Escape Hatches for the Clonal Offspring
of Serpulid Polychaetes

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Abstract. Serpulid polychaetes in the genera *Filograna* and *Salmacina* reproduce asexually by releasing a single bud at a time from their posterior ends into their calcareous tubes. Here I show that buds of *Salmacina amphidentata* gain access to the exterior of these tubes *via* escape hatches built into the tubes by the parent worms. Each escape hatch consists of a hole in the tube blocked by a calcareous disc that is supported in place by an organic membrane. After buds detach from their parents, the calcareous discs are dislodged, and buds begin to form their own tubes from the resulting openings. Repeated bouts of asexual reproduction result in the formation of aggregations of branched tubes. A survey of *Filograna* and *Salmacina* spp. from the Atlantic, Indian, and Pacific oceans suggests that the formation of escape hatches for clonal offspring is common to many members of these genera.

Introduction

Serpulid polychaete worms are common members of hard substratum communities in all of the world's oceans, and are usually easily recognized by their white calcareous tubes. Construction of these tubes begins when planktonic serpulid larvae arrive at benthic settlement sites and undergo metamorphosis. During metamorphosis each juvenile secretes a primary tube of organic material which attaches it to the substratum (*e.g.,* Nott and Parkes, 1975; Carpizo-Ituarte and Hadfield, 1998). It then adds to one end of this primary tube by precipitating calcium carbonate in a pair of anterior glands, suspending the resulting crystals in a fluid organic matrix, and molding this slurry onto the anterior tube margin, where it hardens (Hedley, 1956; Neff, 1971;

Nott and Parkes, 1975; Clark, 1976). By continuing this process of marginal accretion as it grows, each worm forms a simple calcareous tube attached to the substratum and open only at one end, from which the worm extends its suspension-feeding and respiratory tentacles. Though serpulids are not physically attached to their tubes, under normal circumstances they do not leave them, and worms removed from their tubes are unable to build new ones (*e.g.,* Faouzi, 1931). Tube growth requires a pre-existing tube onto which new material can be added, and apparently only newly metamorphosed juveniles are capable of secreting such a structure *de novo,* in the form of the primary tube.

This pattern of tube growth creates two problems for the few species of serpulids that, in addition to producing larvae by sexual reproduction, reproduce asexually. Asexual reproduction is best known in members of the widely distributed genera *Filograna* and *Salmacina* (see Materials and Methods for comments on the systematics of these taxa), though it occurs in members of at least five other genera as well (*Filogranella, Filogranula, Josephella, Rhodopsis,* and *Spiraserpula:* H. ten Hove, Zoological Museum, Amsterdam, The Netherlands, pers. comm.; ten Hove, 1979; Nishi and Yamasu, 1992; Pillai and ten Hove, 1994). Adults of *Filograna* and *Salmacina* spp. reproduce asexually by paratomous fission, releasing a single budded offspring at a time from the posterior end (Malaquin, 1895; Faulkner, 1930; Cresp, 1964). A newly released bud is thus effectively sealed in a tube whose only opening is blocked by the parent worm. How do these asexually derived individuals gain access to the exterior environment to feed, grow, and reproduce? Once they have gained access to the exterior, how do they build their own tubes?

An important clue is that adults of *Filograna* and *Salmacina* spp. occur in conspecific aggregations of branching tubes. Though individual adults of these species are small, rarely exceeding 500 μm in diameter and a few millimeters

in length, aggregations may be composed of hundreds or thousands of individuals and reach dimensions of tens of centimeters (ten Hove, 1979; Nishi and Nishihira, 1997). Observations of aggregation growth and form suggest that asexually derived individuals somehow form tubes that branch off from the tubes of their parents (Benham, 1927; Hanson, 1948). Benham (1927) suggested that new tubes branch from the mouths of parental tubes. This might obtain, for example, if a parent shared its tube opening with a bud while the latter began to build a new tube. In contrast, Hanson (1948) concluded that newly released buds bore holes in the parental tubes to gain access to the exterior and then build their own tubes starting from these newly created openings. Either of these hypotheses would account for both the escape of clonal offspring from the parental tubes and the formation of aggregations of branching tubes. Determining which, if either, is correct requires more detailed observations of the processes of asexual reproduction and tube growth.

