Mediterranean Marine Science

Vol 20, No 2 (2019)

Individualistic patterns in the budding morphology of the Mediterranean demosponge Aplysina aerophoba

JULIO A. DÍAZ, JUANCHO MOVILLA, PERE FERRIOL

doi: 10.12681/mms.19322

Short Communication

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
The journal is available on line at http://www.medit-mar-sc.net
DOI: http://dx.doi.org/10.12681/mms.19332

Individualistic patterns in the budding morphology of the Mediterranean demosponge *Aplysina aerophoba*

Julio A. DÍAZ[1, 2], Juancho MOVILLA[3] and Pere FERRIOL[1]

[1] Interdisciplinary Ecology Group, Biology Department, Universidad de las Islas Baleares, Palma, Spain
[2] Instituto Español de Oceanografía, Centre Oceanogràfic de Balears, Palma de Mallorca, Spain
[3] Instituto Español de Oceanografía, Centre Oceanogràfic de Balears, Estació d'Investigació Jaume Ferrer, Menorca, Spain

Corresponding author: julio.diaz@uib.es

Handling Editor: Eleni VOULTSIADOU

Received: 10 December 2018; Accepted: 18 April 2019; Published on line: 28 May 2019

Abstract

The external morphology of sponges is characterized by high plasticity, generally considered to be shaped by environmental factors, and modulated through complex morphogenetic pathways. This work shows for the first time that explants of the Atlanto-Mediterranean demosponge *Aplysina aerophoba* reared in aquaria under different pH and temperature conditions produce reproductive buds with a phenotype determined by the donor individual. These results suggest, therefore, that genotype may be an important factor controlling different phenotypes in this species.

Keywords: Mediterranean Sea; *Aplysina aerophoba*; Asexual reproduction; Morphogenesis; Sponge budding.

Introduction

Sponges are invertebrates with a relatively simple body design, formed by an external layer, the pinacoderm; an inner layer, the mesohyl; and an aquiferous system composed of canals and choanocyte chambers. The body structure is maintained with a network of supporting elements of organic (collagene fibres) and/or inorganic (calcium carbonate or silicon spicules) nature. This simplicity often results in an ill-defined, amorphous shape, which implies a lack of a clear set of morphological diagnostic characters. Moreover, sponge morphology is influenced by environmental conditions, such as exposure to sunlight, turbidity of water, currents, and depth (Bidder, 1923; Kaandorp & De Kluijver, 1992; McDonald *et al*., 2002); and modulated through complex morphogenetic pathways (Wiens *et al*., 2008). Therefore, the variability of the phylum Porifera challenges the delineation of high and low level taxa. Often, sympatric sibling species with minor morphological differences are masked under a "species complex" name (Blanquer & Uriz, 2008). Conversely, in other cases, high levels of intraspecific plasticity blurs the boundaries between populations and species (Schmitt *et al*., 2005).

Phenotypical differences are often obvious in characteristics such as colour, growing shape or branching pattern and may be related to degree of genetic proximity between conspecific individuals (Solé-Cava & Thorpe, 1986). This was tested on field experiments involving tropical sponges, in which the relationship between the phenotype and genetic identity of individuals was verified with graft histocompatibility bioassays (Hildeman *et al*., 1979; Jokiel *et al*., 1982; Neigel & Avisse, 1983; Neigel & Schmahl, 1984; Wulff, 1986; Wulff, 1991). For instance, Wulff (1986) found that individuals with a more breakable branching morphology (i.e. individuals with narrower branches) were associated to fusion groups with more members than other individuals with more robust branches. These results suggest that the dispersal capacity could not only be inferred from the clonal phenotype but also influenced by it. Then, there must be a central role in phenotypes related to the expression of asexual characters, such as those that modify the morphology of propagules.

