina*, *T. seychellensis* and *T. tenuisclera*. In these species the phagosomes are considered essential for the production of yolk (Gaino et al., 1987; Gaino & Sarà,1994). We retain that in *T. aurantium* the numerous phagosomes may also have an important role in the synthesis of yolk. Lipid inclusions constitute another relevant component of the stored material, likewise observed in other demosponges (Gaino, 1980; Gaino et al., 1987; Sciscioli et al., 1994).

Figure 7–12 TEM micrographs**. Figure 7.** Section showing the perinuclear area of a mature oocyte. This area is devoid of bacteria. (*d*) dictyosomes; (*ev*) ergastoplasmic vesicles; (*n*) nucleus; (*nm*) nuclear membrane; (*y*) yolk. Scale bar: 1 µm.

Figure 8. Perinuclear area. Note the passage of material (*arrow*) from nucleus (*n*) to cytoplasm. (*d*) dictyosomes; (*nm*) nuclear membrane. Scale bar: 1 µm.

Figure 9. Micrograph of the mesohyl of *T. aurantium* showing a large number of bacteria (*b*). Some bacteria are in division (*arrows*). (*ar*) archeocyte. Scale bar: 1µm.

Figure 10. Uptake of mesohyl bacteria (*b*) occuring at the oocyte surface. (*c*) collagen; (*l*) lipid droplets; (*ph*) phagosomes; (*r*) rod-like inclusions; (*y*) yolk. Scale bar: 1 µm.

Figure 11. Detail of ooplasm showing vacuoles with bacteria (*b*), rod-like inclusions (*r*) and yolk inclusions (*y*). Scale bar: 1 µm.

Figure 12. A lophocyte-like cell (*lo*) close to the oocyte surface (*o*). (*b*) bacteria; (*c*) collagen; (*l*) lipid droplets. Scale bar: 2 µm.

Figure 7–12 Micrographies MET. **Figure 7.** Coupe montrant la zone périnucléaire d'un ovocyte mûr. Cette zone est dépourvue de bactéries. (*d*) dictyosomes ; (*ev*) vésicules ergastoplasmiques ; (*n*) noyau ; (*nm*) membrane nucléaire ; (*y*) vitellus. Echelle : 1 µm.

Figure 8. Zone périnucléaire. On remarque l'extrusion de matériel nucléaire (*flèche*). (*d*) dictyosomes ; (*nm*) enveloppe nucléaire. Echelle : 1 µm.

Figure 9. Micrographie du mésohyle de *T. aurantium* montrant un grand nombre de bactéries (*b*). Certaines sont in division (*flèches*). (*ar*) archéocyte. Echelle : 1 µm.

