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Sexual reproduction in demosponges: ecological and evolutive implications

Reproducción sexual en demosponjas:
implicaciones ecológicas y evolutivas

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General discussion:

Herein is presented an integrative discussion of the main results obtained in the present work. Even though there are specific discussions of the results within each chapter, this general discussion intends to contextualize the entire assemblage of gathered data. I will follow approximately the scheme provided during the thesis that will allow a better understanding of the interrelations and implications of each result.

- **Reproductive biology of sponges and its relationship to environmental factors:**

All the mechanisms involved in reproduction of marine animals appear to ensure survival of the young produced, for example, their appearance when food is available, and when temperature, salinity, etc., are favourable (Giese and Pearse 1974). In temperate waters, summer is the season that gathers the most favourable conditions, in terms of temperature and food availability. Therefore, gametogenic cycles of many demosponges in temperate waters are scheduled to finish with larval release at such time (Tuzet and Pavans de Ceccatty 1958; Siribelli 1962; Scalera-Liaci et al. 1971, 1973; Witte et al. 1994; Corriero et al. 1996; Mercurio et al. 2007).

However, not all temperate demosponges fit within this pattern. Among the demosponges in which its timing of reproduction was studied during this thesis, different strategies were found, in terms of the relationship between their gametogenesis and the seawater temperature. Some of them timed their gametogenesis to release their larvae during summer months, as occurred in *Corticium candelabrum*, *Axinella*

damicornis, and *Chondrosia reniformis*. However, the putative clues that triggered gametogenesis in these three particular cases were fairly different. While water warming appeared to be related to the onset of oogenesis in *C. reniformis*, minimum temperatures triggered the oogenesis in *A. damicornis* and massive oocyte production in *C. candelabrum*. Gametogenic cycles of *Raspaciona aculeata* and *Petrosia ficiformis* ended during the last part of autumn or the initial weeks of winter. Such a timing implies that young sponges resulting from the sexual cycle developed in a not-so-favourable season of the year. It seems, however, that some environmental conditions might be more favourable for the young of these two species at this time of the year. The triggering clue that initiated gametogenesis was similar to that of *Chondrosia reniformis* though, since water warming appeared to be the responsible of the onset of gametogenesis in both cases.

In summary, all these different timings revealed a staggered schedule concerning the reproductive cycles of these five Mediterranean demosponges. Such alternation in the sexual cycles might be fit to avoid the intense competition for feeding resources that would occur if every demosponge species released their larvae within the same time window. Such explanation was also postulated for explaining the breeding seasons of species in areas of little seasonal changes (Thorson 1950; Grainger 1959). Moreover, larval release could be scheduled in each case to avoid predation of larvae and early settlers.

The differentiation in the thermal regulation of reproduction in species inhabiting the same habitat has further implications. If sponges react with different intensity to temperature cues and thermal ranges, they would have different responses to global warming, varying with different intensity its reproductive cycles. Such different responses by individual species may affect the dispersal abilities and recruitment that defines the population dynamics of each species. It can also be disrupted their interactions with the same or adjacent trophic levels (Walther et al. 2002), as long-term data on both terrestrial and marine organisms indicate (Fromentin and Planque 1996; Saetre et al. 1999).

Whereas onset and timing of reproduction are intimately related to environmental factors, duration of reproduction depends more on physiological processes than on environmental pressures. Reproductive cycles of the studied species were very different in their duration, although the sponges were subjected to similar