Polymorphism related to the budding process has been described and studied in other demosponges, especially in the genus *Tethya* (Selenka, 1879; Connes, 1967; Corriero *et al.,* 1996; Hammel *et al.*, 2009). However, information regarding the order Verongiida is scarce. Representatives of this group are characterized by exhibiting high levels of phenotypical variation, being able to reproduce either sexually by oviparity or asexually by means of bud production and fragmentation, and being of high ecological and biomedical importance (Bergquist & Cook, 2002). Here, we present a first preliminary observation indicating that a clone-specific genotype drives

budding morphology in the Atlanto-Mediterranean verongiid *Aplysina aerophoba* (Nardo, 1833), made during an experiment designed to test the potential response of this species to future acidification and global warming.

Material and Methods

In February 2018, five large donor individuals of *A. aerophoba,* separated from each other by more than fifty metres, were collected in Alcudia Bay (Mallorca, Spain) and transported to the aquaria facilities of "Jaume Ferrer" Research Station (Menorca, Spain). Twenty-five explants were cut off from each donor individual and distributed in five 90 l aquaria with identical conditions of salinity (37.5 PSU), light exposure (86 lux in a 12:12 h cycle), and water renewal (60 ml/min). Seawater was continuously mixed with submersible pumps – Eheim Aquaball 60 calibrated to 150 l/h – ensuring similar recirculation and water current in all aquaria. Different pH and temperature conditions were applied in each aquarium as experimental treatments: (i) Tank 1, pH 8.1, T 20 °C; (ii) Tank 2, pH 8.1, T 25 °C; (iii) Tank 3, pH 8.1, T 30 °C; (iv) Tank 4, pH 7.8, T 20 °C; (v) Tank 5, pH 7.6, T 20 °C. Temperature conditions were chosen based on natural seasonal trends observed in the collection area, while pH conditions simulated the current global pH in surface waters and the predicted changes for the year 2100, according to different IPCC scenarios (Bates *et al*., 2014). The initial temperature and pH values at the beginning of the experiment (in March 2018) were 17 °C (± 0.5 °C) and 8.1 (± 0.03 pH units, total scale), respectively. Treatment conditions were reached progressively during a one-month acclimation ramp and then kept constant for three months until the end of the experiment, in June 2018.

Each explant was properly tagged and photographed monthly to assess its morphological status and evolution over time. The photographs were always taken with the explants located in the same place; a plastic grill was placed at the bottom of the tanks and, since the sponges were supported on a methacrylate base fitted in the grill, positions were replicable monthly. Maximum length of randomly chosen buds from Tank 1 (n= 43), Tank 2 (n= 46), Tank 3 (n= 40), Tank 4 (n= 43), and Tank 5 (n=39) were measured from pictures taken at the end of the acclimation time (Month 1), and then maximum length of the same buds was measured from pictures taken after one month of experiment (Month 2), using the FIJI software (Schindelin *et al*., 2012). Growth rate was calculated as the difference in total length of the buds between the first and second month. *In situ* photographs of random individuals were also taken in the sampling area during December 2017 and August 2018 (Fig. 1).

Growth rate data were transformed with a root square-function and tested for assumptions of normality and homoscedasticity using the Kolmogorov-Smirnov and Levene tests, respectively. A one-way ANOVA was performed to assess differences between treatments in growth rate of the buds, and means were compared between groups by Tukey's HSD post hoc test. Finally, to identify the dominant factor explaining the variability observed between treatments, a two-way ANOVA, with pH and temperature as fixed factors, was applied. Data were analysed using JMP 9.0.1 software (SAS Institute Inc., Cary, NC, USA).

Results

Morphological patterns emerged at the end of the acclimation ramp, after one month of rearing. At that time, the explants began to produce buds and maintained their