In this paper I report the results of such observations on the serpulid *Salmacina amphidentata*. I show that each bud gains access to the exterior of the parental tube *via* an escape hatch built into the tube for it by the parent worm. It then uses the parental tube as a starting point for building its own tube. A survey of *Filograna* and *Salmacina* spp. from the Atlantic, Indian, and Pacific oceans suggests that this pattern of tube growth, among the most complex known in the serpulids, is widespread among members of these genera. These observations raise novel questions concerning the evolution of both asexual reproduction and tube formation in serpulids, as well as the signals that coordinate these two processes.

Materials and Methods

During the spring and summer of 2000, I collected aggregations of *Salmacina amphidentata* consisting of tens to hundreds of individuals from the intertidal and shallow subtidal zones of the Indian River Lagoon in the vicinity of Fort Pierce Inlet, Florida. Aggregations were very common at these sites, on the undersides of stones and the shells of dead bivalves.

Systematics of Filograna and Salmacina

I identify the Floridian specimens studied here as *Salmacina amphidentata,* but both the generic and specific names applied are tentative. Members of *Filograna* and *Salmacina* have been distinguished by the presence or absence, respectively, of opercula; because this trait may be variable even within species, Zibrowius (1968) transferred *Salmacina* spp. to the older genus *Filograna*. Despite this, some systematists continue to refer to operculate forms as *Filograna* spp. and inoperculate forms as *Salmacina* spp. (*e.g.,* ten Hove and Wolf, 1984), a practice I continue here. The species-

level systematics of these genera are also uncertain (Zibrowius, 1973; ten Hove and Wolf, 1984). Nine species of *Salmacina* have been described (ten Hove, pers. comm.), but most descriptions are based on only a few specimens from single localities, and the degree of variation in what are considered distinguishing traits has usually not been assessed (but see Gee, 1963; Nishi, 1992). Confident identification to species will only be possible following a revision of these taxa. The specimens I studied closely resembled *Salmacina amphidentata* from Jamaica (Jones, 1962). They differed from the description of that species only in their greater maximum number of chaetigers (33 *vs.* 25), greater variation in number of thoracic chaetigers (seven or eight, rarely six, *vs.* seven, rarely six), and larger size of thoracic and abdominal uncini. Voucher specimens have been deposited in the U.S. National Museum of Natural History (#186807).

Observations of asexual reproduction and tube growth

Aggregations of living worms were examined with a dissecting microscope soon after collection to study tube morphology and branching patterns. Detailed observations of asexual reproduction and tube growth were made on worms cultured in the laboratory. Cultures were initiated by chipping individual worms in their tubes from the substrates on which they were collected. I blotted tubes dry and attached them to numbered glass microscope slides with cyanoacrylate glue. Slides were held in open plastic slide boxes submerged in containers of vigorously aerated seawater (salinity 34 ppt) at 25°C. I changed the water in containers every other day, and after each water change added enough cells of *Isochrysis* and *Nannochloris* to color the water slightly. Worms grew rapidly under these conditions, adding new tube material at the anterior end and producing clonal offspring. I examined them daily with a dissecting microscope to study tube growth. New sections of tube typically grew prostrate, attached directly to the glass slides. Because these tubes were incomplete in cross-section, I could follow the events of asexual reproduction in individual worms by looking into tubes through the glass slides. Asexual reproductive stages were observed at higher magnifications by dissecting worms from their tubes and examining them with a compound microscope. I also used time-lapse video to observe tube growth more continuously in two individuals. The growing margins of tubes viewed through a dissecting microscope equipped with a color CCD video camera were recorded on a time-lapse recorder set to record one frame every 0.2 s.

