Systematics, variation, and developmental instability: analysis of spine patterns in ancestrulae of a common bryozoan

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Abstract
Since the species was originally described in 1960, the cheilostomate bryozoan Bugula stolonifera is known to be widely distributed in temperate and sub-tropical regions. Evidence also exists that it is an invasive species that can spread rapidly. Yet there have been no studies of its variability at the level of individual populations. One of the taxonomic characters cited in the diagnoses of Bugula spp. is the spination pattern surrounding the frontal membrane of the ancestrula, the founding member of the colony that arises from the metamorphosis of a larva. Although some authorities have recognized that the number and distribution of ancestrular spines can vary, there has been no systematic study of the extent of this variation. We examined the spination patterns of 11,162 ancestrulae derived from larvae released from 54 colonies collected at Woods Hole, Massachusetts, USA. The formula cited in descriptions of the species was present in only 53% of the ancestrulae. In total, 34 distinct patterns were recorded. Two trends in spine appearance were statistically significant. First, those individuals possessing a spine in the proximal position were more likely to gain an additional spine. Second, those individuals lacking a proximal spine were more likely to lose an additional spine. The significance of these trends remains to be explored. Fourteen second-generation ancestrulae possessing five variant (other than the most common) spination patterns were placed in the field for 29 days. Colonies grown from these ancestrulae were returned to the laboratory and 225 larvae produced by these colonies were collected. The spine patterns of ancestrulae resulting from metamorphosis of these larvae were recorded. Twelve patterns not present among those of the second-generation parents were observed in addition to the five original ones. Although all colonies grown in the field were from variant ancestrulae, 51% of the second-generation ancestrulae possessed the basic pattern. Finally, there were no significant differences between left and right side asymmetries in either the first- or second-generation ancestrulae. From these studies we conclude that spine pattern of ancestrulae is not a reliable taxonomic character, at least in the Woods Hole population of B. stolonifera. Second, there exists considerable variation in spine patterns yet there is no bias to left or right asymmetry. Third, possession of a particular spine pattern is not a heritable feature in one-generation studies. The production of ancestrular spines appears to be strongly influenced by random events occurring in the developmental process.

Keywords: Anatomical variation, ancestrula, Bryozoa, Bugula stolonifera, developmental noise
Introduction

Knowledge of the reproductive biology and ecology of bryozoans has grown such that bryozoans provide increasingly important model systems for broad-based studies of the biology of sessile marine organisms (Woollacott 1999). Three features of bryozoans contribute to their choice as experimental subjects. First, some species are widely distributed, seasonally abundant, and easily accessible to investigators. Second, certain species are conspicuous components of fouling communities and/or are invasive species, giving them an increasingly recognized role in ecosystem dynamics, environmental quality, and economic damage. Third, most species retain their early developmental stages and release short-lived anenteric larvae. It is possible to manipulate larval eclosion in the laboratory to obtain large quantities of larvae of known age. As the larvae are non-feeding and settle in a matter of hours, bryozoans make a favourable choice for studies of many aspects of larval biology, induction of metamorphosis, and success and interactions of post-metamorphic stages. Species of the genus Bugula have received the most attention. One species in particular, B. neritina (Linneaus), has been studied extensively; however, a second species, B. stolonifera Ryland, is growing in importance in research with at present approximately 30 published investigations of its biology exclusive of taxonomic or faunistic studies (Appendix 1).

Bugula neritina and B. stolonifera are distributed worldwide in temperate and sub-tropical regions and are considered to be commonly introduced species. In the case of B. stolonifera, detailed accounts exist of its introduction to Auckland Harbour, North Island, New Zealand and subsequent spread to other locales on North and South Island (Hager 1964; Gordon 1967; Gordon and Mawatari 1992). Evidence also suggests that it is a recent arrival in Nagoya Harbour, Honshu, Japan (Scholz et al. 2003). Assessment of the taxonomic status and genetic structure at the population level is important for the knowledgeable integration of findings derived from studies conducted in different geographical locations or from populations collected at one locale but at different depths. In the case of B. neritina, such progress is now under way (Davidson and Haygood 1999; McGovern and Hellberg 2003; Lim 2004). Similar studies of B. stolonifera, however, have yet to appear. The present study was initiated to evaluate the utility of one commonly reported character in Bugula spp. taxonomy: spination pattern of the founding individual (ancestrula) of the colony.

