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DYNAMICS OF THE COLONIAL CYCLE IN THE ASCIDIAN, *BOTRYLLUS SCHLOSSERI*. THE FATE OF ISOLATED BUDS

Giovanna ZANIOLO, Armando SABBADIN *and* Carla RESOLA

Istituto di Biologia Animale, Università di Padova (Italy).

INTRODUCTION

In the colonial ascidian *Botryllus schlosseri*, the buds are fed by blood conveyed from the parents through a connecting stalk. Both the adults and the older buds are interconnected by a vascular network, which crosses the tunic and opens at the periphery into a collecting *marginal vessel*. The tunic and its vascular system represent the persistent colonial matrix, which may survive for weeks in the absence of zooids, owing to the autonomous contraction of the vascular ampullae branching out from the marginal vessel (BANCROFT, 1899; DE SANTO and DUDLEY, 1969).

The isolated colonial matrix can act as a culture medium for young buds detached from the parents as early as the beginning of organogenesis; by vascularizing the buds, it replaces their parents in the feeding function, and allows the organogenesis to be performed (SABBADIN *et al.*, 1971; 1975).

In the present work we shall consider the fate of grown buds isolated by removal of the parents and by detachment from the vascular network of the tunic.

MATERIALS AND METHODS

Young colonies consisting of one system of zooids were used (Fig. 1), attached to glass slides and reared in aquaria with aerated sea water at a constant temperature of 18⁰C. They were fed on unicellular algae.

Three sets of colonies were operated at the colonial stage 9/8/2 (SABBADIN, 1955): this stage is characterized by adult zooids (st. 9) with their dextral buds showing the onset of heart beat (st. 8); these in turn have bud primordia at the hemisphere stage (st. 2) (Fig. 1). In a first group of 11 colonies (series I), 45 dextral and 14 sinistral buds were detached from the adults which were then removed, each bud remaining connected to the marginal vessel through its *radial vessel* (Fig. 2). In the two other sets of 28 colonies each, after removing the adults and the sinistral buds, the connections of the dextral buds with the marginal vessel were interrupted by cutting through the radial vessel. In one of the groups (series II) the marginal vessel was left intact (Fig. 3); in the other (series III) it was fragmented by removing that portion adjacent to the buds (Fig. 4). There were 142 and 83 buds in series II and III, respectively.

The colonies were examined daily with a Zeiss dissecting microscope, and pictures were taken with a Zeiss Tessovar.

RESULTS

The heart beats in the buds and blood circulation in the tunic revived shortly after the operations in the three experimental series. The remnants of the marginal vessel in the colonies of series III did not reestablish interconnections, and each piece, with its own train of vascular ampullae, functioned as an independent blood system.

The results of the experiments are summarized in Table 1.

In series I the persistent connection of buds with the general circulation allowed them to continue development up to the adult stage, in spite of the absence of the parental zooids. However, all the sinistral buds and many dextral buds were resorbed together with their bud primordia; in the remaining 26 buds, which became adult zooids, only the dextral bud primordium achieved maturity.

In series II and III the buds, which had been isolated from the general circulation, had different fates: (1) some reestablished connection with the marginal vessel and eventually achieved the adult stage with only the dextral bud primordium developed; (2) some formed an independent vascular network in the tunic and, while undergoing regression, developed the dextral bud primordium which joined the newly forming vascular system and became an adult zooid of the following generation; (3) some were resorbed together with their bud primordia, either after

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Fig. 1. — A young colony of *B. schlosseri* consisting of a single system of adult zooids (**a**) and two successive generations of buds (**b1**, **b2**), seen from the ventral side. The adults and the older buds are connected by radial vessels (**r**) to the marginal vessel (**m**). × 22,5.

Fig. 2. — Series I: the adults have been removed; the dextral (**d**) and sinistral (**s**) buds with their bud primordia are connected to the marginal vessel (**m**). × 20.

Fig. 3. — Series II: a portion of a colony showing two buds isolated both from the parental zooids, which were removed, and from the marginal vessel. × 70.