Views of the external morphology of tubes and worms were obtained by scanning electron microscopy (SEM). Tubes recently collected from the field were prepared for SEM by first chipping portions of interest from the substratum. They were fixed for 10 min in 5% formalin in seawa-

ter, rinsed in distilled water, dehydrated in several changes of 100% ethanol, and air-dried. Worms to be prepared for SEM were removed from their tubes and relaxed in a mixture of 7.5% $MgCl_2$ and seawater, then fixed in 5% formalin in seawater for 30 min, rinsed in seawater, and post-fixed in 1% osmium in seawater for 2–4 h. They were rinsed several times in distilled water, dehydrated in an ascending concentration series of ethanol solutions, and critical-point dried with CO_2 as the transitional fluid. Specimens were mounted on stubs with adhesive carbon tabs (Ted Pella, Inc.) and sputter-coated with gold before being viewed with a JEOL 6400V microscope.

I sectioned several tubes in order to examine their internal structure, using modifications of methods suggested by K. Fauchald (National Museum of Natural History, Smithsonian Institution, Washington, DC) and K. Fitzhugh (Los Angeles County Museum of Natural History, Los Angeles, CA). Pieces of tubes recently collected from the field were prepared as for SEM, except that once dehydrated they were transferred through several changes of propylene oxide and embedded in PolyBed 812 resin (Polysciences Inc.). Excess polymerized resin was removed with a Buehler Isomet low-speed saw equipped with a diamond wafering blade, and trimmed blocks were glued to glass petrographic slides with five-minute epoxy resin. Blocks were then sectioned with the low-speed saw and polished to the desired level with a Buehler Ecomet 2 grinder-polisher. Finally, I stained the sectioned surface in a solution of hot azure II and methylene blue (each 0.5%, in 0.5% sodium borate: Richardson *et al.*, 1960) for 1 min, rinsed with distilled water, air-dried, and covered the cut surface with immersion oil and a coverslip for photography.

Tube growth in Filograna *and* Salmacina *spp. from other regions*

I examined patterns of tube growth in aggregations of *Filograna* and *Salmacina* spp. from localities in the Atlantic, Indian, and Pacific oceans. Sources of material included the U.S. National Museum of Natural History and specimens in my own collections.