Bugula stolonifera was described by Ryland (1960). In this classic monograph on British species of Bugula, Ryland discusses 11 anatomical features of importance in systematic analyses of species in this genus. Included in this list is the ancestrula, the individual produced on completion of metamorphosis. All bryozoans are colonial and colonies arise from asexual reproduction of the ancestrula and its descendants. Within the Class Gymnolaemata and Order Cheilostomata that include the genus Bugula, ancestrulae present varying morphologies (e.g. Ryland 1965, Figure 20). In general, ancestrulae have been accorded special significance historically. They can occur as solitary individuals, composite complexes involving multiple individuals derived from precocious budding, compound units derived from simultaneously produced and synchronously developed multiple individuals, or compound-composites (Zimmer and Woollacott 1977). Yet, in spite of numerous studies of ancestrulae across the phylum and the diverse forms they present, a commonly accepted synthesis derived from these investigations has yet to be achieved. As best summarized by Ryland (1976, p 288-289), “The phylogenetic significance of the ancestrular form is difficult to assess”. The utility of the ancestrula in systematic studies remains in doubt as well and as Cook (1985, p 50) concludes,
“(ancestrulae) ... seem to have no wide systematic significance, although specifically they may be important”. In the case of Bugula, it remains standard procedure to include a description of the ancestrula, when available, in the diagnosis of a new species.

One feature of the ancestrulae of Bugula spp. traditionally accorded importance in taxonomy is the absence or presence and, if present, the distribution of spines at the perimeter of the frontal membrane. It is known, however, that spination of autozooids can vary within a species (Ryland 1962) and, similarly, so can the spination of ancestrulae. The extent of this variability, however, has not been previously studied.

In his original description of B. stolonifera which was based on material from south Wales in the UK, Ryland (1960) noted that ancestrulae possess one basal (proximal) and three, or rarely two, spines at each distal angle. This pattern can be abbreviated as a spine formula of 3:3:1 (right distal angle, left distal angle, and proximal or basal position). The same spine formula for ancestrulae of eastern Atlantic specimens in general was recorded by Ryland (1965, Figure 20B), Ryland and Hayward (1977) and Hayward and Ryland (1998). In studies of B. stolonifera from the western Atlantic of the USA, Maturo (1957, 1966, 1968) reports spine formulae of 4-2:2:1. Ramalho et al. (2004) offer a revised diagnosis of the species based on material studied from the northern and southern hemispheres of the Atlantic in which they report a single spine formula of 2:2:1. Bugula stolonifera also occurs in the Pacific and Indian oceans and the Mediterranean and North Seas (see Appendix 2 for details on the distribution of this species). No information is apparently available, however, on spine formulae of ancestrulae from locales other than the Atlantic.

In order to understand more fully any systematic significance of spine patterns in ancestrulae of B. stolonifera, we initiated a study in the summer of 2003 based on material collected over a 7-week period in Eel Pond at Woods Hole, Massachusetts. The results presented here document extensive variation in spine patterns within this single population. We also report two statistically significant trends in these patterns. Finally, preliminary genetic studies indicate that spine pattern may be loosely constrained and that this system may be useful for more general investigations of developmental stability and instability.

Materials and methods
Parent colonies of Bugula stolonifera were collected from the sides of floating docks at Eel Pond, Woods Hole, MA, USA. A total of seven collections were made, one collection for each week extending from 14 July to 25 August 2003, at which point the population density of B. stolonifera drastically decreased along the docks. Each week parent colonies were brought back to the laboratory in Cambridge and maintained in 38-litre recycling tanks containing Eel Pond water. All experiments were conducted at 19°C in a constant temperature room. The release of larvae in this species is triggered by the onset of illumination after a period of dark adaptation (Wendt and Woollacott 1999). Accordingly, tanks were covered with opaque black plastic for approximately 16 h. The following morning each colony was placed in a separate glass dish ~2 cm in front of a sealed light fixture containing two F20T12 Cool White fluorescent bulbs. Irradiance values at the leading edge of the vessels were 77 μmol photons s⁻¹ m⁻². Because small crustaceans and other photosensitive, fast-swimming organisms are also found in amongst the colonies, organisms that accumulated at the lighted edge of the vessel during the first 15 min were discarded. Generally very few B. stolonifera larvae emerged during this interval. Over the next 30 min, larvae were pipetted into separate polystyrene weigh boats, one for each colony. Each container was filled with 2.5 ml of Eel Pond water. These larvae are referred
to as members of Release no. 1 (15–45 min after onset of illumination). A second collection was made in the same manner during the following 30 min and these larvae constitute Release no. 2 (45–75 min after onset of illumination). Percentage larval settlement in each dish was assessed after 4 h. Water was decanted and then each dish was rinsed in Eel Pond water to remove all unsettled larvae from the weigh boats. A triangular, plastic clip was attached to the side of each weigh boat and the weigh boats were clipped into a plastic binder and hung inside a 38 litre recycling tank and maintained for 2 days under a 12 h light–12 h dark regime.