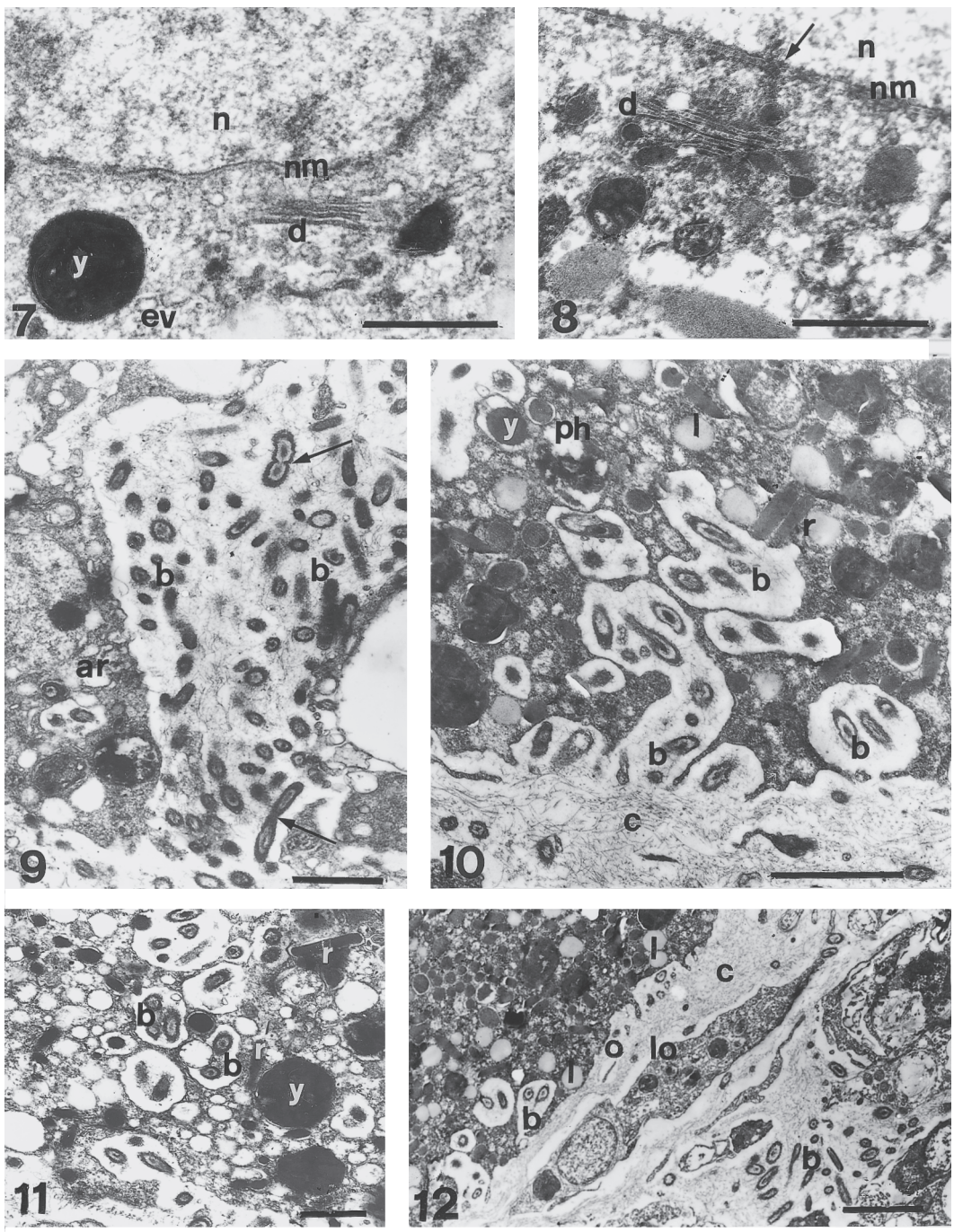
Figure 10. Capture de bactéries (*b*) par la surface de l'ovocyte. (*c*) collagène ; (*l*) globules lipidiques ; (*ph*) phagosomes ; (*r*) inclusions à bâtonnet ; (*y*) vitellus. Echelle : 1 µm.

Figure 11. Détail du cytoplasme ovocytaire montrant des vacuoles avec bactéries (*b*), des inclusions à bâtonnet (*r*) et des inclusions vitellines (*y*). Echelle : 1 µm.

Figure 12. Lophocyte (*lo*) près de l'ovocyte (*o*). (*b*) bactéries ; (*c*) collagène ; (*l*) globules lipidiques. Echelle : 2 µm.

The rod-like inclusions represent a particular kind of inclusion hitherto described only in the genus *Tethya*. In *T. aurantium* they are similar to those observed in *T. citrina* and differ from those of *T. seychellensis* and *T. tenuisclera* owing to the presence in these latter of a specific fibrillar component (Gaino & Sarà, 1994).

The oocytes of *T. aurantium* are immersed in the fibrillar collagen matrix of the sponge body. Fibrils are not arranged to form a coat around each oocyte as is the case of *Scypha ciliata* (Franzen, 1988), *Stelletta grubii* (Sciscioli et al., 1991) and *Geodia cydonium* (Sciscioli et al., 1994). It is noteworthy that in these species the fibrils are partially engulfed by the oocyte and included in numerous large vacuoles arranged peripherically. No images suggest that a similar process occurs in *T. aurantium* in which the phagocytosis by the oocyte is limited to the bacteria associated to the mother sponge.



