RESEARCH ARTICLE

Branch to Colony Trajectory in a Modular Organism: Pattern Formation in the Indo-Pacific Coral *Stylophora pistillata*

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The architecture of the colony in a branching coral is an iterative process in which new layers of calcium carbonate compile atop existing structures that remain unchanged. Colony growth and development, known as astogeny, is believed to be a continuous process, characterized by replication of lower rank unites, polyps, and branches. This study seeks to explore the genetic blueprint of branch-to-colony developmental trajectory in the branching coral *Stylophora pistillata*, within an astogeny period of 1 year. One hundred small branches (initially 2–4 cm long) were sampled from 10 colonies. A year later, 63 remaining colonies were analyzed for their architectural rules by using 15 morphometric parameters. Multivariate statistical tests were preformed. Cluster and two-dimensional nonmetric Multi-Dimensional Scaling analyses revealed that the 10 genotypes could be divided into two major morphometric groups and two intermediate groups, whereas SIMPER analyses (a similarity percentage test) on within-genet similarities showed high similarity between the ramets developed from each of the 10 genotypes. Although, at first, it seemed that different colonies exhibited variable and different architectural designs (each characterized by specific morphometric parameters), a comprehensive analysis revealed that all 10 coral genotypes exhibited a single common developmental plan that was characterized by a continuum of architectural design with several distinct stages. Each stage is marked by its own characteristic morphometric parameters. Changing of developmental rules during the trajectory from branch to coral colony may help the colony to cope better with environmental constraints. *Developmental Dynamics 235: 2111–2121, 2006.* © 2006 Wiley-Liss, Inc.

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INTRODUCTION

Understanding morphogenesis, the generation of the organism's shape, is one of the most fascinating and fundamental subjects in developmental biology (Wolpert, 1996; Kaandorp and Sloot, 1997; Gateño and Rinkevich, 2003). Structuring the body form of sessile colonial organisms is of special interest from two reasons: (1) these organisms are tessellations of basic units (modules) that are arranged in various hierarchical orders, enabling the formation of unlimited structures and body plans (Ryland and Warner, 1986; Kaandorp and Sloot, 1997; Kaandorp and Kubler, 2001; Lasker et al., 2003; Sánchez et al., 2004); (2) body plan in these organisms is one of the major expressed responses to various physical and biological challenges (Ryland and Warner, 1986; Abelson, 1993; Kim and Lasker, 1997; Muko et al., 2000).

The structural hierarchy in complex modular organisms consists of up to three levels of organization. First-order modules, the zooids (in corals, the polyps); second-order modules, grouped zooids arranged in replicated patterns (such as branches in branching corals, or "systems" in botryllid ascidians); and the third-order modules, the daughter colonies or ramets (Ryland and Warner, 1986). The first- and the third-order modules (the zooids and the colony levels) have cap-

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tured the most scientific interest (Rinkevich, 2002; Sánchez and Lasker, 2003).

The roles of the organism's genetic blueprint in shaping structures, as opposed to environmental factors of phenotypes, are important for understanding growth forms of various sessile modular organisms in general, and of corals, in particular (Rinkevich, 2002). Variation of form in coral colonies as a response to environmental conditions has been widely studied. In contrast, only limited scientific endeavour has been devoted to elucidating the rules of body shape and astogeny in coral colonies; rules that are assumed to have a genetic basis (Jackson et al., 1979; Dauget, 1991a; Rinkevich, 2002; Gateño and Rinkevich, 2003; Lasker et al., 2003). Architectural traits, including phenotypic plasticity, may define a suite of morphological responses, influenced by genetics or by epigenetic factors (Rinkevich, 2000, 2002). Even phenotypic plasticity may be controlled by genetics (Pigliucci, 1996; Callahan et al., 1997).

A fundamental requirement for understanding development is comprehension of the relationships between genes and morphologies (Nijhout et al., 1986; Sánchez et al., 2004). Such relationships are elucidated, experimentally, by several methodologies. One of the approaches is based on follow-up protocols of growth patterns of isolated branches or nubbins (fragments in size of a single to few polyps; Shafir et al., 2001). Modification of the developed phenotypes may be analyzed quantitatively to establish common rules.

In the branching coral species *Stylophora pistillata* (as in other coral species; Gateño and Rinkevich, 2003), the developing colony responds to the environment by sets of morphometric rules that "canalize" (sensu Waddington, 1942; Buss and Blackstone, 1991) growth patterns to the observed typical species morphology. This was first demonstrated by Loya (1976), showing that the lost spherical structure in broken *S. pistillata* colonies is regained by a differential growth pattern: fast growth rates in regenerating parts, together with reduced growth rates in the intact branches. The growth of a colony in this species also reveals the existence of feedback mechanisms that "consider" current shapes and future growth within an architectural scheme of a preplanned colonial astogeny. These mechanisms include changes in growth directionality of isogeneic branches that risk contiguity (Rinkevich and Loya, 1985a), the retreat growth phenomenon recorded occasionally between confronting allogeneic branches (Rinkevich and Loya, 1985b), and the process of apical ramification in branches (Rinkevich, 2000, 2002). *S. pistillata* (as probably other branching colonial organisms) possesses, therefore, conserved "morphogenic codes" (Hogan, 1999), sets of species-specific rules that are expressed repeatedly and in harmony to develop the colonial landscape.

THE STUDIED SPECIES

Stylophora pistillata (Esper, 1787) is a widely distributed Indo-Pacific branching coral species characterized by rapid growth rate and variety of color morphs. In the Gulf of Eilat (Red Sea), it is abundant in the lagoon, rear-reef, and reef flats and common in the fore-reef (Loya, 1976). *S. pistillata* is an ecologically important reef builder, forming a complex habitat for various species of crabs, fish, and a variety of cryptic organisms (Rinkevich, 2002).

Astogeny in this species is characterized by axial, rod-like growth form and by integrated developmental processes of branches carrying numerous polyps (each approximates 1 mm in diameter). A colony originates from a settled planula larva that metamorphoses into the primary polyp and starts to deposit a calcareous skeleton within 1-day after metamorphosis. One week thereafter, six more polyps are added peripherally by inter-tentacular budding, forming a circle of polyps around the primary polyp (Fig. 1a). This kind of lateral plate expansion continues until, at some yet unidentified stage, a single ramified branch develops by apical accretion. New branches are then developing, conforming to the basic architectural rules of this species and creating the typical three-dimensional (3D) spherical symmetry.