Parent colonies were returned to a 38-litre tank, the colonies kept separate by wrapping each in labelled, fine-mesh nylon bags. The tank was again covered with opaque black plastic to dark-adapt the colonies for use the next day. Following the same procedure described for the first day, Release no. 3 and Release no. 4 were collected on the second day. Hence, by the end of the two days there were a total of four batches of first-generation larvae collected from each parent colony.

First-generation spine counts

Most *B. stolonifera* larvae completed metamorphosis at 19°C within 48 h after settlement. The completion of metamorphosis was defined as that time at which the ancestrula developed an extendable polypide and began feeding. The number of successful metamorphs was counted and % success of metamorphosis calculated. Only colonies that resulted in ≥100 ancestrulae were included in the final data set. The spine pattern for each ancestrula from these colonies was recorded and the data for each colony were compiled over the four separate releases. There were no significant differences between Release no. 1 + no. 2 and Release no. 3 + no. 4 (paired t test $P=0.086$) or Release no. 1 + no. 3 and Release no. 2 + no. 4 (paired t test $P=0.222$). For the week of 11 August, no ancestrular spine counts were recorded for Release no. 3 or no. 4 as a mishap occurred in sample handling. Nine colonies, however, of the 11 August collection resulted in ≥100 ancestrulae for Release no. 1 and no. 2 and these data were included in our analysis.

Second-generation spine counts

In order to determine if there exists a genetic tendency towards variation in spine numbers, first-generation ancestrulae derived from 14 different parent colonies were returned to the field and permitted to grow until larvae were produced whose metamorphs could be examined for their spination pattern. First-generation ancestrulae containing variant spination patterns were selected for analysis and those lacking the target spination pattern were removed from the weigh boat. Thus, ancestrulae remaining on the weigh boat all possessed the same spine pattern and were marked by circling their position in the container. The selected weigh boats were then glued on opposite sides to plastic binders cut approximately 5 cm in length. String was then threaded through the binders on each side and these were attached into a rectangular 0.31 m × 0.15 m plastic crate. An anchor was attached to the bottom of the crate and a line to the top and the open side of the crate was covered with a fine black mesh that was used to keep out potential predators in the field. New weigh boats were added to the crate each week with a total of 14 weigh boats containing first-generation colonies that successfully grew in the field.

After 29 days in the field for each sample, first-generation colonies were removed and returned to the laboratory. While in the field, wild *B. stolonifera* larvae settled on the dishes.
Hence, colonies not marked at their base were removed so that only the original marked colonies remained. These colonies were dark-adapted overnight and second-generation larvae were obtained the next day following the same procedures used for collecting first-generation larvae from the parent colonies. Again, % induction, % success of metamorphosis, and the spine patterns of ancestrulae were recorded. All data were included for this experiment on the second-generation because <100 larvae were collected from each colony.

Results

A total of 12,809 first-generation larvae were obtained from 54 parent colonies over the course of 7 weeks. The success rates for induction of metamorphosis and for completion of metamorphosis were 96% and 91%, respectively (Table I). The spine pattern for each of the 11,162 successful metamorphs was assessed. Our observations of spine patterns were based on anatomical presence or absence of spines and not on the developmental patterns

Table I. *Bugula stolonifera* larvae and ancestrulae pooled from field-collected parent colonies producing first-generation larvae that gave rise to ≥100 ancestrulae per colony; chi-square tests ($P<0.05$) were conducted to determine if significant differences existed in comparisons. (A) Success of reproduction in first-generation colonies.

| Category | Number | % |
|----------|--------|---|
| Colonies | 54 | – |
| Larvae released | 12,809 | – |
| Larvae initiating metamorphosis | 12,269 | 96 |
| Larvae completing metamorphosis | 11,162 | 91 |

(B) Spination patterns of ancestrulae given as number of spines on the left:right:proximal sides of the frontal membrane.

| Category | Number | % |
|----------|--------|---|
| 3:3:1 | 5954 | 53 |
| Sum of all variants from 3:3:1 pattern | 5208 | 47 |
| Sum of dominant three patterns (3:3:1, 3:3:0, 4:4:1) | 9098 | 82 |

(C) Occurrence of dominant variant pattern (3:3:0) and its association with gain or loss of spine in another position.

| Category | Number | % |
|----------|--------|---|
| Occurrence of 3:3:0 spination pattern
3:3:0 of total ancestrulae (11,162) | 2805 | 25 |
3:3:0 of variant ancestrulae (5208) | 2805 | 54 |
| Consequences of loss or retention of proximal spine on spine pattern
If lose proximal spine, then gain spine in another position | 323 | 45 |
If lose proximal spine, then lose spine in another position | 392 | 55 |
If retain proximal spine, then gain spine in another position | 1003 | 59 |
If retain proximal spine, then lose spine in another position | 708 | 41 |

If lose proximal spine, then significantly more likely to lose spine in another position ($\chi^2=6.659; P<0.0001$). If retain proximal spine, then significantly more likely to lose spine in another position ($\chi^2=50.862; P<0.0001$).
of spine production (Figures 1 and 2). It is possible that a spine pattern of 3:2:1 was a result of two left distal spine losses and one spine gain. Because the individual spine positions on each distal margin cannot be distinguished, a pattern of 3:2:1 was assumed to be the result of one spine loss on the left distal margin.

