MEETING REPORT

JEZ-B MOLECULAR AND DEVELOPMENTAL EVOLUTION WILEY

From the individual to the colony: Marine invertebrates as models to understand levels of biological organization

Laurel S. Hiebert[1,2] (ID) | Leandro M. Vieira[3] | Stefano Tiozzo[4] (ID) | Carl Simpson[5] (ID) | Richard K. Grosberg[6] | Alvaro E. Migotto[1,2] | Andre C. Morandini[1,2] | Federico D. Brown[1,2,7] (ID)

[1]Departamento de Zoologia, Instituto Biociências, Universidade de São Paulo, São Paulo, Brazil

[2]Centro de Biologia Marinha (CEBIMar), Universidade de São Paulo, São Sebastião, Brazil

[3]Laboratório de Estudos de Bryozoa (LAEBry), Departamento de Zoologia, Centro de Biociências, UFPE, Cidade Universitária, Recife, Brazil

[4]Sorbonne Université, CNRS, Laboratoire de Biologie du Développement de Villefranche-sur-mer (LBDV), Paris, France

[5]Department of Geological Sciences and Museum of Natural History, University of Colorado, Colorado, USA

[6]Coastal and Marine Sciences Institute, University of California Davis, Davis, California, USA

[7]Instituto Nacional de Ciência e Tecnologia em Estudos Interdisciplinares e Transdisciplinares em Ecologia e Evolução (IN-TREE), Salvador, Bahia, Brazil

Correspondence
Alvaro E. Migotto, Andre C. Morandini, and Federico D. Brown, Departamento de Zoologia, Instituto Biociências, Universidade de São Paulo, Rua do Matão. Travessa 14. no. 101, 05508-090, São Paulo, Brazil.
Email: aemigott@usp.br and acmorand@ib.usp.br or fdbrown@usp.br

Abstract

The developmental and evolutionary principles of coloniality in marine animals remain largely unexplored. Although many common traits have evolved independently in different groups of colonial animals, questions about their significance for colonial life histories remain unanswered. In 2018 (Nov. 25 - Dec. 8), the inaugural course on the *Evolution of Coloniality and Modularity* took place at the Center for Marine Biology of the University of São Paulo (CEBIMAR-USP), Brazil. During the intensive two-week graduate-level course, we addressed some of the historical ideas about animal coloniality by focal studies in bryozoans, tunicates, cnidarians, and sponges. We discussed many historical hypotheses and ways to test these using both extant and paleontological data, and we carried direct observations of animal colonies in the different phyla to address questions about coloniality. We covered topics related to multi-level selection theory and studied colonial traits, including modular miniaturization, polymorphism, brooding, and allorecognition. Course participants carried out short research projects using local species of animals to address questions on allorecognition and regeneration in ascidians and sponges, fusion and chimerism in anthoathecate hydrozoans, and evolution of polymorphism in bryozoans. Although many questions remain unanswered, this course served as a foundation to continue to develop a developmental and evolutionary synthesis of clonal and modular development in colonial marine organisms.

KEYWORDS
allorecognition, ascidians, bryozoans, budding, clonality, cnidarians, modularity, polymorphism, regeneration, sponges, tunicates

1 | INTRODUCTION

Major transitions in evolution have been associated with nested hierarchical levels of biological organization (Smith & Szathmáry, 1995; Szathmáry & Smith, 1995). Most transitions are rare, such as the origin of multicellularity, or unique, such as the origin of eukaryotes. As a consequence, those major evolutionary transitions can be difficult to study. Transitions that occur in animals (i.e., the evolution of multicellularity, the evolution of sociality, or the evolution of coloniality) offer a unique opportunity to understand how evolutionary processes affect each of these nested levels of organization because the transitions are common and repeat in different lineages and so we can bring the full force of comparative methods to bear on the problem. If a hierarchical organization represents an innate property of vertical complexity—i.e. nested levels of biological organization—in biological systems, organisms may evolve and transition among the distinct levels of organization using some common underlying evolutionary principles. Whereas horizontal levels of complexity –as defined by the parts within a single level of organization such as the number of genes in an organisms (e.g., ca. 30,000 genes in humans) or number of cells in an organism (e.g., ca. 1000 cells in *Caenorhabditis elegans*)– are represented by quantifiable units (Duclos et al., 2019), and consequently represent easy characters to assess for change during evolutionary transitions, the processes acting on the evolution of vertical complexity have only recently started to gather empirical evidence (McShea, 2017). For example, a recent hypothesis suggests the existence of a complexity ratchet mechanism that may be allowing more complex phenotypes to irreversibly evolve, resulting in the evolution of complex solutions to natural selection, which under some circumstances, are favored over simpler ones (Liard et al., 2020; Simpson, 2020, in this issue). Whether these mechanisms can be applied across the different major evolutionary transitions in animals, which affect the levels of biological organization remains to be empirically tested, and colonial organisms that have transitioned among vertical levels of complexity may provide important insights to these newly proposed mechanisms.