environmental stimuli because inhabited a similar habitat. All the studied sponges showed annual gametogenic cycles, characteristic of shallow, temperate seas (Giese and Pearse 1974). Relatively short cycles of oogenesis were developed by *Raspaciona aculeata* (3-5 months) and *Chondrosia reniformis* (3 months). Rapid maturation of oocytes has been described in several other demosponges, as *Aplysilla rosea* (Lévi 1956), *Cinachyra tarentina* (Lepore et al. 2000), *Erylus discophorus* (Scalera-Liaci and Sciscioli 1970), *Haliclona loosanoffi* (Fell 1976a), *Halisarca nahatensis* (Chen 1976), *Iophon piceus* (Ereskovsky 2000) or *Mycale contarenii* (Corriero et al. 1998). Oogenesis in *Axinella damicornis* and *Petrosia ficiformis* lasted for 7-8 months, as also described for *Ircinia fasciculata*, *Ircinia variabilis*, *Pellina semitubulosa*, *Spongia officinalis* (Scalera-Liaci and Sciscioli 1975), *Cliona celata* and *Haliclona oculata* (Wapstra and van Soest 1987), *Halichondria okadai* (Tanaka-Ichiara and Watanabe 1990), and *Halichondria panicea* (Witte and Barthel 1994). *Corticium candelabrum* experienced an unexpected pattern of oogenesis with continuous production of oocytes during the entire year, a rare case for not only sponges but also other invertebrates (Giese and Pearse 1974). However, it appeared that, although new oocytes were produced every month of the year, oocyte maturation was completed in about 7 or 8 months. Some other sponges are known to produce oocytes during most months of the year, as *Halisarca dujardini* (Lévi 1956), *Hippiospongia lachne* (Storr 1964), *Haliclona ecbasis* (Fell 1974), *Ochridaspongia rotunda* (Gilbert and Hadzisce 1977), and *Mycale contarenii* (Corriero et al. 1998). However, the case of *C. candelabrum* is slightly different, since it produces oocytes over the entire year. Unfortunately, the fate of new oocytes that are produced in the months when fertilisation takes place remains unclear. As pointed out before, differences concerning the disparity in the duration of the reproductive cycles depend more on physiological processes undergone by gametes than on environmental pressures. In the particular case of oogenesis, if nurse cells help to complete vitellogenesis, cycles will be shorter than in those cases with auto-synthetic production of yolk, whether it is helped occasionally by nurse cells.

Spermatogenesis was consistently a shorter process at the population level in all the species when compared to oogenesis, as described for most demosponge species (see Reiswig 1983 and Boury-Esnault and Jamieson 1999 for reviews). Nevertheless, its duration ranged from 15 days approximately in *Petrosia ficiformis* to 1-2 months in *A. damicornis*, *R. aculeata* and *C. reniformis*, and 4-5 months in *C. candelabrum*. Previous

studies about the reproductive cycles of *A. damicornis* (Siribelli 1962), *Petrosia ficiformis* (Scalera-Liaci et al. 1973a), and *C. reniformis* (Scalera-Liaci et al.1973b) are consistent with our results. Synchronous spawning events of sperm occur in oviparous species, and resulted, logically, from synchronous spermatogenesis at the population level (Reiswig 1983). This was the case of *Axinella damicornis*, *Raspaciona aculeata,* and *Chondrosia reniformis*. As many other viviparous sponges, *Corticium candelabrum* experiences longer spermatogenesis at the population level compared to oviparous sponges (the rest of the studied species). Also, sperm spawning is asynchronous, extending for several months. Since oocytes are not developing strictly in a synchronous way, sperm spawning may have extended for several months to ensure a high success in fertilisation.

External fertilisation is the most widespread mode of reproduction in the marine environment. Despite the prevalence of this mode of reproduction, little is known about fertilisation of sessile animals under natural conditions (Yund and McCartney 1994). Recent *in situ* studies of fertilisation in both sessile and mobile invertebrates have focused on determinations of the yield of fertilized ova ("female fertilisation success") (Yund and McCartney 1994; Metaxas et al. 2002). In sponges, however, the unique estimate of fertilisation rate was done upon histological preparations of *Xestospongia bergquistia* (Fromont and Bergquist 1994). Similarly, during the current work, estimate of fertilisation rate in *Corticium candelabrum* was performed comparing the average of mature oocytes to the average of produced larvae of a monitored population. Fertilisation rates of both *X. bergquistia* and *C. candelabrum* were surprisingly high (71.4% and 99.3% respectively) when compared with other sessile organisms with external fertilisation (20%) (Levitan 1995).

- **The origin of gametes in demosponges:**

The development of gametes in sponges involves differentiation of somatic cells into gonial cells (Fell 1974, 1983; Reiswig 1983; Simpson 1984; Boury-Esnault and Jamieson 1999). Three different cell types have been postulated as the origin of gametes directly or indirectly, which led Fell (1974) to affirm that "possibly the only common source of all the germ cells produced during the life history of a sponge is the blastomeres of the undifferentiated embryo". Since 1974, no investigations have been

conducted in search for the determination of a germ cell lineage in sponges, but the recent discoveries of vasa and nanos genes in *Hydra* (Torras et al. 2004; Extavour et al. 2005) may encourage researchers to undertake the genetic path in this particular issue.