Within the colonial sphere, upgrowing branches (UGBs; Fig. 1b) are added primarily by dichotomous fission at a branch-tip (Rinkevich and Loya, 1985a; Rinkevich, 2000, 2002). The apex of each UGB axis comprises several contiguous polyps. Growth rates of newly formed dichotomous UGBs differ significantly from those of older branches (Rinkevich, 2000). In addition to the UGBs, many lateral, inward- and outward-facing lateral branches (LBs; Fig. 1b) are formed. Outward facing LBs extend similarly to UGBs, adding more ecological volume to the colony's spherical structure. Inward LBs, that, after a lengthily extension, may touch and fuse with UGBs, cease to grow at a certain point, preventing isogenic fusions (Rinkevich and Loya, 1985a). As in other colonial organisms (Lasker et al., 2003; Sánchez and Lasker, 2003; Sánchez et al., 2004), these developmental patterns may reveal evolutionary fixed robustness of morphological integration.

This study focuses on identification of such a suite of architectural rules for colony pattern formation and branch morphometric forms among colonies of *Stylophora pistillata* from the Red Sea. The rationale here is based on the assertion that there is a genetic component in colony astogeny. If indeed so, the key question for analysis should be, Do different colonial forms result from different colonial growth trajectories or whether the same trajectories overlay on different growth rates/patterns?

RESULTS

All 15 morphometric characters were analyzed in the 63 colonies (Table 1). Using these morphometric characters, a significant difference was recorded between the different genets (ANOSIM; Global R = 0.524; $P < 0.001$). SIMPER analyses (a similarity percentage test) on within-genet similarities showed high similarity between the ramets developed from each of the 10 genets (75–88%, Table 2). The 10 parameters mostly contributing to this similarity were Nx, SI, DI, H, ΔH%, BaL, Sv/Ev, Le, Ev, and Ω (Table 2).

These above 10 morphological parameters (MPs) also split the genotypes into two distinct groups of colo-

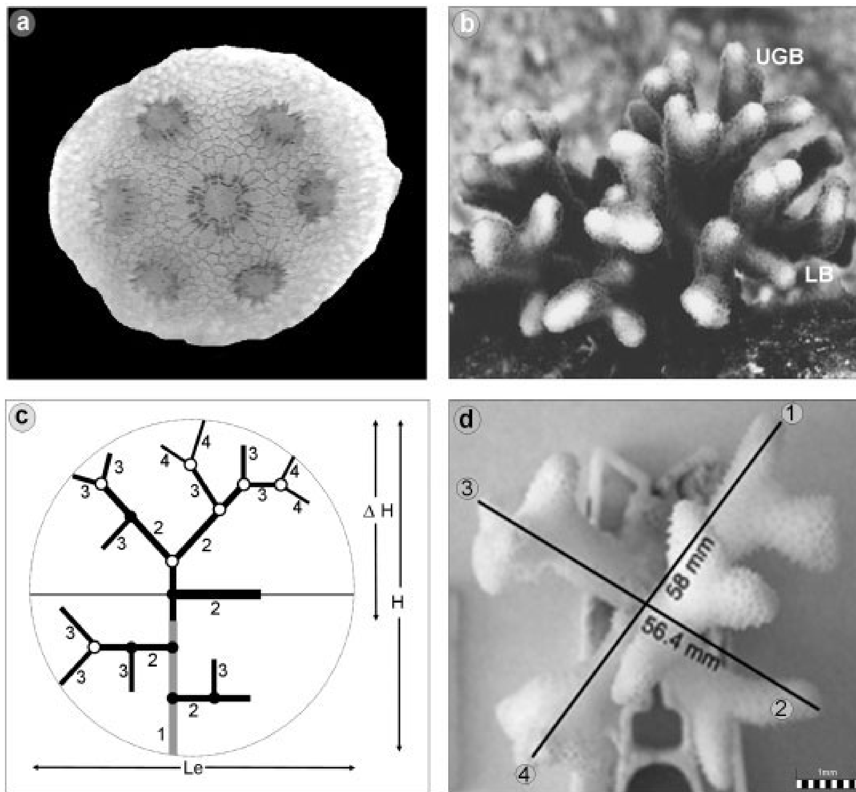

Fig. 1. Architectural structures (a,b) and some morphometric parameters (c,d) of *S. pistillata* colony. **a:** A young colony possessing the primary polyp in the center and six polyps in the periphery, before the development of the first axial branch. **b:** a typical colony revealing the characteristics of branch types (UGBs, LBs, apical bifurcation) and the colony spherical structure. **c:** schematic illustration depicting branch types. White dots represent dichotomous initiations. Black dots mark side initiations. The numbers along branches indicate the Reverse Strahler order of the branch. The stem (the initial part - grey, later on extension - black) is order 1, daughter branches from the stem got order 2, and so on. In this example, $\Omega = 4$ (Abraham, 2001). Le, H and ΔH refer to parameter 12, 2 and 3, respectively in Table 5. The doted circle encompasses the ecological volume (Ev). **d:** measurements of maximal length (axis 1–4) and the perpendicular maximal width (axis 2–3) of a developing colony.

nies. Colonies of the first group (A, B, E, F, H, I, J) were characterized by the parameters N2, SI, Sv/Ev, ΔH%, H, and BaL. Colonies of the second group (C, D, G) were characterized by the parameters Le, N3, DI, Ev, and Ω (Table 2). Thus, each MP contributed to a single group of colonies. The MDS (Multi-Dimensional Scaling) analysis (Fig. 2a) further revealed two distinct groups of genotypes: one group with colonies C, D and G (as above) and a second group of colonies consisting of genotypes H, I and J. Colonies A, E, B, and F were scattered. A Cluster Analysis Tree (Fig. 2b) preformed on the average values for each genet revealed high resemblance between all genotypes at levels <85%. However, at 87% resemblance, the 10 colonies split again into two distinct groups. Group I consisted of colonies H, I, J, A, and E, and group II consisted of colonies F, B, D, C, and G (Fig. 2b). At a level higher than 87% similarity, four morphological subgroups were formed. Genotypes A and E assembled into subgroup Ia; H, I and J into subgroup Ib; F and B into subgroup IIa; and D, C, and G into subgroup IIb (Fig. 2b).