Of these 11,162 ancestrulae, 5954 (53%) had a spine formula of 3:3:1 that has been described earlier and 5208 (47%) were some variant of this pattern. Over the 7-week period, the variants ranged from 37% to 56% with a mean of 45%±6.5 (Figure 3). There was a significant difference in the number of variants to the number of 3:3:1 spine patterns over the 7-week period (χ² = 209.860, P<0.0001). However, the change in percentage of variants did not follow a seasonal trend (Figure 3) nor was the percentage of variants related to the number of ancestrulae produced each week. The 54 colonies were divided into two categories: those collected early in the season (weeks 1–3) and those collected late in the season (weeks 5–7). Since week 4 fell precisely in the middle of the sampling period, the number of colonies for this week, nine, was divided and distributed equally into the early and late categories. Of the 22 colonies that had percentage variants below the mean of 45%, 12 came from early in the season and 10 were from late in the season. There were 30 colonies that contained percentage variants above the mean variants of 45% and 14.5 came from early in the season and 15.5 came from late in the season (the 0.5 is a result of the seven colonies in week 4 that had percentage variants above the mean being divided between the two categories). Two colonies had 45% variants, thus the total equalled 54 colonies. There was no significant difference between the number of colonies above and below the mean collected either early or late in the season (χ² = 1.42, P=0.700). Hence, the change in percentage variants observed was apparently randomly distributed over the 7-week period.

Figure 1. (A) SEM of an ancestrula of *Bugula stolonifera*: this ancestrula has a spine pattern of 4:3:1 (see Figure 2); (B) diagram of the position of left and right distal margins and position of the proximal spine.
The most common variant spine pattern was 3:3:0, lacking the proximal spine, accounting for 54% of the total variants (Table I). A loss of the proximal spine, regardless of distal spine numbers, occurred in 3516 of the ancestrulae or 68% of the total variants. If

![Image of spine patterns](image)

Figure 2. Some common spine pattern formulae and views of the frontal membrane of ancestrulae of *Bugula stolonifera*. All spine patterns are recorded from the viewpoint of the ancestrula right:left:proximal. (A) Diagram of an ancestrula with the typical 3:3:1 spine pattern cited in the text; (B) 3:3:0 variant spine pattern resulting from a loss of the proximal spine; this spine pattern was the most abundant variant spine pattern accounting for 54% of the variant spine patterns; (C) 3:2:1 variant spine pattern resulting from a loss on the left distal margin; (D) 4:3:1 variant spine pattern resulting from a spine gain on the right distal margin.

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![Graph of seasonal trends](image)

Figure 3. Seasonal trends in frequencies of 3:3:1 (black) and variant (grey) spine patterns for the first-generation ancestrulae over the course of the 7-week sampling period from 14 July to 25 August. The ratio of variants to 3:3:1 and % variants are included.
an ancestrula lost the proximal spine, then it was more likely to lose a spine on either the left or right distal margin. However, if the ancestrula retained the proximal spine, then it was more likely to gain a spine on one of the distal margins. Both of these relationships were statistically significant (Table I).

Changes in spine pattern between the left and right distal margins of the ancestrulae occurred with approximately equal frequency: 31% left, 33% right. There was no significant difference between changes on the left or on the right side (Table II). Changes involving one position occurred 74% of the time with significantly fewer changes involving more than one position (Table II). Of the 3516 ancestrulae that lost their proximal spine, 235 (7%) of these gained one spine on either the left or right distal margin, retaining a total of seven spines. Similarly, 23 (1%) ancestrulae lost a spine on either the left or right margin but gained a spine on the opposite margin to maintain a total of six distal margin spines regardless of presence or absence of the proximal spine. No distal margin contained more than four spines and the proximal spine position was never occupied by more than one spine. Hence, no ancestrula had a total of more than nine spines.

Colonies derived from 14 first-generation ancestrulae with variant spination patterns were grown in the field for 29 days (Table III). These colonies were then returned to the laboratory. In sum, they released 262 second-generation larvae and had a 90% success rate for induction of metamorphosis and a 95% success rate for completion of metamorphosis.