Oogonia

In the 4 species whose oogenesis has been studied as part of this thesis, oocytes appeared to be derived from archaeocytes. Oocytes of *Corticium candelabrum* located within the mesohyl, somewhat distant from choanocyte chambers. That situation together with size, morphology, and affinity for stains make us suggest an archaeocyte origin for the oocytes. Oocytes of *Petrosia ficiformis*, *Raspaciona aculeata*, and *Axinella damicornis* appeared to be derived from archaeocytes by the same reasons. Oocytes of *Asbestopluma occidentalis* appeared to be derived similarly from archaeocytes, but in this case such derivation is even more likely, since the sponge lacks choanocytes. It occurs widely that oocytes are derived from archaeocytes within the phylum Porifera, not only in demosponges (Leveaux 1941; Lévi 1956; Saller and Weissenfels 1985; Witte and Barthel 1994), but in hexactinellids (Boury-Esnault et al. 1999) Nevertheless, oocytes appeared to derive from choanocytes in some demosponges (Gaino et al. 1986a) and many calcareans (Sarà 1974), and from pinacocytes in few calcareans (Borojevic 1969). However, definitive conclusions may also rest on genetic data, which are lacking so far.

Spermatogonia

Spermatogonia of *Crambe crambe, Corticium candelabrum*, and *Petrosia ficiformis* appeared to be derived from choanocytes. While in the case of *C. candelabrum* only the size, morphology, and presence of flagellum in the spermatogonia led us to suggest such choanocyte origin, more suggestive observations were performed in the other two cases. Observations of detachment of swollen choanocytes from choanocyte chambers towards the mesohyl in *Crambe crambe*, together with morphology and size, gave us more reasons to postulate their choanocyte origin. Spermatogonia in *Petrosia ficiformis* appeared to be swollen choanocytes that were present in regular choanocyte chambers, somewhat alike to the swollen choanocytes of *Crambe crambe*. In both cases, although not observed, we suggest that the cysts were formed from numerous mitoses and not from the differentiation of the whole chamber, because only few swollen choanocytes appeared to migrate towards the

mesohyl. A choanocyte origin for spermatogonia has been suggested in the majority of sponges (see Reiswig 1983 and Boury-Esnault and Jamieson 1999 for reviews), but as occurred in the case of oocytes, the postulations, although quite plausible, are purely speculative since every study has been undergone upon fixed material.

The case of *Asbestopluma occidentalis* is somehow special. *A. occidentalis* does not posses any flagellated cell. Thus, the archaeocyte origin of spermatogonia arises as the most probable option. Other cells that could have originated spermatogonia are pinacocytes, but the long distance from the pinacoderm to the central shaft were spermatic cysts appeared made this option very improbable. Such archaeocyte origin for spermatogonia has been postulated before, not only for hexactinellids, in which the enucleated nature of choanocytes disqualify them as a probable origin (Boury-Esnault et al. 1999), but for demosponges (Fincher 1940; Lévi 1956; Gaino et al. 1986b). In hexactinellids (Boury-Esnault et al. 1999) and the demosponge *Stylotella heliophil*a (Fincher 1940), spermatogonia cysts are postulated to derive from archaeocyte aggregates or congeries, being the last term coined to refer the aggregates observed in hexactinellids. Analogously, spermatogonia in *A. occidentalis* are the result of cellular differentiations carried out by archaeocyte aggregates or congeries.

- **Oogenesis and oocytes**

Since poriferans lack gonads or specific reproductive ducts, gametes usually locate within the mesohyl of the sponges. Oocytes occur often widespread in the sponge tissue (Fell 1974, 1983; Simpson 1984), surrounded by relatively normal somatic tissue containing choanocyte chambers. However, in some cases, oocytes appear aggregated or clustered in certain parts of the sponge tissue, and enclosed by loose mesohyl (e.g., Lévi 1956; Borojevic 1969; Kaye and Reiswig 1991). We found oocyte clustering in the most superficial part of the subpinacoderm of *Asbestopluma occidentalis*, quite close to the surface (see Chapter 4). Such location and disposition of oocytes may have further implications (see below), since oocytes were fertilised simultaneously. The rest of the studied species located the oocytes homogeneously within the entire mesohyl (see Chapters 2, 3, 6).