The Cluster Analysis results further enabled us to analyze the morphometric characteristic on two operational levels: (1) testing the differences between the two major morphological groups (I and II); and (2) testing the differences between the four morphological subgroups (Ia, Ib, IIa, IIb).

Testing the Differences Between Morphometric Groups I and II

MDS analysis that considered the two assigned morphometric groups (Fig. 3a, performed on all 63 studied colonies) further demonstrated the validity of this clustering. Ramets belonging to morphometric group I were assembled on the left side of the 2D ordination, and ramets from morphological group II were grouped on the right side of the 2D ordination. Some of the ramets were scattered. A significant difference (ANOSIM; Global R = 0.502; $P < 0.001$) was recorded when analyzing morphometric groups I and II.

Simper analysis revealed that the morphometric parameters most contributing to this difference between the two groups were Ev, N3, Sv/Ev, DI, and Ev/nB (Table 3). The parameters Bal and H were the least (<5%) contributing to the dissimilarity. Simper results further revealed a high similarity within the groups (>75%, Table 3). The parameters contributing to this similarity in group I genotypes were N2, H, Sv/Ev, BaL and ΔH%. The parameters contributing to this similarity in group II genotypes were N3, Ev/nB, DI, Le and H (Table 3).

Testing the Differences Between Morphometric Subgroups Ia, Ib, IIa, and IIb

A significant difference (ANOSIM; Global R = 0.556; $P < 0.001$) was recorded when analyzing all morphometric subgroups in a single test. However, pairwise tests (done separately on each subgroup pair combination) showed significant differences only between subgroups I(a) and II(b) (ANOSIM: R = 0.735; $P < 0.001$), and between subgroups I(b) and II(b) (ANOSIM: R = 0.769; $P < 0.001$; Table 4).

MDS analysis (Fig. 3b) that considered the four assigned morphometric groups revealed grouping of the points (colonial ramets) according to suggested clustering. Ramets from morphological subgroup II(b) were assembled at the right side of the 2D ordination and ramets from morphological group I(b) and I(a) were clustered at the left side of the 2D ordination. Ramets from subgroup II(a) were scattered in the middle. The two subgroups (mainly subgroup II(a)) may explain the overlapping points observed when considering only the two main groups (Fig. 3a), as they repre-

TABLE 1. Average (±SD) Values for the 15 Morphometric Parameters (See Table 5; Parameter Nx Is Detailed for N2 and N3) Measured for Each of the 10 *S. pistillata* Genotypes[a]

Morphometric parameter	Genotype (number of ramets)									
H(4)	I(4)	J(7)	A(6)	E(4)	F(6)	B(7)	D(8)	C(9)	G(8)	
H (mm)	39.2 ± 7.1	44.1 ± 4.0	41.8 ± 5.6	44.5 ± 2.1	78.6 ± 6.6	54.9 ± 8.1	56.0 ± 11.4	50.7 ± 8.1	59.5 ± 8.6	62.7 ± 5.9
ΔH (mm)	28.0 ± 5.0	26.8 ± 2.5	28.7 ± 3.9	15.9 ± 2.6	46.6 ± 1.9	39.0 ± 6.4	28.3 ± 3.1	25.2 ± 2.3	27.9 ± 2.7	38.8 ± 3.7
ΔH%	260 ± 71	160 ± 13	220 ± 33	60 ± 17	150 ± 19	250 ± 32	120 ± 42	100 ± 26	90 ± 31	160 ± 25
nB (#)	11.8 ± 5.4	12.0 ± 4.7	9.0 ± 4.6	16.7 ± 5.6	15.0 ± 3.4	21.0 ± 7.6	11.3 ± 7.3	22.3 ± 6.7	30.3 ± 10.8	36.9 ± 13.4
N2 (%)	77 ± 23	80 ± 19	73 ± 8	72 ± 15	94 ± 7	68 ± 21	78 ± 23	41 ± 7	38 ± 7	46 ± 19
N3 (%)	5 ± 11	8 ± 16	12 ± 12	25 ± 16	6 ± 7	20 ± 14	18 ± 21	36 ± 6	43 ± 6	33 ± 9
UGB (%)	24 ± 12	21 ± 8	44 ± 25	11 ± 9	10 ± 4	25 ± 8	27 ± 22	40 ± 11	27 ± 9	23 ± 7
ΔW	2.3 ± 1.6	3.3 ± 1.0	2.1 ± 0.7	2.6 ± 0.5	6.1 ± 1.6	5.0 ± 2.0	3.8 ± 2.2	6.4 ± 2.4	9.3 ± 3.3	13.1 ± 4.7
Ev (cm^3)	14.4 ± 13.5	17.8 ± 9.6	11.1 ± 9.3	24.3 ± 13.2	35.1 ± 12.2	52.4 ± 25.8	46.8 ± 35.6	64.3 ± 40.1	110.9 ± 51.2	136.1 ± 64.8
Ev/nB (cm^3)	1.06 ± 0.51	1.48 ± 0.61	1.09 ± 0.46	1.42 ± 0.46	2.34 ± 0.70	2.45 ± 0.59	4.29 ± 2.26	2.73 ± 1.12	3.53 ± 0.67	3.67 ± 1.21
SI (%)	82 ± 22	87 ± 12	72 ± 18	77 ± 7	97 ± 6	80 ± 16	63 ± 16	58 ± 9	52 ± 11	67 ± 16
DI (%)	18 ± 22	13 ± 12	28 ± 18	23 ± 7	3 ± 6	20 ± 16	37 ± 16	42 ± 9	48 ± 11	33 ± 16
Sv/Ev	0.21 ± 0.08	0.20 ± 0.08	0.36 ± 0.26	0.18 ± 0.05	0.17 ± 0.05	0.09 ± 0.01	0.12 ± 0.04	0.13 ± 0.05	0.11 ± 0.03	0.08 ± 0.01
Le (mm)	22 ± 11	25 ± 4	20 ± 7	30 ± 4	33 ± 4	38 ± 7	35 ± 8	45 ± 8	51 ± 7	55 ± 9
Bal(mm)	8.1 ± 0.9	8.6 ± 1.2	9.6 ± 2.6	7.6 ± 1.1	10.9 ± 0.6	8.6 ± 0.8	13.3 ± 3.1	8.0 ± 0.6	9.4 ± 0.8	8.9 ± 1.2
Ω	3 ± 1	3 ± 1	3 ± 1	3 ± 1	3 ± 1	3 ± 1	3 ± 1	4 ± 0	4 ± 1	4 ± 1

[a]For parameter definitions, see Table 5.

sent an intermediate morphometric stage.