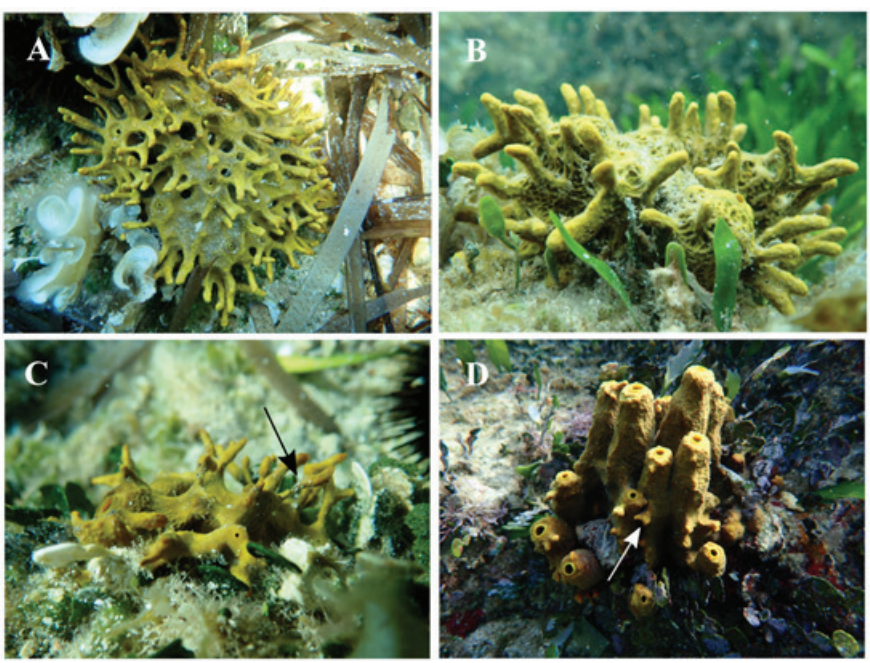

Fig. 1: *In situ* photographs of *A. aerophoba*. A-C, late summer appearance; D, winter appearance. Arrow in C, bud point of fissure; arrow in D, small incipient bud.

unique morphological traits until the end of the experiment, three months later. Differences in form, size, and shape of buds defined five patterns, each one clearly distinguishable macroscopically and only sharing in the clones deriving from the same donor individuals. Variability was expressed in the form of: (i) flattened branches with little hand-like lobules in T clones; (ii) long, elongated, breakable projections in V clones; (iii) single, thick digitations in X clones; (iv) divergent branches that dichotomized from a thin root in Y clones; and (v) finger-like branches that dichotomized from a thick root in Z clones (Fig. 2). It is remarkable that the specific clonal expression of the phenotypes was uniformly observed, regardless of treatment conditions.

Significant differences between treatments were observed in the growth rate of the buds (ANOVA one-way, $F_{4, 209} = 48.7130$, $p < 0.001$). Average growth rate (mean ± standard error of the mean, SE) was 7.9 ± 0.3 mm month^{-1} for buds in Tank 1; 12.8 ± 0.5 mm month^{-1} in Tank 2; 14.1 ± 0.6 mm month^{-1} in Tank 3; 6.8 mm ± 0.4 month^{-1} in Tank 4; and 7.9 ± 0.5 mm month^{-1} in Tank 5. The ANOVA two-way analysis subsequently applied to our results indicated that the significant differences between treatments observed in the growth rate of the buds were due to the effect of temperature ($F_{2,209} = 43.8851$, $p < 0.001$), rather than pH ($F_{2,209} = 0.2346$, $p = 0.0765$).

In addition, during the second half of the experiment, some of the buds were released and adhered to the supporting grid or other surfaces of the aquaria, via newly formed tissue or exposed spongin fibres (Fig. 3).

Discussion

The budding process in *A. aerophoba* has been previously documented, in both natural and *ex situ* conditions (Vacelet, 1959; Gerçe *et al*., 2009). The temperature effect on bud development observed *ex situ* is analogous to what happens with this species in the wild, where summer conditions promote the formation of buds (Fig. 1, A-C) and, presumably, later autumn storms may contribute to asexual dispersal by breaking and transporting the buds generated (Battershill & Bergquist, 1990). However, how bud polymorphism affects demographic processes and traits such as asexual dispersal, success in the establishment of new colonies, or genetic variability in populations, remains unknown.