The phagocytosis of bacteria by the oocytes has been repeatedly documented in sponges (Gallissian & Vacelet, 1976; Gaino, 1980; Sciscioli et al., 1989, 1991, 1994; Kaye, 1991; Gaino & Sarà, 1994). In *T. aurantium*, the engulfing of bacteria is particularly intense since the whole cell surface has an active role in their uptake and incorporation. In addition, bacteria are not taken up individually but in groups, so that each vacuole contains several microrganisms, a process resulting in a massive incorporation of bacteria in the oocytes. On this account, the behaviour of sponge gametes does not differ significantly from that observed in the mesohyl cells of many sponge species, which are able to take advantage of the associated microrganism for their nutrition. Bacteria phagocytosed by the oocyte of *T. aurantium* may perform a similar trophic role, as hypothesized for these gametes in other sponge species or deduced from the altered morphology of bacteria inside vacuoles (Gaino, 1980; Sciscioli et al., 1989, 1991, 1994; Gaino & Sarà, 1994), although we did not observe such alteration in *T. aurantium*. The presence of bacteria in oocytes could also be a normal process for transferring symbionts from generation to generation as known in other species (Gallissian & Vacelet, 1976; Gaino, 1980; Sciscioli et al., 1989, 1991, 1994; Gaino & Sarà, 1994). In *T. aurantium*, this assumption is supported by the occurrence of intact bacteria inside the cytoplasmic vacuoles of oocytes.

Most of the data on oocyte-bacteria association support the notion that the ability of the oocyte to entrap bacteria is restricted to free bacteria, dispersed in the sponge mesohyl. Indeed, in *Petrosia ficiformis* where bacteria are included in highly specialized cells (Vacelet & Donadey, 1977; Bigliardi et al., 1993), no such microrganisms are observed in the ooplasm (Lepore et al., 1995).

The bulk of ultrastructural data on the developing oocytes of *T. citrina, T. tenuisclera, T. seychellensis* and *T. aurantium* allow us to compare the organization of their gametes. *T. citrina* stands out among the other species owing to the occurrence of a peripheral cortex. In *T. seychellensis* the cell surface has a layered appearance resulting from the accumulation of membranes associated with cytoplasmic vacuoles. No cortical differentiation has been observed in the remaining species. However, we cannot exclude that these apparent differences could be related to different stages of development. The acquisition of a cortical structure could be a relevant step in the gamete differentiation, leading to a protective envelope that enhances oocyte survival after spawning. Lipid droplets, phagosome-like granules and rod-like bodies are constant cytoplasmic components of the developing sponge oocytes. Nevertheless, their shape, size and appearance vary from species to species, thereby suggesting specific metabolic activities.

Acknowledgements

This study was in large part supported by funds from the Italian Ministry of University and Research.

References

Bavestrello G., Calcinai B., Ceccati L., Cerrano C. & Sarà M. 2000. Skeletal development in two species of *Tethya* (Porifera, Demospongiae). *The Italian Journal of Zoology,* **67** : 241-244.

Bigliardi E., Sciscioli M. & Lepore E. 1993. Interactions between prokaryotic and eukaryotic cells in sponge endocytobiosis. *Endocytobiosis & Cell Research,* **9**: 215-221.

Corriero G., Balduzzi A. & Sarà M. 1989. Ecological differences in the distribution of two *Tethya* (Porifera, Demospongiae) species coexisting in a Mediterranean coastal lagoon. *Pubblicazioni della Stazione Zoologica di Napoli (Marine Ecology)*, **10** (4): 303-315.

Corriero G., Sarà M. & Vaccaro P. 1996. Sexual and asexual reproduction in two species of *Tethya* (Porifera: Demospongiae) from a Mediterranean coastal lagoon. *Marine Biology,* **126**: 175-181.

Franzen W. 1988. Oogenesis and larval development of *Scypha ciliata* (Porifera, Calcarea). *Zoomorphology,* **107**: 349-357.