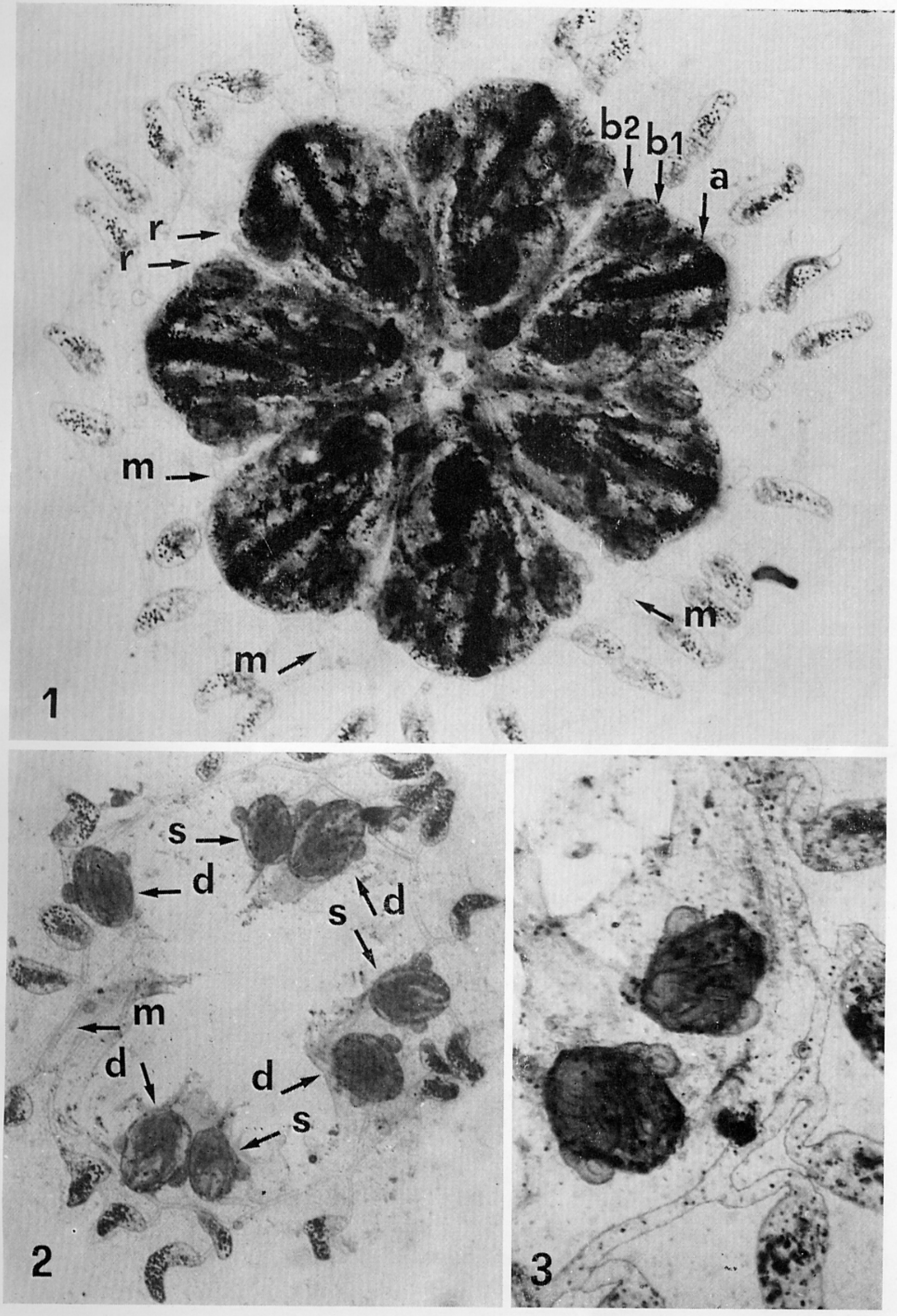

b2
b1
a
r
r
m
m
m
1
s
d
d
s
m
d
d
s
2
3

Table 1

Buds and their bud primordia isolated within the colonial matrix. The buds resorbed were (*a*), or were not (*b*), connected with the general circulation. The buds which regressed constructed their own vascular system in the tunic, independent of the general system; they did not reach maturity but, like the matured buds, each developed a bud primordium.