Simper analysis results revealed that the parameters contributing to the dissimilarity between these subgroups were, in combination I(a) vs. II(b), the parameters Ev, N3, DI, nB, and UGB; in combination I(b) vs. II(b), the parameters Ev, N3, DI, nB, and Sv/Ev (Table 2). Simper results further revealed within subgroups high similarity (75–82%; Table 3). The parameters most contributing to this similarity in subgroups I(a) and I(b) were N2, SI, Sv/Ev, and BaL. The parameters contributing to the similarity in subgroup II(a) were N2, Ev/nB, H, ΔH%, and BaL. The parameters contributing to the similarity in subgroup II(b) were N3, DI, Le, Ω, and Ev (Table 3).

Architectural Characteristics

Subgroup I(b) colonies included genotypes H, I, and J (Figs. 2b, 4a). This subgroup was characterized by distinctive up-growth rate (ΔH% = 160–260%), by intensive side initiation (SI% = 72–87%) and, after 1 year of growth, by final branch order 2 (N2% = 73–80%). The last two parameters indicated that most new branches developed on the first branch and perpendicular to it. The ratio of skeletal volume to ecological volume was high (Sv/Ev = 0.20–0.36), the volume of the skeleton was approximately one third of the total ecological volume (indicating limited space volume between the branches). Ev (ecological volume), one of the parameters that had not appeared in the SIMPER test as most contributing to the similarity within the group, was small (Ev = 11–18 mm^3, compared with 64–136 mm^3 in group II(b)); nB (average number of branches) was low (nB = 11, compared with nB = 30 in group II(b) colonies). Colonies of subgroup I(b) did not add significantly to the colony ecological volume, resulting in low ecological volume per branch (Ev/nB).

Subgroup II(b) colonies (genotypes C, D, G; Figs. 2b, 4b) exhibited contrasting architectural characteristics to subgroup I(b). After 1 year of growth, these colonies increased significantly their ecological volumes through a wide lateral dimension (Le = 45–55 mm, compared with Le =

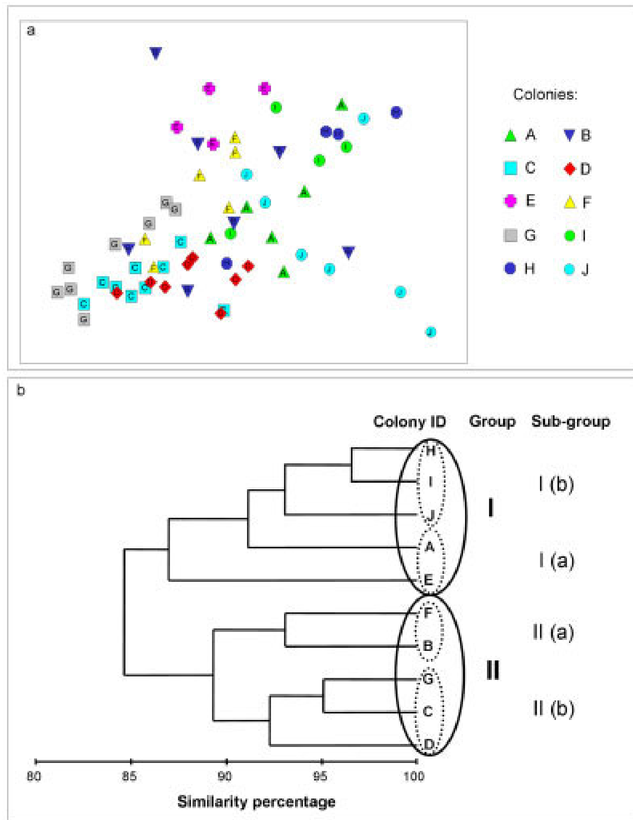

Fig. 2. Morphospace analyses performed on the genet level. **a:** MDS analysis performed on the studied *S. pistillata* genotypes. **b:** cluster analysis (PRIMER - Bray Curtis algorithm) of the 10 studied *S. pistillata* genotypes. The major groups (I, II) are circled by solid line, the subgroups' colonies - by dashed line.

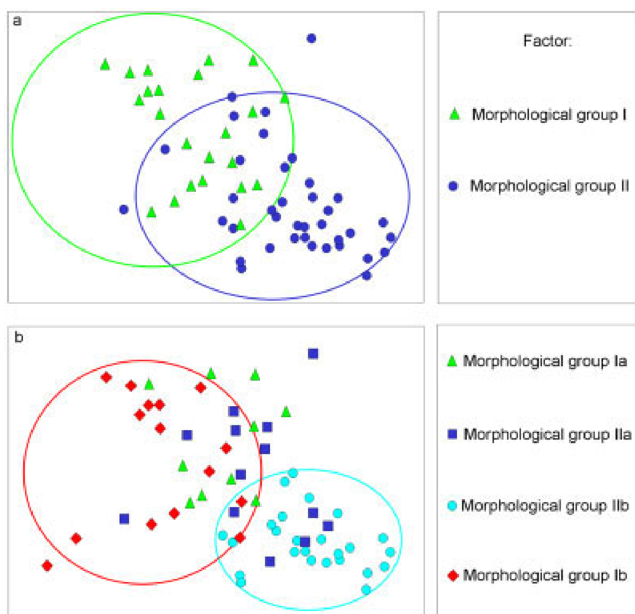

Fig. 3. Two-dimensional MDS analyses done on the ramet level. **a:** Testing the two main morphological groups I and II. **b:** testing the four morphological sub-groups: I(a), I(b), II(a), and II(b).