Results

Morphology of Salmacina amphidentata *from the Indian River Lagoon*

Knowledge of the morphology of both aggregations and individual worms is needed to understand patterns of asexual reproduction and tube growth. Small aggregations (10–1000 individuals) of *Salmacina amphidentata* are common in the shallow waters of the Indian River Lagoon on the undersides of hard objects like empty bivalve shells and small stones (Fig. 1A). Tubes are white, 250–400 μm in outside diameter, with walls 20–50 μm thick. They are
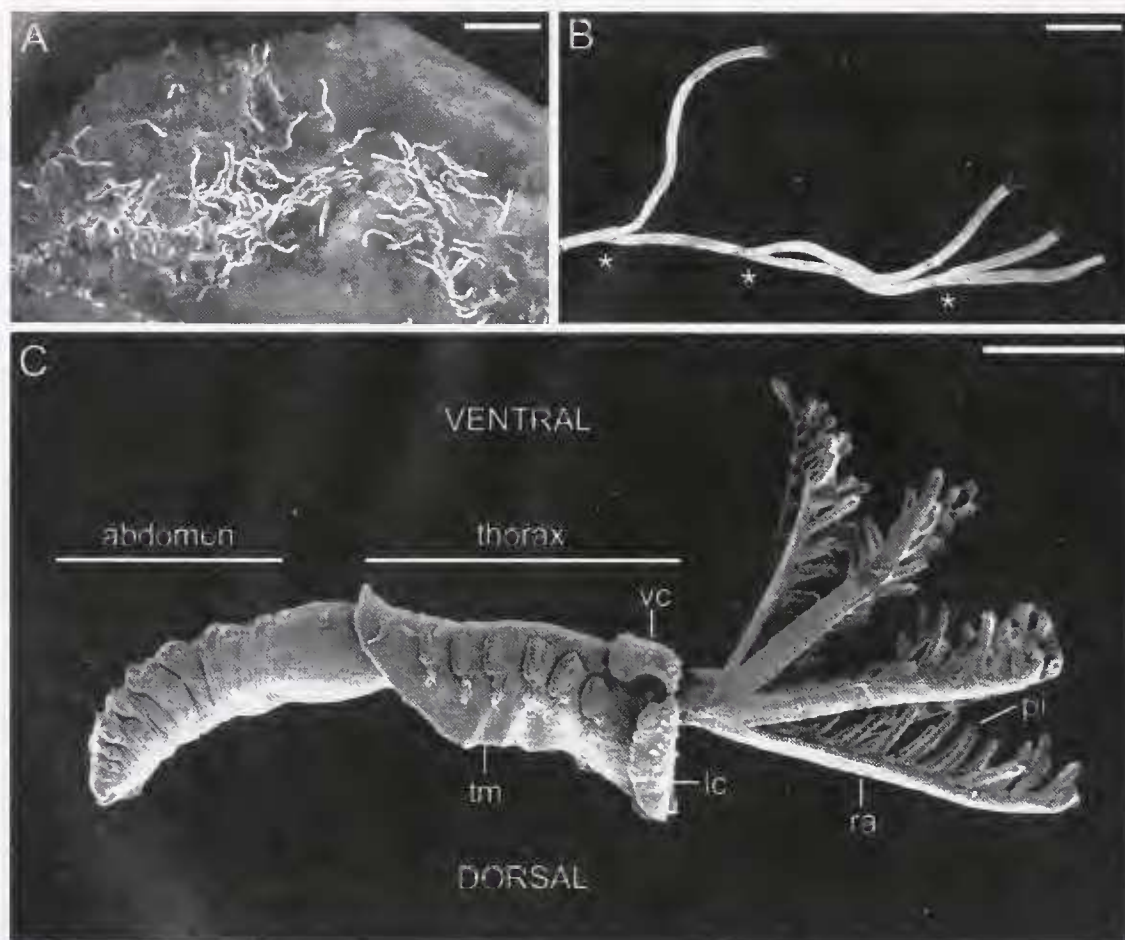
generally attached to the substratum along most of their lengths. Individual tubes can be up to several centimeters long, but older regions are usually eroded away or covered with other encrusting organisms, including sponges, hydrozoans, and bryozoans. Though the overall form of aggregations is often obscured by these other organisms, with careful examination it is possible to see that the tubes of *S. amphidentata* branch periodically. Branching is more easily seen in aggregations of worms raised in the laboratory (Fig. 1B).

Tubes are built and inhabited by worms 1.5–4 mm in length and 200–350 μm in diameter. Their bodies are divided into three regions—an anterior thorax, a median achaetigerous region, and a posterior abdomen (Fig. 1C). The anterior end of the thorax bears eight main feeding tentacles, or radioles, each of which bears side branches (pinnules) arranged in alternating rows. The tips of the radioles are not at all or sometimes very slightly inflated, but never bear structures resembling the opercula of *Filograna* spp. Behind the radioles the thorax is encircled by a collar of tissue. The collar is divided by deep incisions into one ventral and two lateral lobes. The lateral lobes of the collar are continuous with two dorsal flaps of tissue, the thoracic membranes, that extend the entire length of the thorax. The thorax is composed of seven or eight (rarely six) chaetigerous segments, of which the first is associated with the collar and bears elongate chaetae (capillary or fin-and-blade) dorsally. The remaining thoracic segments bear elongate chaetae of several kinds dorsally, and uncini (short, hooked chaetae) ventrally.