In natural conditions, where sponges are exposed to currents and waves, budding architecture could be essential to determine recruitment success. On the one hand, thinner buds are more prone to breakage than thicker ones. On the other hand, heterogeneity in sizes and forms may influence the dispersion capacity of propagules in the water column by affecting characteristics such as den-

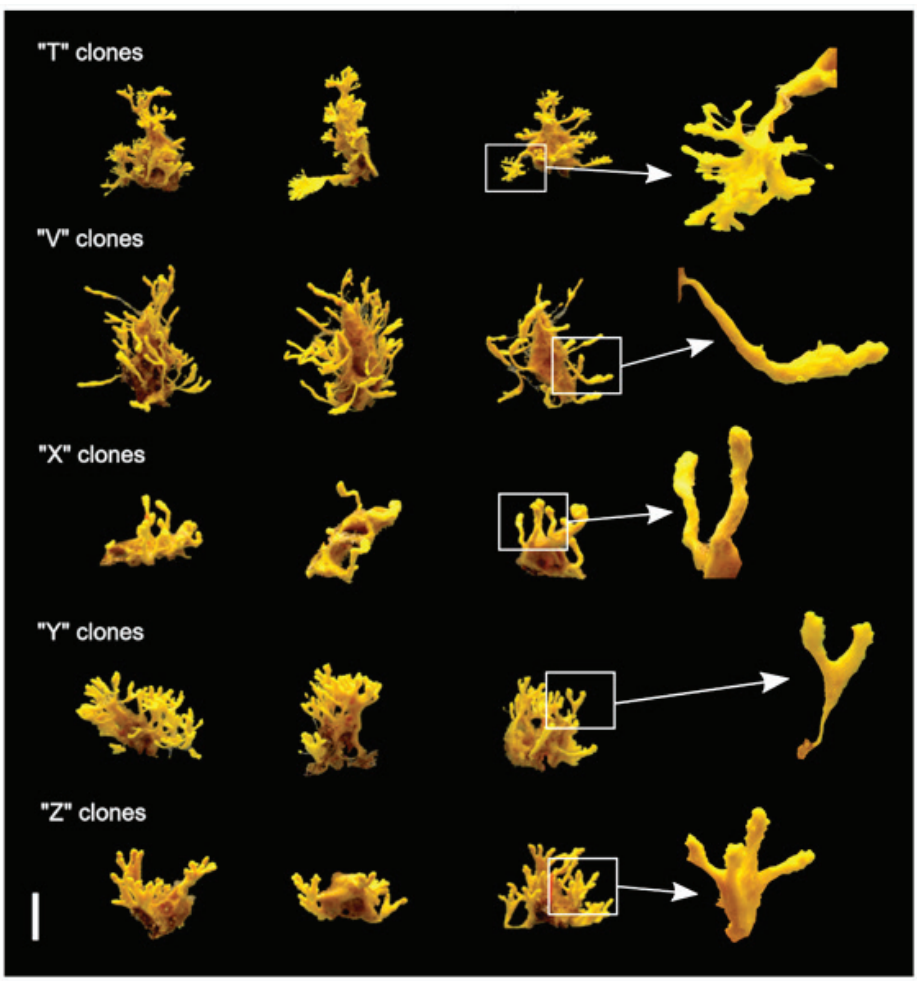

Fig. 2: Budding patterns shown by the different explants reared in aquaria. Letters indicate the donor individual. Scale bar, 3 cm.