The evolutionary, developmental, and biological consequences of clonality—vegetative propagation—and colonial life histories in animals deserve special attention. The biology of clonal organisms has been studied in plants, protists, and fungi, but less studied in animals. What are the consequences of coloniality on the fitness of an organism? Why do some colonial clades evolve division of labor, but not others? What are the links between organismal modularity and phenotypic plasticity? In animals, clonal propagation by fission and regenerative developmental processes have received some attention; however, the study of budding processes and animal coloniality are scarce and have only been studied in a handful of species. How do embryological developmental mechanisms contrast to post-embryonic/adult developmental processes in colonial species?

To seek a better understanding of the evolutionary and biological aspects of coloniality as a life history strategy, we brought together in 2018 (November 25—December 8) specialists and students to undertake the challenge of synthesizing and integrating data of the biology (physiology, ecology, and development) and evolution (genetics of clonality and selection) of colonial marine animals in a 2-week intensive graduate-level course entitled *Evolution of Coloniality and Modularity (ECM)*. The first edition of the course included 8 instructors from 3 different countries (5 Brazil, 2 United States, and 1 France), and 11 students from Brazil (6), Russia (3), and United States (2) (Figure 1). A resulting product of this inaugural course was the publication of this Special Issue, where several


FIGURE 1 Inaugural international course on "Evolution of Coloniality and Modularity" that took place at the Center for Marine Biology, University of São Paulo (CEBIMar), São Sebastião, Brazil, on November 25–December 8, 2018. (a) Course flyer. Credits: L. Hiebert (design), A. Migotto (flyer photographs), Cifonauta—Marine Biology Image Database—http://cifonauta.cebimar.usp.br/. (b) Course participants from left to right: A. Pauda (UFRJ, BR), B. Luz (UFPA, BR), C. Vaga (USP, BR), E. Belikova (SPBU, RU), R. Weinberg (SFSU, US), A. Kvach (SPBU, RU), C. Hernandez (SFSU, US), W. Kutyumov (SPBU, RU), S. Tiozzo (CNRS, FR), J. Lawley (USP, BR), L. Hiebert (USP, BR), R. Grosberg (UC Davis, US), F. Brown (USP, BR), C. Simpson (U. Colorado, US), A. Migotto (CEBIMAR-USP, BR), L. Vieira (UFPE, BR), E. Gamero (USP, BR), and A. Morandini (USP, BR, not shown) [Color figure can be viewed at wileyonlinelibrary.com]

instructors have contributed. This theoretical and practical course took advantage of the infrastructure—easy access to many marine colonial animals—provided at the Center for Marine Biology of the University of São Paulo (CEBIMAR-USP). Another focus of the course was to progressively accumulate knowledge of experimental methodologies or specialized techniques to investigate metazoan coloniality, and eventually enable us to continue in-depth studies on fundamental aspects of the life cycles of common colonial marine species of the Brazilian South Atlantic, as well as to gradually generate a local critical mass of highly skilled researchers to explore and disseminate research on animal coloniality. The next edition of the course is tentatively scheduled for December 2021. We are looking forward to welcoming new students and instructors, and their challenging approaches to expand our understanding of the fascinating life of colonial animals.