Series	Buds						Bud primordia matured No.
	total No.		resorbed No. (*a*)	resorbed No. (*b*)	regressed No.	matured No.	
I	dextral	45	19	—	—	26 (*57.77%*)	26 (*57.77%*)
	sinistral	14	14	—	—		
II	dextral	142	36	17	31	58 (*40.84%*)	89 (*62.67%*)
III	dextral	83	—	51	29	3 (*3.61%*)	32 (*38.55%*)

reconnection with the marginal vessel (a), or during the formation of their own vascular system (b).

An external vascularization prooved to be a necessary condition for buds or their bud primordia to survive and reach the adult stage.

The marginal vessel reestablished connection with the majority of the buds of series II, in which case its integrity had been maintained, and

Fig. 4. — Series III: a bud isolated both from the parental zooid and from the marginal vessel which was interrupted in the portion facing the bud. × 42,5.

Fig. 5. — Series II: the marginal vessel is directing a collateral (**c**) towards an isolated bud. × 51,2.

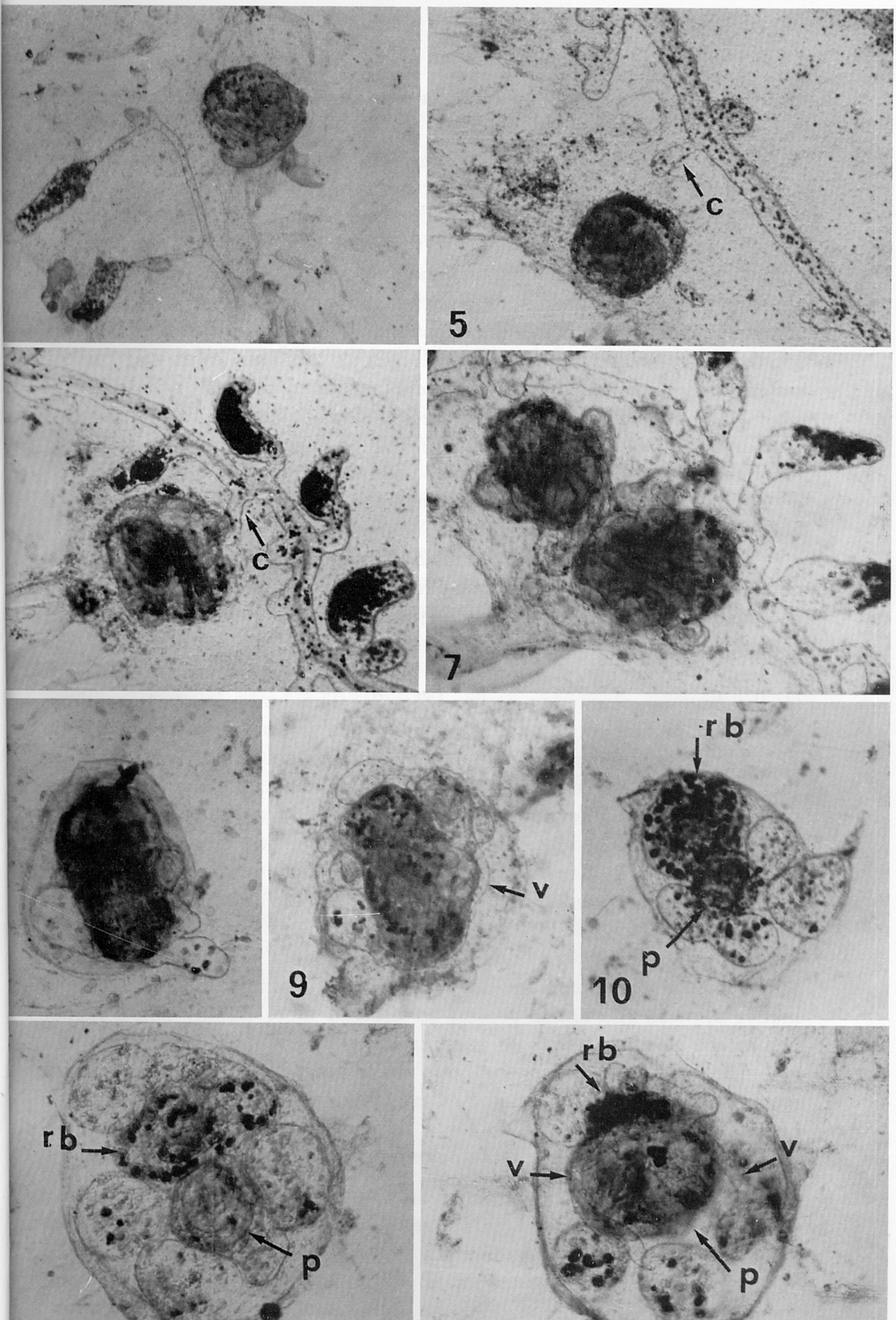
Fig. 6. — Series II: a previously isolated bud reached on its right side by a collateral (**c**) of the marginal vessel. × 66.