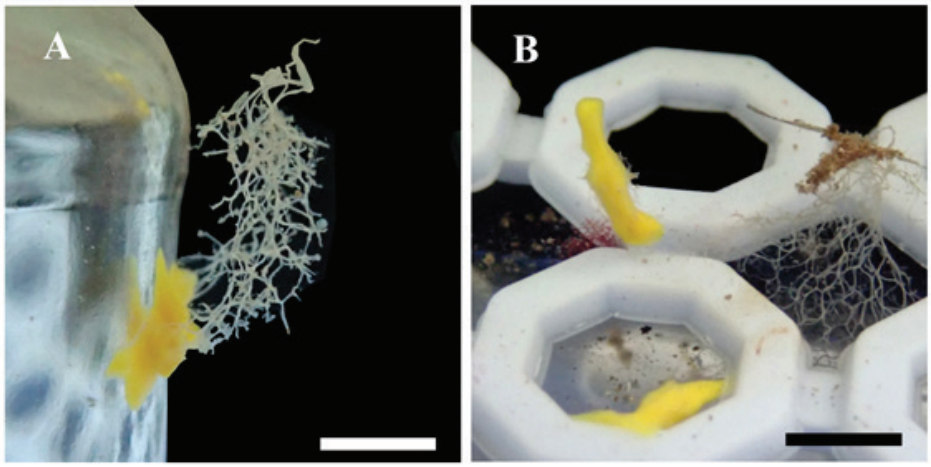

Fig. 3: Broken buds adhered to different elements of the experimental tanks, with newly formed tissue in A, and adhered to a grid via exposed spongin fibres in B. Scale bar in A and B, 1 cm.

sity, volume, effective surface and, consequently, buoyancy. Finally, differences in bud morphology could determine settlement efficiency by increasing or decreasing the tendency of buds to adhere to certain substrates.

The distinct phenotypes of buds in our study were uniformly observed regardless of the aquaria treatment conditions, suggesting that in *A. aerophoba*, the morphology of buds could have an intrinsic genetic factor. Correlation between donor individual and clone morphology has been previously reported in other demosponge species, with clonal-diagnostic traits, like colour and branching pattern, proposed as indicators of physiologically independent individuals (Neigel & Schmahl, 1984; Wulff, 1986). However, this is the first time that a phenotype related to an asexual reproductive process has been observed in *A. aerophoba*, a fact that may provide insights into the study of reproduction, population dynamics, and morphogenesis of the group. The use of controlled aquaria allowed us to eliminate the effects of biotic and abiotic factors that affect sponge growth and to study phenotype expression without the bias induced by these factors. Further research will determine how polymorphic the budding phenotypes are in natural populations, and how this affects dispersion success and the population structure of the species.

Acknowledgements

This work was co-funded by the *Direcció General d'Innovació i Recerca del Govern de les Illes Balears* through the special action AAEE038/2017 (MultiCaB) and FEDER. The authors are grateful to M. Elena Cefalì, Ignacio Bolado, Guillem Mateu, Xavier Busquets, Joan Cabot, and Albert Fuster for their help and field assistance. The Research Station "Jaume Ferrer" is co-funded by the *Govern de les Illes Balears* and the Spanish Oceanographic Institute. We are also grateful to the *Laboratorio de Investigaciones Marinas y Acuicultura* (LIMIA) for their technical support and to Dr Nona Agawin for lending the trolley. The authors would also like to thank the two anonymous reviewers for their comments and suggestions that improved the original manuscript.

References

Bates, N.R., Astor, Y.M., Church, M.J., Currie, K., Dore, J. E., *et al.* 2014. A Time-Series View of Changing Surface Ocean Chemistry Due to Ocean Uptake of Anthropogenic CO_2 and Ocean Acidification. *Oceanography*, 27 (1), 126-141.

Battershill, C.N., Bergquist, P.R., 1990. The influence of storms on asexual reproduction, recruitment, and survivorship of sponges. New perspectives in sponge biology. Smithsonian Institution Press, Washington, DC, 397-403

Bergquist, P.R., Cook, S.D.C., 2002. Order Verongida. p. 1081-1096. In: *Systema Porifera: A Guide to the Classification of Sponges*, Vol. I. Hooper J. N. A., & Van Soest R. W. M., (Eds). Kluwer Academic/Plenum Publishers, New York.

Bidder, G.P., 1923. The relation of the form of a sponge to its currents. *The Quarterly Journal of Microscopical Science*, 67, 293-323.

Blanquer, A., Uriz, M.J., 2008. 'A posteriori' searching for phenotypic characters to describe new cryptic species of sponges revealed by molecular markers (Dictyonellidae: Scopalina). *Invertebrate Systematics*, 22 (5), 489-502.