Using a theoretical and practical approach and based on the principle of problem-based learning (PBL) the inaugural course focused on the study of fundamental aspects of life cycle evolution of colonial marine animals. Course activities included lectures, collecting trips, demonstrations of laboratory, and analytical methods to study colonial animals, including preparation of cultures, documentation of asexual and clonal development using microphotographs or illustrations. The first part was composed of readings of primary literature followed by group discussions on definitions of coloniality, modularity, functional morphology, and genetics of clonality. We also covered topics related to multilevel selection theory, and the evolutionary consequences of coloniality, including miniaturization, polymorphism, allorecognition, and brooding. The second part was dedicated to practical activities and experiments, with instructors proposing different questions, related to a major challenge in the study of coloniality. Course instructors provided background on the biology of specific taxa that were studied in the course, including cnidarians, bryozoans, tunicates, and sponges. Course participants collected data and recorded observations (mini-projects), based on local fauna, to answer those primary questions. A few research topics that were selected by students during the course for their mini-projects included: allorecognition and regeneration in ascidians and sponges, coloniality in anthoathecate hydrozoans, and polymorphism in bryozoans (Figure 2). In the following sections, we will report on some of the projects, arranged by topic, providing a general background on the research questions, commenting on the approaches and animal models used, and providing prospects for future studies.
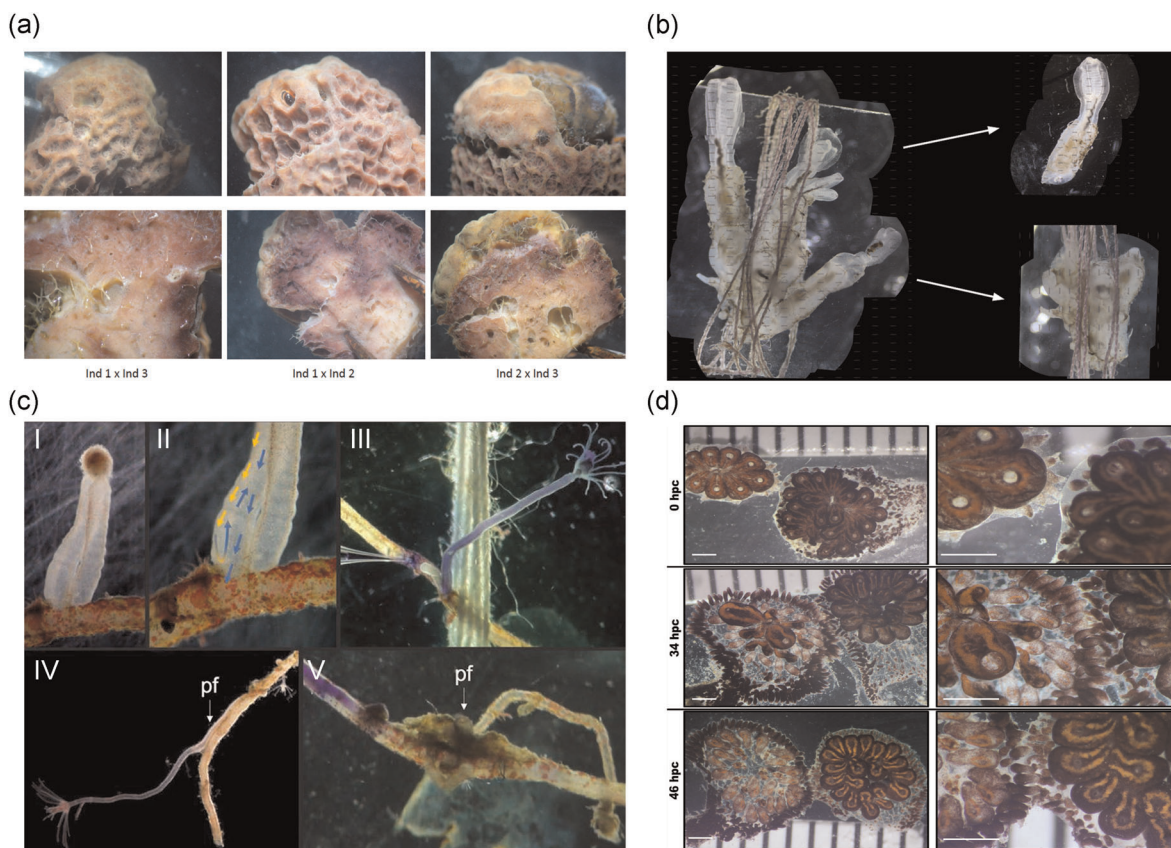
FIGURE 2 Images produced by the students as examples of some of the projects developed by them during the course. (a) Allorecognition of adult individuals of the demosponge *Aplysina fulva*, by A. Padua; (b) regeneration in two colonial ascidians, *Clavelina oblonga* and *Perophora viridis*, by V. Kutyumov, A. Kvach, and E. Belikova; (c) return to coloniality: an example from Hydrozoa, by C. Vaga and B. Luz; (d) visual characterization of allogeneic interactions in botryllids, by R. Weinberg and C. Hernandez [Color figure can be viewed at wileyonlinelibrary.com]