Posterior to the thorax is a short achaetigerous body region, and behind this is the abdomen. In the abdomen the positions of the chaetae are reversed relative to those of the thorax—here, the uncini are dorsal and elongate chaetae are ventral. This pattern of chaetal inversion is found in all serpulids, as well as in the related sabellariids and sabellids (Fitzhugh, 1989). The abdomen is variable in length, and is composed of from 5 to 25 chaetigerous segments. Finally, the terminal end of the abdomen is capped by the pygidium, which bears the anus.

Asexual reproduction and tube growth

Worms raised in the laboratory grew rapidly, adding to their tubes at rates of up to several millimeters a day. The process of normal tube growth in *Salmacina amphidentata* was similar to that described in other serpulids (*e.g.*, Hedley, 1956, 1958; Neff, 1971). Worms added to the anterior margins of their tubes while they were actively feeding, with the radioles extended out of the tube opening and the three lobes of the collar folded back over the tube margin. The ventral collar lobe bore two lateral pores on the surface that was in contact with the tube margin (not shown); I assumed that these were the openings of the calcium-secret-
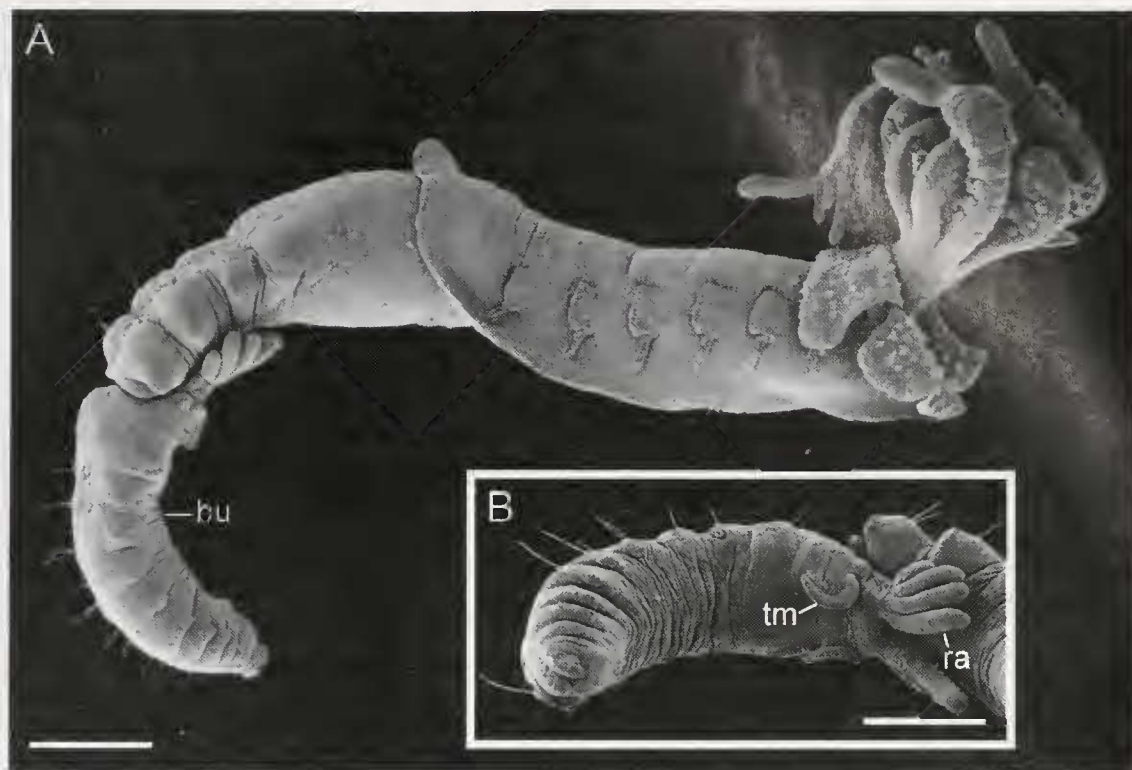
Figure 1. Morphology of aggregations and individuals of *Salmacina amphidentata*. (A) Aggregation of tubes growing attached to the shell of a dead bivalve. Scale bar = 6 mm. (B) Detail of an aggregation cultured in the laboratory on a glass microscope slide, showing periodic branching of the tubes (asterisks). The feeding tentacles (radioles) of three individuals are extended from their tube openings. Scale bar = 2.5 mm. (C) An adult *S. amphidentata* in lateral view. Between the anterior thorax and the posterior abdomen is a short achaetigerous region. The elongate chaetae of the first thoracic chaetiger (the "collar chaetae") are obscured by the lateral lobe of the collar. Scale bar = 250 μm. lc = lateral lobe of the collar, pi = pinnules, ra = radioles, tm = thoracic membrane, vc = ventral lobe of the collar.