20–25 mm in subgroup I(b)). The growth was also characterized by high dichotomous branch initiations (DI = 33–48%, compared with 13–28% in subgroup I(b)) and a high percentage of order 3 branches (N3 = 23–40%, compared with 5–12% in subgroup I(b)). As the colonies easily bifurcated, they created higher orders of branches, with the parameter $\Omega = 4$ (Table 1). It is interesting to note that a significant difference was found in the weight added between I(b) and II(b) genotypes ($\Delta W = 2.1$–3.3 gr in subgroup I(b) and 6.4–13.1 gr in subgroup II(b); Fig. 5). This outcome corresponded to the significant differences recorded in the number of new branches added (nB = 11 in subgroup I(b) and 30 in subgroup II(b)), for the total new length branch added (Tl = 91.34 mm in subgroup I(b) and 261 mm in subgroup II(b)) and to the intensive bifurcate growth (Bp = 8.27 in subgroup I(b) and 20.8 in subgroup II(b)).

The other two subgroups of genotypes I(a) (colonies A, E) and II(a) (colonies B, F; Fig. 2b) represented two intermediate architectural stages (Fig. 4c,d). Colonies of subgroup I(a) were closely related to colonies of subgroup I(b), with high rates of side initiation (SI = 77–97%) and high percentages of order 2 branches (N2 = 72–94%). They revealed a moderate ratio of skeletal volume to total ecological volume (Sv/Ev = 0.17), lower than of subgroup I(b) colonies (Sv/Ev = 0.20–0.36). In this intermediate group, the 1-year height parameter (H = 44.5–78.6 mm) was the most representative parameter as opposed to the percentage of height added, characteristic to subgroup I(b) colonies. The most prominent parameters that failed to show in the SIMPER results were the increased lateral dimension (Le = 30–33mm) that was higher than in subgroup I(b) and the increased average number of branches (nB = 15–16 as compared with 9–12 in subgroup I(b) colonies). When comparing their architectural characteristics to subgroup I(b) colonies, subgroup I(a) colonies showed a faster rate of lateral extension, an increase in the number of branches, more height, and a decrease in the ratio of skeletal-to-ecological volume, revealing more space between the branches.

TABLE 2. SIMPER Outcomes for Similarity Among Ramets of the Same Genet[a]

Genotype (N)	Within colony average similarity (%)	Five parameters most contributing to the similarity				
H (4)	76.17	N2	Bal	SI	Sv/Ev	ΔH%
I (4)	81.09	N2	Bal	SI	Sv/Ev	ΔH%
J (7)	75.80	N2	UGB	SI	Sv/Ev	ΔH%
A (6)	83.42	N2	H	SI	Sv/Ev	Ω
E (4)	88.03	N2	H	SI	Bal	ΔH%
F (6)	84.11	N2	H	SI	ΔW	ΔH%
B (7)	74.74	N2	H	DI	Bal	Ev/nB
G (8)	83.81	ΔW	Ev	nB	Le	N3
C (9)	86.28	Ω	Ev	DI	Le	N3
D (8)	85.99	Ω	UGB	DI	Le	N3

[a]For parameter definitions, see Table 5.

TABLE 3. SIMPER Analysis of the Five Parameters Most Contributing to the Similarity (S) or Dissimilarity (D) Between and Within the Morphometric Groups[a]

Level of analysis(groups)	Average dissimilarity (D) or similarity (S)	Five most contributing parameters				
I vs. II	D = 34.04	EV	N3	Sv/Ev	DI	EV/nB
I(a) vs. II(b)	D = 30.91	EV	N3	UGB	DI	nB
I(b) vs. II(b)	D = 36.44	EV	N3	Sv/Ev	DI	nB
Within I	S = 75.50	N2	Sv/Ev	H	Bal	ΔH%
Within II	S = 78.20	N3	Ev/nB	H	Le	DI
Within I(a)	S = 80.01	N2	SI	Sv/Ev	H	Bal
Within I(b)	S = 76.94	N2	SI	Sv/Ev	ΔH%	Bal
Within II(a)	S = 75.26	N2	Ev/nB	H	ΔH%	Bal
Within II(b)	S = 82.88	N3	DI	Le	Ω	Ev

[a]For parameter definitions, see Table 5.

TABLE 4. Pair-wise Test Results for Dissimilarity of Developmental Patterns Between the Subgroups

Subgroups			Pair-wise R statistic	Significance level ($P = 0.05$)
I(a)	vs.	I(b)	0.272	0.002
II(a)	vs.	II(b)	0.411	0.000
I(a)	vs.	II(a)	0.236	0.003
I(a)	vs.	II(b)	0.735	0.000
I(b)	vs.	II(a)	0.307	0.000
I(b)	vs.	II(b)	0.769	0.000

Parameters that were unchanged in both subgroups were the appearance of branches from order 2 only and the significant side branch initiations (Fig. 4a,c).

Subpopulation II(a) colonies (genotypes B and F; Fig. 2b) were morphologically similar to subpopulation II(b) and I(b) genotypes (Fig. 3d). This intermediate-group colony was characterized by distinctive colony height (H = 55.5mm) similar to colonies of subpopulation II(b), high percentages of height added (ΔH% = 185%), high percentages of branches from order 2 (N2 = 73%) and high lateral branch initiations (SI = 72%), resembling colonies of subpopulation I(b). It resembled subpopulation II(b) also in the high average of ecological volume per branch (Ev/nB = 3.4 mm^3). From the parameters not showing in the SIMPER results, the parameters Le (lateral dimension = 37 mm) and Sv/Ev (ratio skeletal volume to ecological volume = 0.11) were similar to subpopulation II(b). The architectural signature of this intermediate group represented a transformation from up-growth form to increasing of ecological space by expansion in the lateral dimension and by adding more branches to the colony. However, the branching system was still simple and most branches were of order 2.