This study corroborated that the ultrastructure of oocytes is quite similar among demosponges, as noted by Fell (1983). Differences affected only to the oocyte size, and

quantity and composition of the yolk. Despite the different time required for growth, early-stage and mature oocytes studied in the current work were strongly similar in their appearance, content, and inner organization. The earliest recognizable oocytes were frequently slightly amoeboid; but became more or less ovoid or spherical as they grew on (see Chapter 1, 2, 3, 4, and 6), as known for other sponges (Fell 1983; Simpson 1984). Microvilli were usually observed in the surface of oocytes of *Axinella damicornis*, *Raspaciona aculeata*, and *Petrosia ficiformis*, also described for many other sponges (Fell 1983) and numerous invertebrates and vertebrates (Nørrevang 1968). The fate of those microvilli after fertilization remains unclear, but may be withdrawn after spawning like in many other animals (Nørrevang 1968).

All oocytes were nucleolate and showed a perinuclear region (of variable wideness, depending on the species) devoid of yolk inclusions and usually containing dictyosomes. Proteins and lipids constitute the major part of the organic reserves of the yolk (Adiyodi and Subramonian 1983). Yolk platelets appeared to show a remarkable uniformity in structure throughout the animal kingdom (Nørrevang 1968). The mature yolk platelet consists of a dense core, often embedded in a less dense matrix, and is surrounded by a membrane. Similar yolk inclusions occurred in *Raspaciona aculeata* and *Corticium candelabrum*, even though the membrane was not very obvious in both cases. However, yolk inclusions in *Axinella damicornis* and *Petrosia ficiformis* differed much from the structure mentioned above. Both types of yolk platelets were membrane-bound. But, whereas heterogeneous yolk platelets, similar to those of *P. ficiformis* have been reported in a number of cases (Fell 1983; Gaino et al. 1987; Gaino and Sarà 1994) -even though the multilayered yolk is characteristic of *Petrosia ficiformis*-, yolk granules similar to those of *A. damicornis* have never been observed to date. Nonetheless, as a role, yolk inclusions were very abundant in all the studied species, occupying most of the cytoplasm of the oocyte. There is little information in the literature available about the process of vitellogenesis in sponges. As developing oocytes endocytoses and/or synthesize yolk materials and their precursors, yolk accumulate in their cytoplasm. Auto-synthesis of yolk, as that described for instance in *Aplysina cavernicola* (Gallissian and Vacelet 1976), has been suggested to occur at some level in all the studied species. In *Axinella damicornis*, yolk appeared to be synthesized by digestion of endocytosed bacteria (see Chapter 6). The auto-synthetic yolk production is generally considered a primitive type of vitellogenesis, and it is

found in the less evolved members of each phylum (Adiyodi and Subramonian 1983; Eckelbarger 1994). Auto- and hetero-synthetic (yolk supplied by nurse or vitelline cells) types of vitellogenesis can occur in the same organism (Gremigni 1983). Yolk or their precursors derived in a large portion from nurse cells in *C. candelabrum*, *P. ficiformis*, *A. occidentalis,* and *Raspaciona aculeata*. In all these cases, contribution of auto-synthesis of yolk can not be ruled out. Oviparous sponges had traditionally been considered to have small eggs with exclusively auto-synthesized yolk (Fell 1983), which regard them as "ancestral" or "primitive". Lévi (1956) suggested that oviparity in sponges is the ancestral condition, as also postulated in many other animal groups (e.g., Fraipont et al. 1996). Nevertheless, this assumption appears to conflict with the output of some genetic approaches (e.g., Borchiellini et al. 2004). However, *Petrosia ficiformis* and *Raspaciona aculeata* are oviparous sponges with relatively large eggs, which combine auto- and hetero-synthetic production of yolk. Therefore, generalizations for the phylum are hard to be formulated.