2 | STUDYING REGENERATION AND BUDDING TO UNDERSTAND THE EVOLUTION OF COLONIALITY

The ECM course focused exclusively on marine invertebrates, where colonial species are relatively abundant. On the basis of the widespread phylogenetic distribution of marine colonial forms, coloniality is likely to have evolved independently from solitary ancestors and within many separate phyla (Hiebert et al., 2020, in this issue; Maynard-Smith, 1978). How these transitions repeatedly occurred in evolutionary history is an unanswered question. A current view is that coloniality may have evolved from incomplete asexual reproduction; that is, colonies arose from a solitary ancestor that gained the ability to asexually-reproduce (i.e., clone itself) and subsequently stopped the process before the individuals completely separated from each other (Beklemishev, 1969; Boardman et al., 1973; Jackson, 1977). Much of this thinking could perhaps be traced back to Beklemishev (1969), who described stages in the origins of coloniality, from relatively independent modules to more integrated forms with enhanced connection between each new module (Beklemishev, 1969). This origin story for colonies—one of increasing complexity and interconnection between originally clonal animals—is treated as an assumption, often being used to define colonial organisms themselves. However, there is no strong evidence that incomplete cloning underlies every evolutionary transition to coloniality (Hiebert et al. 2020, in this issue).

One group of ECM students, V. Kutyumov, A. Kvach, and E. Belikova (Saint Petersburg State University), examined an alternative hypothesis to explain the evolution of coloniality based on the origin of differential somatic growth between major body parts (Marfenin, 1993). The transition from solitary to colonial may have come about due to constraints that limit the size of the feeding structures, for example, with no similar constraints on the basal structures. Thus, the basal region could maintain normal somatic growth while the anterior end would only be able to increase in size by another form of somatic growth—the addition of repeated units in a process akin to metamerism as described in the evolution of some segmented animals and plants. In this scenario, coloniality would have arisen in a solitary ancestor by the sprouting of multiple anterior ends, equipped with feeding organs, and allowing for indefinite growth of the other (the basal) end. Thus, this "somatic growth" hypothesis for the origin of colonies does not invoke any type of asexual reproduction.

In their project, the students postulated that coloniality within animals could have evolved in taxa with high regenerative capacity. Mechanisms for regeneration have been theorized to have been recruited/modified in the origins of agametic development (Sköld & Obst, 2011). Thus, in the context of the "somatic growth" hypothesis, the ancestor may have had a high regenerative ability to replace the upper portion of the body, which could have led to the evolutionary potential for adding additional upper regions. If this was true, then one might predict that current colonial forms would have a higher regenerative capacity for replacing anterior ends from the base, but

not for regrowing basal structures from the anterior ends. The working group tested this prediction by performing basic regeneration experiments in two species of colonial ascidians: *Clavelina oblonga* and *Perophora viridis*.

The mechanisms in the transition to coloniality are essential for understanding the homologies of the colonial organisms with their solitary counterparts. For example, according to the "incomplete asexual reproduction" hypothesis, zooids/polyps would be homologous to the whole body of the solitary ancestor. However, according to the "somatic growth" hypothesis, the zooids/polyps would be homologous to the upper portion of the ancestor, while the basal structure of the colony as a whole, would be homologous to the basal region of the ancestor. Further research could test for regenerative potentials of distinct body parts or distinct regions of the colony to reconstruct ancestral traits.

3 | ALLORECOGNITION: LIVING IN A WORLD OF CELLULAR FUSION AND REJECTION

Permanent competition for space characterizes the life of sessile marine organisms. At least in one phase in their life cycle, many marine invertebrates—such as sponges, bryozoans, cnidarians, or ascidians—live on hard substrates either on the seafloor or on floating objects. When a species comes into contact with another sessile species, they generally compete to deter the growth of the allospecific. However, when two members of the same species come into contact, they can grow side by side without any interference, deter the growth of the conspecific, or fuse. Many of the rules and genetic bases that govern self- versus nonself recognition of conspecifics were originally untangled in the colonial ascidian *Botryllus schlosseri* and in the hydrozoan *Hydractinia symbiolongicarpus*, and most recently in the demosponge *Amphimedon queenslandica* (for recent review see Nicotra, 2019).