DISCUSSION

In this study, we identified two "types" of architectural strategies, represented by two subpopulations of *S. pistillata* genotypes, in addition to two intermediate groups. These architectural designs described the detailed

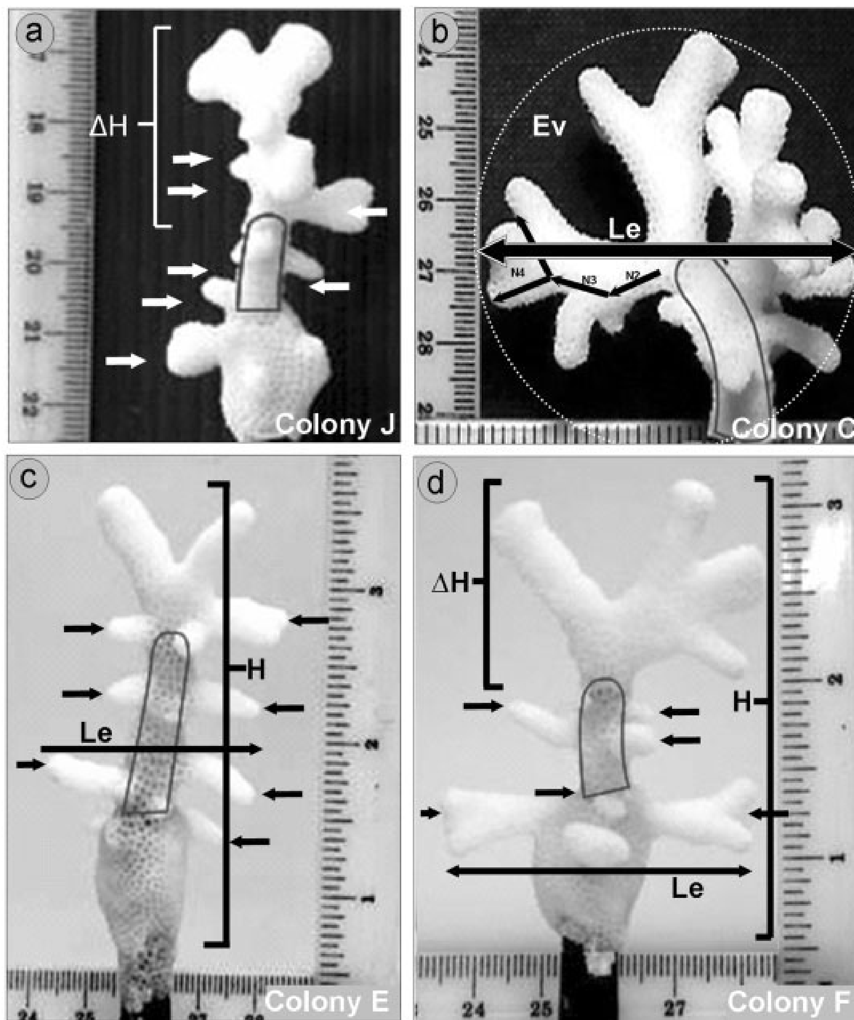

Fig. 4. Examples of typical 1-year architectural growth of the four designated subpopulation patterns. Black line marks delineate the couture of each branch fragment a year ago. **a:** Subpopulation I(b), colony J. Arrows point to side initiated branches, belonging to order 2. ΔH is the added height to the colony after 1 year of growth. **b:** Subpopulation II(b), colony C. Arrows point to the initiation of new order of branches (N2, N3, N4). Le is total lateral extension. Ev is the ecological volume. **c:** Subpopulation I(a), colony E. Arrows point to side initiated branches of order 2. H is the height of the colony after 1 year of growth. Le indicates the total lateral extension of the colony. **d:** Subpopulation II(a), colony F. ΔH is the added height to the colony after 1 year of growth.

trajectory of branch to colony trajectory, during an astogeny window of 1 year. Each of the above architectural designs was characterized by specific morphometric parameters. However, a comprehensive analysis of all characteristics attributed to the four subgroups revealed that they might not have presented different growth strategists but a continuum of a single developmental process (Fig. 5). Colonies that had the fastest growth rates showed, after 1 year of development, the characters of subgroup II(b) genotypes, whereas the slowest growing colonies were subgroup I(b) genotypes (Fig. 5). Colonies with average growth rates were in intermediate phases. It is suggested, therefore, that during the development of a branch fragment to a colony, the fragment "transformed" from one developmental design to the other (stages in Fig. 5) and the differences between the two extreme morphological subgroups reflected differences between developmental stages caused by different growth rates of the genotypes, within the experimental window of 1 year and not necessarily different developmental strategies.

Whereas the colony astogeny in *S. pistillata* is a continuous developmental plan, we can define at least three early distinct phases, as schematically illustrated in Figure 5. After 1 year of growth, slow growing *S. pistillata* colonies will present the "time 0 to phase X" (Fig. 5) pattern of development as group I(b) colonies (H, I and J; Figs. 2b, 4a). These colonies will show fast vertical growth, initiation of many side branches of order 2, minimal added ecological volume and limited average ecological volume per branch, exhibited by a high ratio of skeletal to ecological volume (Table 1). The two intermediate subgroups (I(a), II(a)) depict the developmental characteristics illustrated by moving from phase X to phase X+1 (Fig. 5). During this developmental step, the branches reduce vertical growth rates and intensify lateral extension that lead to an increased ecological volume (Table 1). Thereafter, the initiation of a higher order of branches is enhanced by bifurcation of UGBs and LGs and the development of a more complex branching system. The skeletal to ecological volume ratio decreases, revealing the formation of more space between the branches. Fast-growing *S. pistillata* colonies (group II(b)) show, after 1 year of growth, the characteristics illustrated by moving from phase X+1 to phase X+2 (Fig. 5). They reveal the same rate of vertical extension as in slow growing colonies, but increased values for all other growth parameters. Above architectural stages of development are only part of the repertoire (not yet completely elucidated) of developmental stages in the astogeny from branch to well-developed colony. Studying the development of colony for longer periods will probably reveal additional developmental stages.