Table II. Changes from 3:3:1 pattern in spination of first-generation Bugula stolonifera ancestrulae and comparisons of changes between sides and loss and gain of spines; chi-square tests ($\alpha=0.05$) were conducted to determine if significant differences existed in comparisons. (A) Number of positions in which changes (additions or subtractions) occurred.

| Category                | Number | %  |
|-------------------------|--------|----|
| One position change     | 3849   | 74 |
| Two position change     | 1047   | 20 |
| Three position change   | 260    | 5  |
| Four position change    | 9      | 0.2|
| Five position change    | 4      | 0.1|
| Six position change     | 39     | 0.7|

(B) Right versus left side changes.

| Category                        | Number | %  |
|---------------------------------|--------|----|
| Simultaneous left and right side changes | 892    | 27 |
| Total left side change          | 1616   | 31 |
| Total right side change         | 1679   | 32 |

No significant difference between changes on right and left sides ($\chi^2=1.205; P=0.272$).

(C) Gain versus loss changes.

| Category | Number | %  |
|----------|--------|----|
| Right loss | 790    | 15 |
| Left loss  | 804    | 15 |
| Right gain | 889    | 17 |
| Left gain  | 860    | 17 |

No significant difference between loss on right or left side ($\chi^2=0.123; P=0.726$). No significant difference between gain on right or left side ($\chi^2=0.481; P=0.488$).
Table III. Ancestrulae of *Bugula stolonifera* derived from the larvae produced by first-generation colonies grown in the field from variant ancestrulae; these data are grouped as second-generation ancestrulae being similar or dissimilar in spination pattern to the first-generation ancestrulae from which they were derived. (A) Success of second-generation colonies transplanted back to the field.

| Category                                      | Number | %  |
|-----------------------------------------------|--------|----|
| Second-generation colonies grown in the field | 14     |    |
| Larvae released                               | 262    |    |
| Larvae initiating metamorphosis               | 235    | 90 |
| Larvae completing metamorphosis of those initiated | 225    | 95 |

(B) Spination pattern of ancestrulae derived from larvae produced by second-generation colonies that were grown from ancestrulae with variant spination patterns.

| Category | Number | % |
|----------|--------|---|
| 3:3:1    | 114    | 51|
| Variants | 109    | 49|

(C) Similarity of second-generation ancestrulae in spine pattern to that of parent first-generation ancestrulae.

| Parent         | Like parent | % | Not like parent | % |
|----------------|-------------|---|-----------------|---|
| Total          | 51          | 23| 172             | 77|
| 3:3:0          | 49          | 45| 61              | 55|
| >6L & R        | 2           | 2 | 85              | 98|
| <6L & R        | 0           | 0 | 26              | 100|

>6L & R, ancestrulae with more than six spines on the left and right sides; <6L & R, ancestrulae with less than six spines on left and right sides.

Ancestrulae founding the second-generation colonies had one of five variant spine formulae: 3:3:0, 3:2:1, 4:3:1, 3:4:1, 2:2:1. Larvae derived from these colonies, however, produced ancestrulae of 17 distinct spine patterns, the five original spine patterns represented in the second-generation ancestrulae and an additional 12 patterns. Although no colonies were grown from 3:3:1 ancestrulae, 114 (51%) of the second-generation ancestrulae resulted in the 3:3:1 pattern and 109 (49%) resulted in a variant of this pattern. Whereas variants in the first generation occurred in 46% of the ancestrulae, in the second generation, which was derived exclusively from variant ancestrulae, variants increased over those in the first generation by only 3%. There was no significant difference in the ratio of 3:3:1 to variant spine patterns between the first and second generations ($\chi^2 = 0.433; P = 0.510$) even though the first generation was derived from a random sample of the wild population and the second generation was derived solely from variant colonies of the first generation. The second-generation ancestrulae were grouped into three categories: >6 spines left and right (110 ancestrulae), <6 spines left and right (87 ancestrulae) and 3:3:0 (26 ancestrulae). Of the total 223 ancestrulae, only 51 (23%) were in the same category as their parent colony ancestrula (Table IV). Hence, an overwhelming number of second-generation ancestrulae did not match the spination pattern of the first-generation ancestrula from which they were derived.

We examined for symmetry versus asymmetry in the spine patterns of the right versus left distal margins (Table IV). In the case of the first-generation ancestrulae (Table IV(A)),...
symmetrical spine patterns occurred in 9617 ancestrulae (86%). Of these, 858 (9%) were symmetrical but possessed a spine pattern other than 3:3:1 or 3:3:0 (i.e. 2:2:1, 4:4:1, 4:4:0, 2:2:0, 1:1:1, 1:1:0, 0:0:1). Asymmetry was found in 1545 of the first-generation ancestrulae (14%). Of these, 53% resulted from changes on the left distal margin and 49% from changes on the right distal margin. There was no significant difference between these numbers. Asymmetry resulting from simultaneous changes on the left and right sides occurred for 2% of the ancestrulae.