Comparative studies of allorecognition in several species of botryllid ascidian colonies have shown that the site of recognition (i.e., outer tunic, tunic, ampullar systems, or vasculature of the colonies) vary considerably among species; where cells in the outer tunic would be derived, and cells of the vasculature would be ancestral (Cohen et al. 1998; Saito & Watanabe 1982). Taking advantage of the great diversity of colonial ascidian species on the northern coast of São Paulo state (SE Brazil), course participants R. Weinberg and C. Hernandez (San Francisco State University, SFSU) set out to continue to validate these observations on three additional botryllids, as well as in two sister-group species (i.e., *Symplegma rubra* and *S. brakenhielmi)*. Identifying the cell types and sites of allorecognition in this group may reveal interesting scenarios for how ancestral immune cells may have been co-opted for colonial-specific functions, such as allorecognition (Cohen et al. 1998). Other colonial-specific characters—where immune cells are presumably involved— that vary among species of botryllids and *Symplegma* include oviparity versus viviparity (Okuyama et al., 2002) and synchrony versus

asynchrony in the cycles of zooid replacement (Gutierrez & Brown, 2017). Mapping variable characters in the phylogeny of this group (for a phylogeny of Brazilian botryllids and *Symplegma;* see Ferreira, 2007) has important implications for understanding the directionality of character transitions during the evolution of higher orders of biological organization.

Although the colonies of hydrozoans are typically produced by a continuous process of budding new polyps from older ones that remain physically connected, recent research in the hydrozoan *Ectopleura larynx* demonstrated that after an initial budding phase the full-sized colony is achieved by the aggregation and fusion of non-clonal-produced polyps (Chang et al., 2018). In this species, sexually produced embryos are brooded within female gonophores, which develop into a unique dispersive larval stage: the actinula. These larvae are ready to settle as soon as released, many of them settling on the parental colony. At the point of attachment, the epithelia of the just-settled polyp and the parental colony fuse resulting in a common gastrovascular cavity shared throughout the entire colony (Nawrocki & Cartwright, 2012). This process results in the formation of a chimeric colony, containing genetically distinct polyps (Chang et al. 2018). One group of ECM students (C. F. Vaga and B. L. P. Luz from USP) investigated whether chimerism was common in a cryptogenic congeneric hydrozoan species: *Ectopleura crocea.* Not being able to carry out a genetic approach during the short period of the course, they decided to document by direct observations the fusion events of adult polyps with actinulae, comparing actinulae from the same and different parental colonies. Preference of offspring to settle on parental colonies (philopatry) may indicate that allorecognition mechanisms may be operating. Whether fusion events in *E. crocea* resulted in complete chimerism as in *E. larynx* or resulted only in the partial fusion of two or more colonies was also evaluated by the group by direct observation of each fusion event. Fusion events could either involve the fusion and formation of a common gastrovascular cavity suggesting complete chimerism or alternatively occur only at the level of the perisarc (exoskeleton) suggesting partial fusion. The study of these traits has implications at the evolutionary, developmental, and ecological levels and is thus essential to understanding coloniality in Hydrozoa.

Although recurrent reports of fusion, followed by the formation of chimeric sponges—or alternatively rejection—have been documented in the literature for demosponges in four distinct orders (i.e., Haplosclerida, Axinellida, Poecilosclerida, and Astrophorida) (Hildemann et al., 1980; Buscema & van de Vyver, 1983; Fernandez-Busquets & Burger, 1999; Gaino et al., 1999), the extent of fusion events or the level of variation in the time of fusion observed within conspecifics has raised questions about tissue or cell type-specific control in allorecognition. Using transplantation experiments in the demosponge *Aplysina fulva*, course participant A. Padua (Universidade Federal do Rio de Janeiro, UFRJ) set out to test how different regions (i.e., tissues and cell types) of the sponge responded to fusion events. These set of experiments are interesting not only because they will allow evaluating whether distinct regions of the sponge show different levels of allorecognition responses but also

because this species belongs to the Verongida, an order of demosponges that presents an organic collagen fibrous skeleton, rather than the inorganic spicules of siliceous demosponges. It would be intriguing to continue to investigate how skeletal components affect allorecognition in sponges, perhaps combining experiments of members of different groups of sponges.

4 | DIVISION OF LABOR AND POLYMORPHISM IN ANIMAL COLONIES: WHY DO SOME COLONIAL CLADES EVOLVE DIVISION OF LABOR, BUT NOT OTHERS?