Mitochondria appeared in large clusters in *P. ficiformis* and in small ones in *R. aculeata*. Both models had been described before in other demosponges (see Fell 1983 and Simpson 1984 for reviews). Mitochondrial clusters, from few units to an immense number of them, are known to occur in oocytes of both invertebrates and vertebrates (Nørrevang 1968), mainly because of the great energetic demand derived from the intense synthetic activity.

Polar bodies were observed only in *Corticium candelabrum* (by electron microscopy, see Chapter 2) and in *Petrosia ficiformis* (by light microscopy, see Chapter 3). Whereas polar bodies had been found using light microscopy in many cases (e.g., Lévi 1950; Tuzet and Paris 1965), they had remained unseen by electron microscopy to date, and still require further investigation.

- **Spermatogenesis and sperm**

The spermatozoa of sponges are diverse in both size and general morphology (Reiswig 1983). However, general reviews concerning the evolution of sperm morphology (Fawcett 1970; Baccetti 1982, 1984, 1986; Hodgson 1986) consistently regard sponge sperm as having round or conical shape, large nucleus, and few large mitochondria, all features that would correspond to a "primitive" status among sperm

morphologies. Even though Fell (1974) and Reiswig (1983) summarized several features that are supposed to appear in "modified" or "derived" spermatozoans (such as acrosomes, or modified shapes of the sperm head), such characteristics have been later unconsidered in many general reviews. More recently, "modified" sperm morphologies have been well described by TEM in a number of cases. Tripepi et al. (1984) observed an extremely elongated and V-shaped spermatid in *Crambe crambe*; likewise, Barthel and Detmer (1990) found an elongate V-shaped sperm in *Halichondria panicea*. Anakina and Drozdov (2001) described a non-flagellated sperm in *Leucosolenia complicata*. Such morphologies are explained by the fact that sperm morphology is preferentially related to the fertilisation mode and not to phylogenetic position (Franzen 1956; Fawcett 1970). However, so far the unique fertilisation mechanism known in sponges is irremediably mediated by a carrier cell (usually a choanocyte) (Fell 1989).

During the course of this thesis we have found both "primitive" and "modified" sperm morphologies. Among primitive sperms, several structures have appeared that are not regarded as primitive. The sperm of *Corticium candelabrum* and *Petrosia ficiformis* fitted into the "primitive" type of the sperm classification. They both were round cells with a large nucleus and few large mitochondria. However, both sperms also showed "derived" features: acrosomal (or proacrosomal) vesicles in *P. ficiformis*, and a true acrosome in *C. candelabrum*. Whereas the proacrosomal vesicles of the sperm of *P. ficiformis* were similar to the vesicles of cnidarians (Hinsch and Clark 1972; Boury-Esnault and Jamieson 1999) and other sponges, such as *Suberites massa* (Diaz and Connes 1980) and *Hymeniacidon caruncula* (Reiswig 1989), the sperm of *C. candelabrum* bore a true C-shaped acrosome, as many other homosclerophorids (Baccetti et al. 1986; Boury-Esnault and Jamieson 1999). The sperm morphology of *Corticium candelabrum* fitted entirely with the fertilisation mechanism, which in this case is mediated by a carrier cell (see Chapter 2). The sperm only needs to swim in the seawater, and thereafter be "captured" by the carrier cell (which is a choanocyte). Likely, and although *P. ficiformis* showed external fertilisation, the round shape of its sperm corresponds to the most common morphology found among organisms that show fertilisation in the seawater (Fawcett 1970).

"Modified" sperm morphologies were found in the species *Crambe crambe* and *Asbestopluma occidentalis*, both belonging to the order Poecilosclerida. The sperm of *C. crambe* was elongated and V-shaped, with a lengthened nucleus containing coiled-

compacted chromatin, a single mitochondrion, and an intracytoplasmic channel ("ciliary pit") that harboured the initial part of the flagellum. The flagellum was anchored to a basal body from which also a long cross-striated rootlet arose. The spermatozoon also showed a conical acrosome in the tip of the head surmounted over a subacrosomal rod. It was surprising when we found that the sperm of the phoronid *Phoronopsis harmeri* (Reunov and Klepal 2004) was extremely similar, revealing an exceptional case of adaptive convergence within the metazoans.