ing glands, as in other serpulids (Hedley, 1956). Time-lapse video sequences revealed that calcareous material was slowly added to the tube margin underneath the collar flaps, resulting in an increase in tube length. Growth was not continuous, but resulted from bouts of feeding and tube deposition that lasted for a few minutes and were interrupted by retraction of the worms into their tubes for variable lengths of time.

In addition to adding to their tubes, worms raised in the laboratory reproduced asexually, with most adults releasing from one to four buds per month. (They did not reproduce sexually during this 6-month study, though a few aggregations collected from the field during this period contained sexually mature adults and larvae brooded in the parental tubes.) The events of asexual reproduction in *Salmacina amphidentata* were very similar to those described in other species of *Filograna* and *Salmacina* (*e.g.,* Malaquin, 1895; Faulkner, 1930; Cresp, 1964). Briefly, the first overt sign of asexual reproduction in an adult worm was the appearance of a dense accumulation of bright orange granules in the coelomic cavities of a variable number (5–15) of its posteriormost abdominal segments. Soon after this accumulation of granules appeared, two dorsolateral groups of rudimentary radioles, four on each side, developed on the anteriormost abdominal segment that contained orange granules; these radioles marked the anterior end of the new bud (Fig. 2A, B). At this stage, radioles were simple unciliated cylinders and bore no pinnules. The rudiments of the thoracic membranes appeared at this time as well, as two small dorsolateral flaps of tissue posterior to the radioles (Fig. 2B). The demarcation between the body regions of the bud became evident soon after with the shedding of the origi-