In the case of second-generation ancestrulae, 26 of the 223 ancestrulae possessed asymmetrical spine patterns. Of the 197 that were symmetrical, only nine (5%) had a pattern other than 3:3:1 or 3:3:0 (i.e. 2:2:1, 4:4:1, 4:4:0, 2:2:0, 1:1:1, 1:1:0, 0:0:1). Asymmetry was found in 26 of the first-generation ancestrulae (14%). Of these, 53% resulted from changes on the left distal margin and 49% from changes on the right distal margin. There was no significant difference between these numbers. Asymmetry resulting from simultaneous changes on the left and right sides occurred for 2% of the ancestrulae.

**Discussion**

We observed in a sample of 11,162 ancestrulae of *Bugula stolonifera* that the most frequently cited spine formula of 3:3:1 occurred in only 53% of ancestrulae. The remaining 47% were distributed among 33 distinct patterns. Eighty-two per cent of the total variability, however, was accounted for by the three most common patterns (3:3:1, 3:3:0, 4:4:1). The total number of patterns is likely greater than the 34 we report as our observations were based exclusively on the presence or absence of spines and not on development of the complement of spines.
The underlying sources of this phenotypic variability are unknown at present. A convenient point of departure, however, is to break down the determinants of spine pattern into three components: genetic, environmental, and historical. Other studies of bryozoans point the way to directions of future work. First, Maturo (1973) studied offspring from known maternal colonies of two morphotypes of *Parasmittina nitida* (Verrill) and documented fidelity of anatomical features between offspring and mother. Second, the size of individual zooids is also considered a character of taxonomic importance (e.g. Jackson and Cheetham 1990). In studies notably by Okamura (1987) and Hunter and Hughes (1994), variation observed in zooid size within natural populations was resolved in laboratory studies into mostly environmental and to a lesser extent genetic causes. Third, Cheetham et al. (1993, 1994, 1995) provide an elegant series of quantitative investigations on the genetics of phenotypic evolution. Finally, Hageman et al. (1999) studied skeletal morphology by partitioning variance between genetic, environmental, and interactions between genetic and environmental factors. Their findings supported a strong correlation between genotype and morphology. These findings also document that a large percentage of the variation could not be accounted for within parameters of their model, however, suggesting that additional refinements are needed to describe the overall variability they observed.

We studied inheritance of spine pattern by growing an F2 generation in the field from F1 colonies with ancestrulae of known spination. Our findings document that a tight coupling in spine distribution does not occur between mother and offspring. Second, we found a linkage between presence or absence of the proximal spine and addition or loss of spines at the distal margins in the F1 generation set of ancestrulae. Larvae and metamorphs were cultured under laboratory conditions designed so that all developed at the same temperature and light regime. Obvious sources of environmentally induced change were consequently reduced to a minimum. Our results indicate that presence or absence of an individual spine may not be autonomously determined, but that higher-order organization may exist that governs the development of the overall complement of spines.

Though our experiments growing the F1 generation were designed to reduce environmental variation, we cannot exclude the possibility that some unidentified environmental parameter existed in such a fashion as to affect spination, as is known to occur in some other bryozoans. For example, Hageman et al. (1999) identified a “tank effect” among what seemed to be identical environments in their laboratory study of phenotypic variation in growth of *Electra pilosa* (Linnaeus). In *Conopeum reticulum* (Linnaeus) (Hutchins 1941), spination of zooids is known to vary with the salinity of water in which they occur. Third, Stebbing (1973a, 1973b) documented in a quantitative analysis that zooids in a single colony of *Electra pilosa* (Linnaeus) can possess different spination patterns. Those at the margin of a colony where that colony was adjacent to a competitor for space possessed long-spined zooids whereas others did not, thus supporting the earlier conclusion by Marcus (1926) based on qualitative observation that such spines may prevent overgrowth by a dominant competitor. Fourth, evidence is clear that in the case of *Membranipora membranacea* (Linnaeus) spination of autozooids can be phenotypically plastic. Yoshioka (1982) first demonstrated that spine production is induced in this species by the presence of a predatory nudibranch. Harvall (1984, 1986, 1991, 1998) and Harvall and Padilla (1990) have revealed in detail the complex nature of this association, its ecological underpinnings, and taxonomic implications. The studies cited above point clearly to the phenotypically variable expression of spination in adult zooids of other
anascan cheilostomates. It is tempting to extend these findings to the process of spine development in ancestrulae. Although the ancestrular spines become obscured as colony development progresses, the potential for spines to have a protective role in ancestrular and early colony life seems apparent. Little is known, however, about the dynamics of interspecific competition or predation on ancestrulae and newly established colonies. Analysis of the extent of phenotypic plasticity in ancestrular spination gained from laboratory studies by varying known stressors would be an important direction in future studies.