Fig. 7. — The two buds of Fig. 3 reconnected to the marginal vessel. × 81,2.

Figs. 8, 9. — Series III: two initial steps in the formation of an independent vascular system by an isolated bud. Fig. 8: vascular ampullae branching out from the bud (× 75). Fig. 9: two days later, the stalks of the ampullae are forming a major vessel (**v**) encircling the bud. (× 60).

Fig. 10. — Series III: a regressing bud (**rb**) with its train of vascular ampullae; bud primordium (**p**) developing. (× 100).

Figs. 11, 12. — Series III: two steps in the formation of an independent vascular system by a regressing bud (**rb**). Fig. 11: vascular ampullae proliferated from the bud; bud primordium (**p**) developing (× 72,8). Fig. 12: five days later, the regressing bud has been almost resorbed, and the bud primordium has developed further; a major circumferential vessel (**v**) is forming. (× 72,8).


5
c
c
7
v
9
rb
p
10
rb
p
rb
v
v
p
12

with only 3 buds of series III, when it had been fragmented. In this last series the formation of new vascular networks normally occurred.

The new vessel reconnecting the bud with the general circulation always arose as a collateral of the marginal vessel adjacent to the bud (Fig. 5 & 7). After reaching the bud it opened into the lacunae of the mantle. The bud appeared to polarize the course of the vessel, which always joined the bud on the right side (Fig. 6), where the heart is located, even when this entailed a longer route. This point was further tested with some additional operations performed on buds with reversed *situs viscerum*, with the heart on the left side, and in this case it was this side attained by the new vessel.

The reconnection of the buds with the marginal vessel required an average of 36 hrs. During this time the development of the bud and bud primordia did not progress.

The formation by the buds of independent vascular systems required an average of two weeks. In many respects it resembled the formation in the metamorphosing larva of the primordium of the general vascular system of the tunic. A variable number of ampullar extrusions of the epidermis arose (Fig. 8, 10 & 11) and progressed outwards, connected to the bud by elongating stalks (Fig. 9). The buds, in the meantime, underwent regression; the internal structures gradually disappeared (Fig. 12). However the heart continued to beat for about 10 days, pumping the blood into the newly forming vascular network. The dextral bud primordium started developing after some days of stasis and, at the time the maternal bud became a structureless vesicle, had reached stage 7 at which time, as in normal development, a radial vessel arose from its subendostylar sinus and joined an ampulla or an ampullar stalk. Later, the fusion of ampullae and ampullar peduncles resulted in a major vessel encircling the developing bud (Fig. 9 & 12), with new ampullae proliferating at the periphery. In series II the less severely modified colonial matrix allowed the entire process to proceed more rapidly and a higher percentage of successful cases was registered.

In both series when the buds developing inside the new vascular system attained filtering stage 9, their own bud primordia had not progressed beyond stage 4 or 5; for a time, only two instead of three generations were coexistent, as in colonies newly founded by the oozooids.

DISCUSSION

The dynamics of the colonial cycle in *B. schlosseri* is governed by two basic phenomena: the synchronous development of the individuals of the same generation, in a strict correlation with the developmental stages of the other coexisting generations; the competition among the contemporary individuals as well as between those of the other coexisting generations. These phenomena are influenced by both intrinsic factors and the environment.

The initiation of bud primordia is confined to the stage 7 of the younger generation, which normally coincides with the disappearance of the older generation and the attainment of the adult stage by the intermediate generation. The blastogenic potentialities of zooids are asymmetrical, those of the right side being greater; the bud primordium on the right side splits into two or three buds more frequently than that on the left side; some of the buds often fail to fulfil development and whenever only one bud per side survives, it is always that on the left side to be resorbed. The normally three coexisting generations may be reduced to two under unfavourable conditions since both the resorption of the adult generation and the maturation of the following generation occur earlier than normal. On the other hand the generations can be increased to four, their life span being considerably lenghtened, by experimentally reducing the number of individuals per generation (SABBADIN, 1958; 1973).