The literature (Bell, 1986; Rosen, 1986; Dauget, 1991b; Abraham, 2001; Kaandorp and Kubler, 2001; Merks, 2003; Sánchez et al., 2004) considers the development of modular organisms, including coral colonies, as an iterative process. In this process, a colony is being constructed by adding identical modules through the same pathway of development (Jackson, 1979; Dauget, 1991b; Lasker et al., 2003; Sánchez and Lasker, 2003; Sánchez et al., 2004). According to this tenet, the basic architectural rules remain the same during the entire development. However, the *S. pistillata*

1-year window of development studied here revealed several sets of architectural modes, each with its own specific rules. Although, the growth rules appeared to have changed, the changes seemed to represent a predictable and continuous process. Similarly, Le Tissier (1988) divided the growth of *Pocillopora damicornis* branches into seven categories, but stated that these categories exhibited a single continuous process, found to be similar in corals growing even under different environmental conditions. These outcomes recall gorgonians astogeny (Weinbauer and Velimirov, 1995, 1998), where the colony grows in a way that reduces the ratio between colony height and colony width, a strategy aiming to decelerate the perpendicular growth of the colony into the high water column (in which drag forces from the currents are stronger; Lesser et al., 1994; Bruno and Edmunds, 1998).

This rationale also applies to the developmental processes leading to increased total ecological volume and the tendency to increase the average ecological volume per branch. The resultant space between branches is highly relevant for the heterotrophic nutrition of the colony (Weinbauer and Velimirov, 1995, 1998). The skeletal volume to ecological volume ratio reflects the relative space between coral branches. In a developing *S. pistillata* colony, we found that an initial ratio of the skeletal volume to the total ecological volume (0.17–0.36; represented in this study by colonies A, E, H, I, J) was reduced to 0.08–0.13, in more developed stages (colonies D, C, and G). Bruno and Edmunds (1998) described a similar phenomenon in colonies of the coral species *Madracis mirabilis*. This strategy of colony architecture maintains efficient space between branches, which may also be important for reducing the influence of the boundary layer around each branch (Patterson, 1984), improving respiratory and metabolic rates (Bruno and Edmunds, 1998).

The above results suggest that *S. pistillata* colonies possess a species-specific genetic basis for colony architecture. Differences in colonial form are attributed to changes in growth rate. Astogeny in *S. pistillata* has several morphometric stages, each characterized by different morphometric parameters, revealing the important role of the species's genetic blueprint in colonial growth. In the past, scientists tried to explore the importance of fixed colonial landscape rules by simulating growth patterns that orchestrate the complex structure of a colony into mathematical language (Bell, 1986; Kaandorp and Sloot, 1997; Kaandorp and Kubler, 2001; Kaandorp et al., 2005) by pre-employing architectural models to branching forms of colonies (Dauget, 1991a,b) or by applying physical attributes to morphologies (Abelson, 1993; Kim and Lasker, 1997; Muko et al., 2000). The above studies reveal different possible explanations for pattern formation of coral colonies. However, the present study illuminates, for the first time, the importance of changes in architectural rules and depicts the need for detailed follow-up observations in elucidating astogeny in colonial forms.

EXPERIMENTAL PROCEDURES

General

Experiments were conducted in Eilat, Israel, in front of the H. Steinitz Marine Biology Laboratory. Ten adult *S. pistillata* colonies (marked by letters A to J), 15–20 cm diameter each, were carefully chiseled out from a depth of 7 m. Each colony represented a single genet, as colonial fragments of this species in Eilat do not develop to ma-

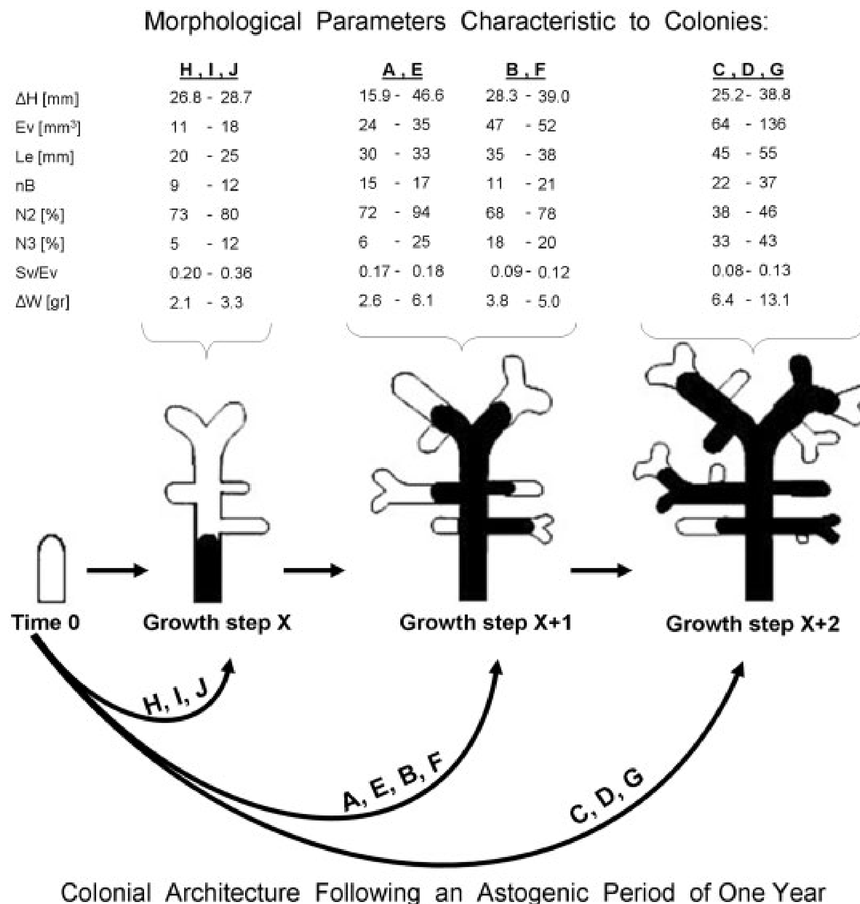

Fig. 5. A schematic illustration of architectural trajectory from single *S. pistillata* branches into small colonies. The 1-year "window" of development reveals, at least, four different major architectural stages of growth (0, X, X+1, X+2) and three connecting phases to which the 10 *S. pistillata* genotypes are assigned. Phase 0 to X is characterized by fast lateral growth and many new side initiations. Phase X to X+1 is characterized by an increase in ecological volume, by extending through lateral dimension, and bifurcation of branches. Phase X+1 to X+2 is characterized by an augmentation of the ecological volume, by bifurcation of older branches to new generation of branches, while lateral elongation of the colony is reduced. The morphological parameters characteristic to each step are detailed.