Many colonial marine invertebrates undergo asexual budding processes resulting in the formation of physically connected modular animals. This modular architecture has important consequences for the evolutionary potential of the colony and provides advantages for sharing resources (Harvell 1994; Hiebert et al. 2020; Hughes, 2005). Colonial animals often show functional variation of modules, resulting in polymorphs, with phenotypically distinct zooids. Among colonial invertebrates, bryozoans comprise the only phylum with almost exclusively colonial representatives and include many taxa with a high degree of colony polymorphism. Despite an independent origin of bryozoan polymorphs, morphological categories have been assigned based on structural and functional similarities (Lidgard et al., 2012). Bryozoan polymorphs that have different functions other than feeding are called heterozooids, and among these avicularia (zooids for active defense) are the most common example with structural similarities that evolved independently multiple times (Carter et al., 2010; Lidgard et al., 2012; Schack et al., 2018). In cheilostome bryozoans, the most common modules, the autozooids (i.e., feeding zooids), may have specialized adventitious structures, including the composite frontal shield (Gordon, 2000) and spines (Lidgard et al., 2012; Vieira et al. 2014), which are considered to derive from highly modified zooids. Thus, cheilostomes comprise a complex modular system that regulates structural differentiation at the levels of the colony and zooids. It is likely that the diversity of polymorphs and whole body structures found in cheilostomes could have evolved in response to predation (Lidgard et al., 2012). Polymorphs specialized for sexual reproduction are also quite variable in cheilostomes, with changes in polypide and/or cystide structures. For example, ovicells—that serve as brooding chambers in cheilostomes—are considered specialized reproductive heterozooids if they derive from modified spines (e.g., Simpson et al., 2017), but have also been considered as body-wall outgrowths rather than heterozooids (Ostrovsky, 2013). Simpson (2012) observed that reproductive specialization is a prerequisite for further functional specialization of heterozooids in bryozoans (and all other colonial groups), and that if all zooids have a reproductive capacity, the evolution of new functional zooids never occurred.

To evaluate how the functional morphology of bryozoan colonies affects polymorphism, one group of ECM course students

(E. Gamero-Mora, J. W. Lawley from USP) set out to investigate the relationships between levels of polymorphism (i.e., polymorph types) to (i) levels of parental care, and (ii) colony form. The group compared photographic records provided by the instructors of 86 species of cheilostome bryozoans. First, because parental care in bryozoans presumably increases the fitness of the offspring but is rather energetically costly (Ostrovsky 2013), the group examined whether the number and area of ovicells—as a proxy to parental investment—was related to the levels of polymorphism (i.e., number of polymorph types) in different bryozoan species. Second, they investigated the relationship between different colony forms (encrusting vs. erect) and the number of polymorph types. Because of the high levels of variability in the number and types of polymorphs found in bryozoan species, comparative studies by direct observation of photographic records could potentially reveal important morpho-functional relationships with important implications to the evolution of polymorphs.

5 | PERSPECTIVES

Colonial animals represent a unique possibility to expand our understanding of the processes of evolution acting at different levels of biological organization. Several colonial traits—such as an ability to bud and regenerate, the potential to live as chimeric organisms with the related potential for allorecognition, and the specialization of functional modules that have been explored in this inaugural course—have evolved convergently many times in different groups of animals (Alié et al., 2020, in this issue; Blackstone & Jasker, 2003; Brown & Swalla, 2012). Many of these traits have been described and studied with different degrees of detail in the different groups of colonial animals, but much work remains to be done to understand the basic principles of these convergent events across colonial groups of animals. Evolutionary theories at large rely on studies conducted in unitary (or solitary) organisms that are vastly restricted in describing the evolutionary change in solely sexually reproducing populations of organisms. It is about time to expand our understanding of the evolutionary processes acting on organisms with facultative clonal or asexual reproduction.