The sperm of *A. occidentalis* was equally elongated but not V-shaped. It showed also a lengthened nucleus with the apical area transformed into a hammer-head structure that provided a swollen appearance to the spermatozoon's tip. The nucleus was surmounted by few small acrosomal vesicles. The initial portion of the flagellum was also harboured in a ciliary pit. Not only was the sperm morphology of *A. occidentalis* surprising, but the cyst structure. Sperm was produced in cysts that were progressively enveloped by many layers of follicle cells. Cysts, as suggested by Vacelet (1996) and Vacelet and Boury-Esnault (1996), were supposed to be released intact and captured by a neighbour sponge, similarly to prey capture. Once trapped, bacteriocytes of the sponge (by means of enzymes secreted by bacteria) are hypothesized to disssolve the enveloping cells, so that the sperm is set free in the mesohyl of the to-be-fertilised sponge. As the layer underneath the pinacoderm is characterized by a great accumulation of collagen, the elongated sperm morphology is postulated to facilitate the progression in such a viscous and dense medium. The "massive" release of sperm that may occur inside the body of the sponge can explain the simultaneous fertilisation of an oocyte cluster. Similarly, the modified sperm morphology of *C. crambe* may be and adaptation for fertilisation alternative to that of carrier cells: the "V" shape (which is also an elongated shape) could enhance sperm movement through the viscous mesohyl. In such viscous medium, rotational abilities may improve the advance of the sperm. For that purpose, presumably, the proximal portion of the axoneme crosses the head of the sperm, favouring rotation of the whole V-shaped sperm when moving forward.

Even though the morphology of sperm is related more with fertilisation mechanisms than to phylogenetic position, the species-specific sperm morphology could be useful as a tool for an unequivocal identification of very similar species (Hodgson 1986).

The occurrence of "derived" sperm shapes and "derived" features within "primitive" sperms of such basal and simple metazoans starkly reflects the awesome potential of sponges, which is often unveiled and unconsidered because of the need of making generalizations to cover the wide range of the metazoan variety of morphologies and processes.

In most animals, the totality of sperm produced is not spawned, and sperm remnants are usually phagocytosed by somatic cells (Buckland-Nicks and Chia 1986; Pacey and Bentley 1992; Jørgensen and Lützen 1997; Kalachev and Reunov 2005), generally called Sertoli cells. Not only unspawned sperm is phagocytosed by Sertoli cells or Sertoli-like cells, but abnormal sperm (O'Donovan and Abraham 1987; Griswold 1995, 1998; Nakanishi and Shiratsuchi 2004). Aberrant or abnormal sperm has been rarely documented in sponges, with the exception of two-headed sperm of *Chondrilla australiensis* (Usher et al. 2004), and, in such case, no mechanisms of resorption of those sperm were observed. Motile phagocytic cells (MPCs) were found in spermatic cysts of *Raspaciona aculeata* and *Petrosia ficiformis* (see Chapter 7), engulfing sperm cells prior or after spawning. The complexity exhibited in the relationship between Sertoli cells and sperm cells in other organisms (e.g., Buckland-Nicks and Chia 1986; Griswold 1995) was not found in the studied sponges. However, MPCs in sponges appeared as the first step to achieve such complex relationship, functioning as simple removers of unspawned or abnormal sperm in those basal metazoans. Tracking the transformation of that mechanism in something more complex remain still very arduous, since scarce information about motile phagocytic cells -or Sertoli-like cells- in charge of elimination of sperm is available for many invertebrates.

- **Development in the studied sponges:**

This thesis did not directly deal with embryonic development. Nevertheless, the finding of direct development in *Petrosia ficiformis* was an important and intriguing discovery (see Chapter 3). There are very few cases reported of sponges in which the juveniles do not derive from a free-swimming larva (Watanabe 1978; Sarà et al. 2002). The reasons why these three particular species avoid the larval phase remain unknown, and further studies about the ecological and evolutive implications are required.

To conclude, poriferans conform to a very simple body plan (Bergquist 1978) but possess several complex features (Vacelet 1999), that are often unconsidered by general audience. Such complex features, together with simple structures or behaviours, appear at the cytological or histological level, as reported in the current work, as, for instance, hetero-synthetic vitellogenesis, true acrosomes, modified morphologies of sperm, or the ability of elimination of sperm excess or remnants by Sertoli-like cells. Therefore, assumptions and generalizations regarding the phylum Porifera must be approached with caution.