The integrated pattern of individuals and generations, and the capacity of regulating the number of individuals per generation and the number of coexisting generations, in dependence on the environmental conditions, are mediated by the vascular connections between the individuals. According to WATANABE (1953) « the asexual reproductive phases of the zooids are probably regulated by some growth promoting substances circulating with blood ». It may be that this holds true both for the timing and the intensity of asexual reproduction. Both these phenomena have been affected by our experimental interventions.

The extirpation of the adults resulted in a great decrease of growth in all the three experimental series. Many buds were resorbed with their bud primordia and the surviving ones developed only the dextral bud primordium. This was the case for all the sinistral and a high percentage of dextral buds in series I; a similar percentage of resorption was encountered in those buds of series II which rejoined the general circulation. Many of the regressing buds which constructed their own vascular system contributed to the next generation by developing dextral bud primordia.

The development of these primordia was not timed to that of the primordia of the buds connected to the common circulation; moreover, their filtering stage preceded the budding stage 7 of the older buds, so that for a time the new functional colonial units consisted of two generations only.

On the other hand the connection with an external blood circulation prooved necessary for bud growing and maturing. Under normal conditions the connection is assured by the radial vessel of each bud; it originates at stage 7 from an extrusion of the subendostylar sinus, paralleled by an opposite extrusion from the adjacent marginal vessel. These two vascular evaginations later fuse at their distal end. In series II and III the interruption of the radial vessel at stage 8 was followed by the resorption of its remnant. No regeneration of this vessel by the bud took place, but in many cases a new vessel arose as a mere collateral of the marginal vessel. In series III, in which the portions of the marginal vessel adjacent the buds had been cut off, only occasionally a new radial vessel arose from one of the more distant, lateral fragments. All these observations support the assumption (BERRILL, 1961) that an inductive stimulus on the marginal vessel arises from the bud and diffuses a limited distance. The fact that the bud seems to polarize the course of the vessel which reaches it always on the side where the heart is located, also falls in line with the above assumption. It thas been shown (SABBADIN, ZANIOLO and MAJONE, 1975) that bud primordia isolated or transplanted within the colonial matrix are also effective in inducing the formation of a collateral of the colonial vascular network. It is worthwhile noting that in this case it is the vessel emptying into the bud primordium which determines its polarity and symmetry.

The buds of series II and III which failed to reconnect with the general circulation underwent a slow regression, in the end becoming structureless vesicles engorged with blood cells. However, many of them succeeded in constructing an external blood system to which they transferred the task of feeding their bud primordia. These joined the newly forming vascular system by generating a normal radial vessel. If the entire process had not sufficiently progressed at the time of cessation of heart beat in the maternal bud, the bud primordia were resorbed.

The previously unknown capacity of the bud to construct its own external vascular system is a paramount expression of regulative powers at the individual level; however, it is programmed to assure the survival and continuity of the colony. The blastozooid takes upon itself a task which in normal development is reserved to the metamorphosing larva, from which it differs in many respects but whose genetic complement it shares.

SUMMARY

In three series of colonies of *B. schlosseri* the grown buds with their bud primordia were detached from the parental zooids which were then removed. The connections of the bud with the marginal vessel in the tunic were either maintained (series I) or severed, the vessel being either preserved intact (series II) or fragmented (series III).

The extirpation of the adults resulted in a considerable decrease of the growth rate in the three series: many buds were resorbed together with their bud primordia and the surviving ones developed only the dextral bud primordium.

The majority of buds in series II reestablished connection with the general circulation through a radial vessel arising as a collateral of the marginal vessel; this occurred only occasionally in series III. In both these series the buds, which did not rejoin the general circulation, while regressing showed the capacity to construct external vascular networks which allowed the development of their bud primordia. In this way independent colonial units formed inside the same colonial matrix each with its own temporal growth pattern.

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Present address of the authors. — Istituto di Biologia Animale, Via Loredan 10 - 35100 Padova (Italy).

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