The third component in analysis of variation is the influence of historical factors. Much of the early literature on ancestrulae derives from knowledge that the ancestrula is generally a solitary individual from which the colony is asexually derived. As such, the ancestrula was accorded special attention in the context of phylogeny and recapitulation. One morphotype of ancestrula termed the “tata” (resembling the anascan calloporids) appears multiple times in the complex grade referred to as the “ascophoran cheilostomates” (Ryland 1976). The tata is characterized by a nearly symmetrical array of spines arising from the edge of the frontal membrane. A similar-appearing frontal membrane–spine complex is present in the anascan buguloid Rhabdozoum wilsoni Hincks (Cook and Bock 1994). It is possible that the ancestrulae of Bugula spp. may evidence a tata pattern reflecting some shared evolutionary history. In contrast to the view that ancestrulae possess conserved properties is the fact that ancestrulae of differing types may co-occur within a single genus as documented in the case of Hippothoa (Celleporella) (Ryland and Gordon 1977). Moreover, in some species, ancestrulae form not as solitary individuals, but may develop as composite, compound, and composite–compound units (Zimmer and Woollacott, 1977). Finally, the ancestrula does not attach directly to a substrate in all species. Scrupocellarids, for example, form attachment by way of rhizoids, root-like highly modified non-feeding zooids (Silen 1980). These latter two examples, however, are presumably derived states. As indicated in the Introduction, the connection between ancestrula type and phylogeny is obscure at best. But one cannot discount the importance of historical factors in shaping ancestrula form regardless of whether they have useful phylogenetic content.

Two general conclusions arise from our study of variability of spine patterns. First, on average there exists no bias to left or right in spite of the fact that there is considerable variation in the population. Second, no heritability was documented of particular patterns in offspring of a given individual. Because of this lack of correlation between patterns, spine production in ancestrulae of B. stolonifera appears to be subject to random developmental noise as has been documented in other cases beginning with the classic work on the ocelli-bristle system in Drosophila subobscura Collin by Smith and Sondhi (1960).

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Appendix 1. Studies on the biology of Bugula stolonifera exclusive of taxonomic and faunistic reports

This appendix includes only studies published from 1960, the date of Ryland’s original description of the species, to the present. We have made no attempt to incorporate material from previous studies that are likely attributable to B. stolonifera.

| Subject area                                      | Geographical source of material | Reference                                      |
|--------------------------------------------------|---------------------------------|------------------------------------------------|
| Biomechanics and functional morphology           | UK, Wales                       | Riisgård and Manríquez (1997)                  |
|                                                  | USA, California                 | Okamura (1984, 1990)                           |
|                                                  | USA, North Carolina             | McKinney et al. (1986)                         |
|                                                  | USA, Massachusetts              | Kaufmann (1968, 1971)                          |
| Natural products chemistry and ecology           | USA, Delaware                   | McGovern and Hellberg (2003)                   |
|                                                  | USA, New York                   | Colon-Urban et al. (1991)                      |
|                                                  | USA, Massachusetts              | Lim (2004)                                     |
| Molecular and biochemical systematics            | North Sea, English Channel,     | d’Hondt et al. (2003)                          |
|                                                  | Bay of Biscay                    |                                                |
|                                                  | USA, Massachusetts              | Lim (2004)                                     |
| Larval anatomy                                   | USA, Massachusetts              | Hughes and Woollacott (1980)                   |
|                                                  | USA, Massachusetts              | Reed et al. (1988)                             |
| Laboratory studies                               | Germany, Kiel                    | Jebram (1968, 1977)                            |
|                                                  | USA, Massachusetts              | Bertrand and Woollacott (2003)                 |
|                                                  | USA, Massachusetts              | Brancato and Woollacott (1983)                 |
|                                                  | USA, Massachusetts              | Lim (2004)                                     |
|                                                  | USA, Massachusetts              | Pires and Woollacott (1983)                     |
|                                                  | USA, Massachusetts              | Rodgers and Woollacott (this study)            |
|                                                  | USA, Massachusetts              | Wendt (2000)                                   |
|                                                  | USA, Massachusetts              | Wendt and Woollacott (1995, 1999)              |
|                                                  | USA, Massachusetts              | Woollacott (1980)                              |
|                                                  | USA, Massachusetts              | Woollacott et al. (1989)                       |
| Field studies                                    | UK, Wales                       | Naylor (1965)                                  |
|                                                  | Mediterranean                   | Gautier (1962), Geraci and Relini (1970)       |
|                                                  | Turkey                          | Kocak and Kucukşezgin (2000)                   |
|                                                  | USA, Florida                    | Young and Cameron (1989)                       |
|                                                  | USA, Massachusetts              | Grosberg (1981)                                |
|                                                  | USA, Massachusetts              | Patzkowsky (1988)                              |
|                                                  | USA, New Hampshire              | Winston (1977)                                 |
|                                                  | Australia                       | Keough and Raimondi (1995, 1996)               |
|                                                  | China                           | Xixing et al. (2001)                           |