Connes, R., 1967. Structure et developpement des bourgeons chez l'éponge siliceuse Tethya lyncurium Lamarck. Recherches experimentales et cytologiques. *Archives de zoologie Expérimentale et générale*, 108, 157-195.

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 (2), 175-181.

Gerçe, B., Schwartz, T., Voigt, M., Rühle, S., Kirchen, S. *et al.,* 2009. Morphological, bacterial, and secondary metabolite changes of Aplysina aerophoba upon long-term maintenance under artificial conditions. *Microbial ecology*, 58 (4), 865.

Hammel, J.U., Herzen, J., Beckmann, F., Nickel, M., 2009. Sponge budding is a spatiotemporal morphological patterning process: Insights from synchrotron radiation-based x-ray microtomography into the asexual reproduction of Tethya wilhelma. *Frontiers in zoology*, 6 (1), 19.

Hildemann, W.H., Johnson, I.S., Jokiel, P.L., 1979. Immunocompetence in the lowest metazoan phylum: transplantation immunity in sponges. *Science*, 204 (4391), 420-422.

Jokiel, P.L., Hildemann, W.H., Bigger, C.H., 1982. Frequency of intercolony graft acceptance or rejection as a measure of population structure in the sponge Callyspongia diffusa. *Marine Biology,* 71 (2), 135-139.

Kaandorp, J.A., De Kluijver, M. J., 1992. Verification of fractal growth models of the sponge Haliclona oculata (Porifera) with transplantation experiments. *Marine Biology*, 113 (1), 133-143.

McDonald, J.I., Hooper, J.N., McGuinness, K.A., 2002. Environmentally influenced variability in the morphology of Cinachyrella australiensis (Carter 1886) (Porifera: Spirophorida: Tetillidae). *Marine and Freshwater Research*, 53 (1), 79-84.

Neigel, J. E., Avise, J.C., 1983. Histocompatibility bioassays of population structure in marine sponges: Clonal structure in Verongia longissima and lotrochota birotulata. *Journal of Heredity*, 74 (3), 134-140.

Neigel, J.E., Schmahl, G.P., 1984. Phenotypic variation within histocompatibility-defined clones of marine sponges. *Science*, 224, 413-415.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M. *et al*., 2012. Fiji: An open-source platform for biological image analysis. *Nature Methods*, 9 (7), 676-682.

Schmitt, S., Hentschel, U., Zea, S., Dandekar, T., Wolf, M., 2005. ITS-2 and 18S rRNA gene phylogeny of Aplysinidae (Verongida, Demospongiae). *Journal of Molecular Evolution*, 60 (3), 327-336.

Selenka, E., 1879. Ueber einen Kieselschwamm von achtstrahligem Bau, und über Entwicklung der Schwammknospen. *Zeitschrift für wissenschaftliche Zoologie*, 33, 467-476.

Solé-Cava, A.M., Thorpe, J.P., 1986. Genetic differentiation between morphotypes of the marine sponge Suberites ficus (Demospongiae: Hadromerida). *Marine Biology*, 93 (2), 247-253.

Vacelet, J., 1959. Répartition générale des éponges et systématique des éponges cornées de la région de Marseille et de quelques stations méditérrannéennes. *Recueil des Travaux de la Station marine d'Endoume,* 16, 39-101.

Wiens, M., Belikov, S.I., Kaluzhnaya, O.V., Adell, T., Schröder, H. C. *et al*., 2008. Regional and modular expression of morphogenetic factors in the demosponge Lubomirskia baicalensis. *Micron*, 39 (4), 447-460.

Wulff, J.L., 1986. Variation in clone structure of fragmenting coral reef sponges. *Biological Journal of the Linnean Society. Linnean Society of London*, 27, 311-330.

Wulff, J.L., 1991. Asexual fragmentation, genotype success, and population dynamics of erect branching sponges. *Journal of Experimental Marine Biology and Ecology*, 149 (2), 227-247.