Because animal coloniality remains a poorly studied field of biology, we hope that future courses serve as a venue to bring together the best specialists working in the different groups of colonial organisms or doing research on the topic on a more theoretical level. Over the years, some of us hope to continue organizing periodical courses at CEBIMar with the intention of advancing our understanding of the evolutionary and biological consequences of coloniality as a life history trait in animals. In the meantime, the processes —specifically the evolutionary, developmental, and ecological processes—responsible for organismal transitions across the different levels of biological organization remain incomplete.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/jez.b.23044.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Laurel S. Hiebert https://orcid.org/0000-0001-7756-1066
Stefano Tiozzo http://orcid.org/0000-0003-2822-2783
Carl Simpson http://orcid.org/0000-0003-0719-4437
Federico D. Brown https://orcid.org/0000-0001-9250-5011

REFERENCES

Alié, A., Hiebert, L. S., Scelzo, M., & Tiozzo, S. (2020, March 19). The eventful history of nonembryonic development in tunicates. *Journal of Experimental Zoology Part B Molecular and Developmental Evolution*. Advance online publication. https://doi.org/10.1002/jez.b.22940

Beklemishev, W. (1969). *Principles of comparative anatomy of invertebrates. 1. Promorphology*. University of Chicago Press.

Blackstone, N. W., & Jasker, B. D. (2003). Phylogenetic considerations of clonality, coloniality, and mode of germline development in animals. *Journal of Experimental Zoology Part B Molecular and Development Evolution*, 297, 35–47. https://doi.org/10.1002/jez.b.16

Boardman, R. S., Cheetham, A. H., Oliver, W. A., Jr., America, G. S., & Society, P. (1973). Animal colonies, development and function through time. In Dowden, Hutchinson & Ross, & Pa Stroudsburg (Eds.),. (pp. v–ix).

Brown, F. D., & Swalla, B. J. (2012). Evolution and development of budding by stem cells: Ascidian coloniality as a case study. *Developmental Biology*, 369, 151–162. https://doi.org/10.1016/j.ydbio.2012.05.038

Buscema, M., & Van de Vyver, G. (1983). Variability of allograft rejection processes in Axinella verrucosa. *Developmental and Comparative Immunology*, 7, 613–616.

Carter, M. C., Gordon, D. P., & Gardner, J. P. A. (2010). Polymorphism and variation in modular animals: Morphometric and density analyses of bryozoan avicularia. *Marine Ecology Progress Series*, 399, 117–130. https://doi.org/10.3354/meps08348

Chang, S. E., Orive, M. E., & Cartwright, P. (2018). Nonclonal coloniality: Genetically chimeric colonies through fusion of sexually produced polyps in the hydrozoan Ectopleura larynx. *Evolution Letters*, 2(4), 442–455.

Cohen, C. S., Saito, Y., & Weissman, I. L. (1998). Evolution of allorecognition in botryllid ascidians inferred from a molecular phylogeny. *Evolution, 52*, 746–756.

Duclos, K. K., Hendrikse, J. L., & Jamniczky, H. A. (2019). Investigating the evolution and development of biological complexity under the framework of epigenetics. *Evolution & Development, 21*, 247–264.

Fernàndez-Busquets, X., & Burger, M. M. (1999). Cell adhesion and histocompatibility in sponges. *Microscopy Research and Technique, 44*, 204–218. https://doi.org/10.1002/(SICI)1097-0029(19990215)44:4%3C204::AID-JEMT2%3E3.0.CO;2-I

Ferreira, G. F. A. (2007). Os Botryllinae (Tunicata: Ascidiacea) da Costa Tropical Brasileira. Thesis Universidade Federal do Ceará. pp. 92.

Gaino, E., Bavestrello, G., & Magnino, G. (1999). Self/non-self recognition in sponges. *Italian Journal of Zoology, 66*, 299–315. https://doi.org/10.1080/11250009909356270

Gordon, D. (2000). Towards a phylogeny of the cheilostomes— Morphological models of frontal wall/shield evolution. In C. Herrera, Amalia & Jackson, & B. C. Jeremy (Eds.), *In Proceedings of the 11th International Bryozoology Association Conference* (pp. 17–37). Smithsonian Tropical Research Institute.

Gutierrez, S., & Brown, F. D. (2017). Vascular budding in Symplegma brakenhielmi and the evolution of coloniality in styelid ascidians. *Developmental Biology, 423*, 152–169. https://doi.org/10.1016/j.ydbio.2017.01.012

Harvell, C. D. (1994). The evolution of polymorphism in colonial invertebrates and social insects. *Quarterly Review of Biology, 69*, 155–185.

Hiebert, L. S., Simpson, C., & Tiozzo, S. (2020). Coloniality, clonality, and modularity in animals: The elephant in the room. *Journal of Experimental Zoology Part B Molecular and Development Evolution.* Advance online publication. https://doi.org/10.1002/jez.b.22944

Hildemann, W. H., Bigger, C. H., Johnston, I. S., & Jokiel, P. L. (1980). Characteristics of transplantation immunity in the sponge, *Callyspongia diffusa. Transplantation, 30*(5), 362–367.