Gaino E. 1980. Indagine ultrastrutturale sugli ovociti maturi di *Chondrilla nucula* Schmidt (Porifera, Demospongiae). *Cahiers de Biologie Marine*, **21**: 11-22.

Gaino E., Burlando B., Buffa P. & Sarà M. 1987. Ultrastructural study of the mature egg of *Tethya citrina* Sarà and Melone (Porifera, Demospongiae). *Gamete Research,* **16**: 259-265.

Gaino E. & Sarà M. 1994. An ultrastructural comparative study of the eggs of two species of *Tethya* (Porifera, Demospongiae). *Invertebrate Reproduction and Development,* **26** (2): 99-106.

Gallissian M. F. & Vacelet J. 1976. Ultrastructure de quelques stades de l'ovogenèse de spongiaires du genre *Verongia* (Dictyoceratida). *Annales des Sciences Naturelles - Zoologie et Biologie Animale,* Série **18**: 381-404.

Kaye H. R. 1991. Sexual reproduction in four Caribbean commercial sponges. II. Oogenesis and transfer of bacterial symbionts. *International Journal of Invertebrate Reproduction,* **19** (1): 13-24.

Lepore E., Sciscioli M., Gherardi M. & Scalera Liaci L. 1995. The ultrastructure of the mature oocyte and the nurse cells of the ceractinomorpha *Petrosia ficiformis. Cahiers de Biologie Marine,* **36**: 15-20.

Lepore E., Sciscioli M., Scalera Liaci L., Santarelli G. & Gaino E. 2000. Sexual reproduction of *Cinachyra tarentina* (Porifera, Demospongiae). *The Italian Journal of Zoology,* **67**: 153-158.

Sarà M. & Gaino E. 1987. Interspecific variation in arrangement and morphology of micrasters of *Tethya* species (Porifera, Demospongiae). *Zoomorphology,* **107**: 313-317.

Sarà M. & Melone N. 1965. Una nuova specie del genere *Tethya, T. citrina* sp. n. del Mediterraneo (Porifera, Demospongiae). *Atti della Società Peloritana di Scienze Fisiche, Matematiche e Naturali,* (Suppl.) **11**: 123-138.

Sarà M., Mensi P., Manconi R., Bavestrello G. & Balletto E. 1989. Genetic variability in Mediterranean populations of

Tethya (Porifera, Demospongiae). In *Reproduction, genetics and distributions of marine organisms* (Ryland J. S., Tyler P. A. eds), pp. 293-298. Olsen & Olsen: Fredensborg.

Scalera Liaci L., **Sciscioli M., Papa O. & Lepore E. 1971.** Raffronto tra i cicli sessuali di *Tethya aurantium* (Pallas) Gray e *Tethya citrina* Sarà, Melone (Porifera, Hadromerina). Analisi statistica. *Atti della Società Peloritana di Scienze Fisiche*, *Matematiche e Naturali,* **17**: 287-298.

Sciscioli M., **Lepore E., Gherardi M. & Scalera Liaci L. 1994.** Transfer of symbiotic bacteria in the mature oocyte of *Geodia cydonium* (Porifera Demospongiae): an ultrastructural study. *Cahiers de Biologie Marine,* **35**: 471-478.

Sciscioli M., **Scalera Liaci L., Lepore E. & Gherardi M. 1989.** Indagine ultrastrutturale sugli ovociti di *Erylus discophorus* (Schimdt) (Porifera, Tetractinellida). *Oebalia,* **15**-2 N.S.: 939-941.

Sciscioli M., **Scalera Liaci L., Lepore E., Gherardi M. & Simpson T. L. 1991.** Ultrastructural study of the mature egg of the marine sponge *Stelletta grubii* (Porifera Demospongiae). *Molecular Reproduction and Development,* **28**: 346-350.

Vacelet J. & Donadey C. 1977. Electron microscope study of the association between some sponges and bacteria. *Journal of Experimental Marine Biology and Ecology,* **30**: 301-314.

Watanabe Y. 1978. The development of two species of *Tetilla* (Demospongiae). *Natural Science Report of the Ochanomizu University*, **29**: 71-106.