Appendix 2. Geographical distribution of Bugula stolonifera

This appendix includes only studies published from 1960, the date of Ryland’s original description of the species, to the present. We have made no attempt to incorporate material from previous studies that are likely attributable to B. stolonifera.
| Location                        | Reference                                                                 |
|--------------------------------|---------------------------------------------------------------------------|
| Indian Ocean                   |                                                                           |
| Pakistan, Arabian Sea          | Karim (1971), Javed and Tirmizi (1993)                                    |
| India, Bay of Bengal           | Ganapati and Rao (1968), Satyanarayana and Ganapati (1978)                |
| Western Australia, Shark Bay   | Wyatt et al. (2005)                                                      |
| Southern Ocean                 |                                                                           |
| Australia, Adelaide            | Brock (1985)                                                              |
| Pacific Ocean                  |                                                                           |
| New Zealand                    | Hager (1964), Gordon (1967), Gordon and Mawatari (1992)                   |
| Australia, Southeastern        | Keough and Raimondi (1995)                                                |
| China, South China Sea         | Xixing et al. (2001) and references therein                               |
| Japan, Inland Sea, Nagoya      | Scholz et al. (2003)                                                      |
| USA, Hawaii                    | Soule et al. (1987)                                                       |
| USA, Berkeley, California      | Okamura (1984)                                                            |
| USA, Southern California       | Soule et al. (2001)                                                       |
| Panama                         | Powell (1971)                                                             |
| Atlantic Ocean                 |                                                                           |
| Argentina, Mar del Plata       | Excoffon et al. (1999)                                                    |
| Brazil, Rio de Janeiro State   | Alvarez et al. (1986), Ramalho and Muricy (2003a, 2003b), Ramalho et al. (2004) |
| USA, Florida, Indian River Lagoon | Winston (1982, 1995), Young and Cameron (1989), Okamura (1990)             |
| USA, N and S of Cape Hatteras  | Maturo (1968)                                                             |
| USA, North Carolina, Bogue Sound | McKinney et al. (1986)                                                    |
| USA, Delaware                  | McGovern and Hellberg (2003)                                              |
| USA, Brooklyn, New York        | Colon-Urban et al. (1991)                                                 |
| USA, Woods Hole, Massachusetts | Kaufmann (1971) and numerous others                                       |
| USA, Great Bay, New Hampshire  | Winston (1977)                                                            |
| USA, Atlantic coast            | Maturo (1966)                                                             |
| Netherlands, North Sea         | d’Hondt and Cadée (1994), d’Hondt et al. (2003)                           |
| Belgium, North Sea             | Kerckhof (2000) and references therein                                    |
| Ireland, Côb, Cork             | Ryland (1960)                                                             |
| British Isles, Southwest       | Ryland (1960), Naylor (1965), Hayward (1976), Ryland and Hayward (1977), Hayward and Ryland (1998), Riisgård and Manriquez (1997) |
| France, English Channel and Strait of Dover | Fey (1971), d’Hondt et al. (2003)                                         |
| France, Bay of Biscay          | Prenant and Bobin (1966)                                                  |
| Spain, Abra de Bilbao          | Alvarez et al. (1986)                                                     |
| Temperate North Atlantic       | Ryland and Hayward (1991)                                                 |
| Liberia                        | Pulpeiro (1983)                                                           |
| Ghana                          | Cook (1985)                                                               |
| Caribbean Sea                  |                                                                           |
| Jamaica, Kingston              | Creary (2003)                                                             |
| Mediterranean Sea              |                                                                           |
| Italy, Ligurian Sea            | Geraci and Relini (1970)                                                  |
| Italy, Tyrrhenian Sea          | Ryland (1962), Carrada et al. (1965), Carrada and Occhioni Ambrogi (1979), Occhioni Ambrogi (1980) |
| Italy, Adriatic Sea            | Carrada and Occhioni Ambrogi (1979), Occhioni Ambrogi (1980, 1982, 1985), Occhioni Ambrogi and d’Hondt (1981) |
| Italy and Sardinia             | Occhioni Ambrogi (1981)                                                   |
| Turkey, Aegean Sea             | Kocak and Kucuksezgin (2000)                                              |
| Western Mediterranean          | Gautier (1962), Zabala and Maluquer (1988)                                |