Hughes, R. N. (2005). Lessons in modularity: The evolutionary ecology of colonial invertebrates. *Scientia Marina, 69*(Suppl. 1), 169–179.

Jackson, J. B. C. (1977). Competition on marine hard substrata: The adaptive significance of solitary and colonial strategies. *The American Naturalist, 111*(980), 743–767.

Lidgard, A., Parsons, D., Rouzaud-Cornabas, J., & Beslon, G.(2020). The complexity ratchet: Stronger than selection, stronger than evolvability, weaker than robustness. *Artificial Life, 26*(1), 38–57. https://doi.org/10.1162/artl_a_00312

Lidgard, A., Carter, M. C., Dick, M. H., Gordon, D. P., & Ostrovsky, A. N. (2012). Division of labor and recurrent evolution of polymorphisms in a group of colonial animals. *Evolutionary Ecology, 26*(2), 233–257. https://doi.org/10.1007/s10682-011-9513-7

Marfenin, N. N. (1993). The Phenomenon of Coloniality. Moscow: Moscow University Press. 237 pages. (in Russian with English summary).

Maynard-Smith, J. (1978). *The evolution of sex.* Cambridge University Press.

McShea, D. W. (2017). Evolution of Complexity. In: Nuno de la Rosa L., Müller G.(eds.), *Evolutionary Developmental Biology.* Springer, Cham. https://doi.org/10.1007/978-3-319-33038-9_123-1

Nawrocki, A. M., & Cartwright, P. (2012). A novel mode of colony formation in a hydrozoan through fusion of sexually generated individuals. *Current Biology, 22*(9), 825–829.

Nicotra, M. L. (2019). Invertebrate allorecognition. *Current Biology, 29*, R463–R467. https://doi.org/10.1016/j.cub.2019.03.039

Okuyama, M., Saito, Y., & Hirose, E. (2002). Fusion between in-compatible colonies of a viviparous ascidian, Botrylloides lentus. *Invertebrate Biology 121*, 163–169.

Ostrovsky, A. (2013). Evolution of sexual reproduction in marine invertebrates: Example of gymnolaemate bryozoans. *Evolution of Sexual Reproduction in Marine Invertebrates* (pp. 1–356). Springer Netherlands, Dordrecht. Available from: http://doi.org/10.1007/978-94-007-7146-8

Saito, Y., & Watanabe, H. (1982). Colony specificity in the compound ascidian, Botryllus scalaris. *Proceedings of the Japan Academy, 58B*, 105–108.

Schack, C. R., Gordon, D. P., & Ryan, K. G. (2018). Modularity is the mother of invention: A review of polymorphism in bryozoans. *Biological Reviews, 94*(3), 773–809. https://doi.org/10.1111/brv.12478

Simpson, C. (2012). The evolutionary history of division of labour. *Proceedings of the Royal Society B-Biological Sciences, 279*(1726), 116–121. https://doi.org/10.1098/rspb.2011.0766

Simpson, C. (2020). An ecological driver for the macroevolution of morphological polymorphism within colonial invertebrates. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, Advance online publication. https://doi.org/10.1002/jez.b.22955

Simpson, C., Jackson, J. B. C., & Herrera-Cubilla, A. (2017). Evolutionary determinants of morphological polymorphism in colonial animals. *The American Naturalist, 190*(1), 17–28. https://doi.org/10.1086/691789

Sköld, H. N., & Obst, M. (2011). Potential for clonal animals in longevity and ageing studies. *Biogerontology, 12*, 387–396.

Maynard Smith, J., & Szathmary, E. (1997). The major transitions in evolution (pp. 360). Oxford University Press, Available from: https://oxford.universitypressscholarship.com/10.1093/oso/9780198502944.001.0001/isbn-9780198502944

Szathmáry, E., & Smith, J. M. (1995). The major evolutionary transitions. *Nature, 374*, 227–232.

Vieira, L. M., Spencer-Jones, M. E., Winston, J. E., Migotto, A. E., & Marques, A. C. (2014). Evidence for polyphyly of the genus Scrupocellaria (Bryozoa: Candidae) based on a phylogenetic analysis of morphological characters. *PLOS One, 9*(4), e95296. https://doi.org/10.1371/journal.pone.0095296