TABLE 5. List of Morphometric Parameters Considered to Represent Architectural Components in the Development of New Colonies From Isolated Branches[a]

Way of measuring/calculating	Description	Morphometric character	
Each branch length was measured by digital calipers to the nearest 0.01 mm. Averages were obtained by summing all lengths and dividing by the total number of branches (Kim and Lasker, 1997).	Branch average	BaL	1
Measured from the substrate to the highest ramets' point (Jackson, 1979).	Height (mm)	H	2
(H-Ho); Vertical growth added after 1 yr.	Height added (mm)	ΔH	3
The initial height (H0) was measured from the substrate to the alizarin mark (Rinkevich, 2000). Percentage of height added is calculated as: (H-H0)/H0.	Height added (%)	ΔH%	4
Total number of branches, including the initial branch (Kim and Lasker, 1997, Muko et al., 2000).	Total no. of branches	nB	5
The number of branches from each order as part of the total number of branches (Reverse Strahler Order method; Abraham, 2001).	Branches order (%)	% Nx = N1-N4	6
The number of branch bifurcations divided by the total number of branches.	Dichotomous branches (%)	%DI	7
The number of lateral branches divided by the total number of branches.	Lateral branches (%)	%SI	8
UGBs divided by the total number of branches.	Up-growing branches (%)	%UGB	9
Sum of skeletal and space between the branches volumes. πHr^2; r = width + length/4. Width and length, see Fig. 1d.	Ecological volume (mm^3)	Ev	10
Sum of all branch volumes (each branch is calculated as a cylinder) divided by the ecological volume.	Skeletal to ecological volumes ratio	Sv/Ev	11
The ecological volume divided by the total number of branches, providing an average ecological volume per branch that includes the volume of the branch and surrounding space.	Average ecological volume per branch (mm^3)	Ev/nB	12
Colony width, the axis between two LB in the colony (Fig. 1c).	Lateral dimension (mm)	Le	13
W-W0; W0 = Initial weight, obtained by removing all branches developed above alizarin (Rinkevich, 2000), W= Weight after 1 yr.	Weight added (gr)	ΔW	14
According to the Reverse Strahler Order method (Abraham, 2001); numbers represent the highest order that a specific colony reached.	The order of colony complexity	Ω	15

[a]UGB, up-growing branches; LB, lateral branch.

ture adult colonies (Rinkevich, unpublished observations) Colonies were incubated in transparent plastic bags with alizarin Red S solution for 12 hr in situ (15 ppm; following that of Rinkevich, 2000). During labeling, tips of branches, the major site for calcification, were stained with red dye. Then, new branches grew as white calcium carbonate areas above the red lines. After 2 weeks of postlabeling acclimation, 10 single-tip branches of 2–4 cm each were removed from each colony by wire cutters and attached with plastic clips to underwater nursery tables that were placed at a depth of 7 m, under identical in situ conditions. Sixty-three fragments survived the entire duration of the experiment (1 year) and developed into small colonies. At that stage, colonies were brought to the laboratory and their tissues were removed by immersion in household bleach for 24 hr (Rinkevich and Loya, 1984).

Morphological Measurements

Fifteen MPs (Table 5; Fig. 1c,d) from each of the 63 colonies were measured and analyzed. These MPs were subjected to Draftsman plot analysis (Shaish, 2004) to find and omit pairs of parameters sharing a high degree of correlation with each other ($P > 0.95$), meaning that they did not contribute additional information to the data analysis (Clarke and Ainsworth, 1993). The measurements were taken with digital calipers to the nearest 0.01 mm, and data were stored using EXCEL software. The morphometric parameters describe either (a) the colony level, such as initial height, added height, width, length, ecological volume, total number of branches, the ratio of skeletal volume to ecological volume, average volume per branch and Ω; or (b) the branch level, such as branch average length, up-growing or lateral-growing branches, total bifur-

cations, and branch order (Table 5; Fig. 1c,d). We characterized branch order using the Reverse Strahler order system (Abraham, 2001), by which branches are ranked on the basis of the number of branching events from the original, primary branch (Fig. 1c; Lasker et al., 2003). The first initiated branch (the stem) is ranked 1, each secondary branch is ranked 2, branches that grow from the secondary branches are ranked 3, and so on (Fig. 1c).

Analyses

Data analysis was performed by multivariate analysis methods using PRIMER 5 software (Clarke, 1993; Clarke and Ainsworth, 1993). Data were standardized by rescaling each category, which ensured that each of the characters contributed equally to the overall information on the colony's morphological structure. A Bray–Curtis similarity matrix was used to compare the similarity between the 10 genotypes.

We used two analyses to test the null hypothesis that the architecture of each developed colony, as represented by the morphometric characters, is similar to other colonies in the same genet and differ from other genets: (1) ANOSIM reveals the similarity between ramets of the same genet or between different genets by the value of R. When $R > 0.5$, the genets but not the ramets within a genet, differ from each other; when $R < 0.5$, also the genets do not differ from each other (Clarke, 1993); (2) SIMPER test shows the percentage of similarity between ramets of the same genet and the morphological parameters that contribute to this similarity. We used the SIMPER test also to assess the contribution of each parameter to the similarity between ramets of a specific genet or the dissimilarity between pairs of genets. This assessment was done by isolating each parameter in each colony and calculating the percentages that each parameter contributed to the overall similarity/dissimilarity. We chose to present the five most contributing parameters to the similarity/dissimilarity between the tested groups (Clarke, 1993). In addition, two-dimensional nonmetric MDS algorithm was used on the Bray–Curtis similarity matrix to see weather the genets were grouped together. In this analysis, the genets were represented by points on a two-dimensional graph. The closer the points were, the higher was their similarity (Clarke and Ainsworth, 1993). We also used the hierarchical agglomerative clustering analysis method, in which samples were organized in a tree-shaped diagram, according to their level of similarity (Clarke and Ainsworth, 1993).

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