Figure 2. Asexual reproduction by posterior budding in *Salmacina amphidentata*. (A) Lateral view of an individual with developing bud fixed on the day that it built an escape hatch in the tube. Scale bar = 200 μm. (B) Dorsal view of the developing bud. The bud's thoracic chaetae have not yet appeared. Note the rudimentary radioles and thoracic membranes. Scale bar = 250 μm. bu = bud, ra = radioles, tm = thoracic membrane.
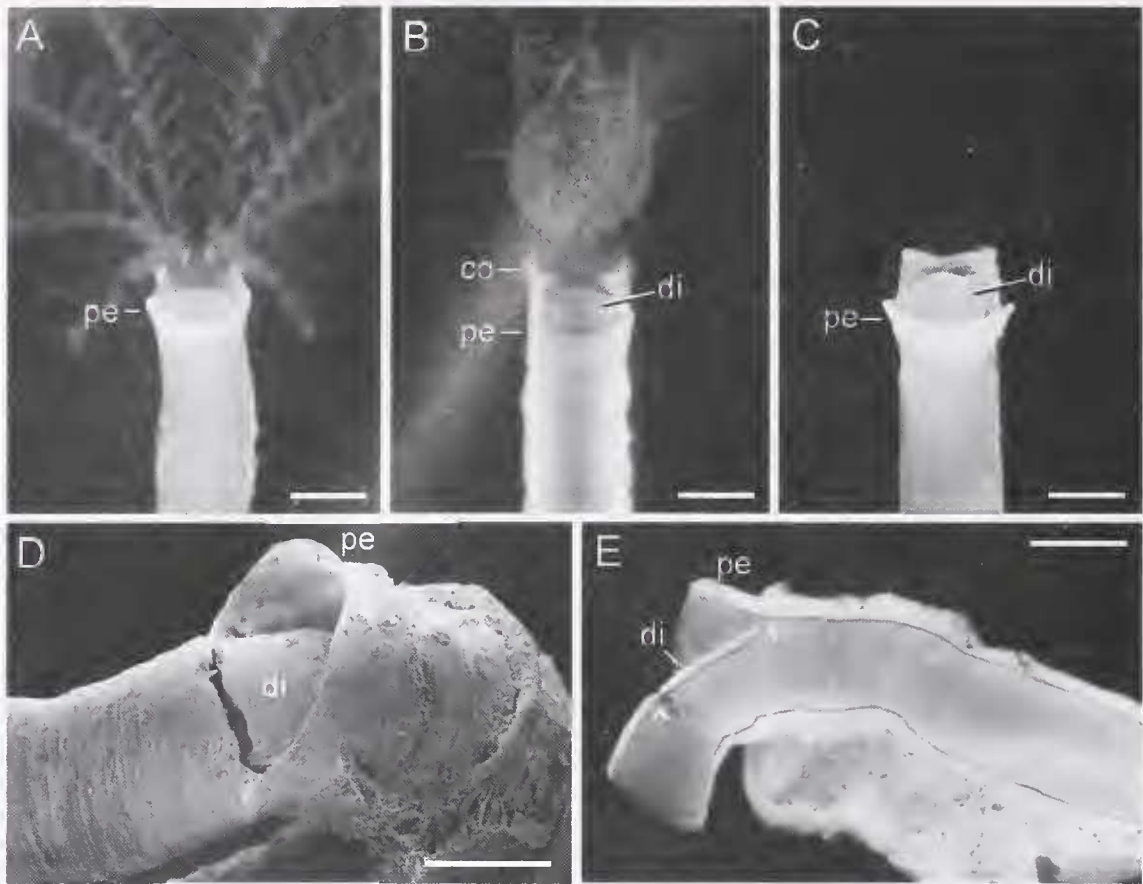
nally adult abdominal chaetae in the first segment of the bud, and the sequential development (anterior to posterior) of three thoracic chaetigers from this segment. The first thoracic chaetiger bore only elongate chaetae dorsally. The next two thoracic chaetigers, which developed soon after the first, bore both dorsal elongate chaetae and ventral uncini. Posterior to these new thoracic chaetigers, the segments retained the abdominal configuration of chaetae present when the segments belonged to the adult (*i.e.*, uncini were dorsal). During this time, the gut of the bud remained continuous with that of the adult, and algal cells ingested by the adult passed through the developing bud. Though I did not follow the timing of early events of asexual reproduction in detail, the period from the initial appearance of orange granules in the posterior of an adult to the stage illustrated in Figure 2 took no more than 4 days.

At this stage in development—when the bud had formed eight radioles, the rudiments of the thoracic membrane, and from zero to three thoracic chaetigers—the parent worm altered its pattern of tube growth in a striking way. Instead of continuing to form a normal cylindrical tube, it built a small trumpet-shaped flare, or peristome, in the tube opening. Immediately distal to the peristome, the adult continued to form a cylindrical tube, but built into its uppermost

surface a circular or ovoid hole of about the adult worm's diameter. The hole was almost completely blocked with a calcareous disc slightly smaller than the hole in diameter (Fig. 3A–D). The disc was supported in the hole by a very thin layer of organic material (~1–3 μm: Fig. 3E) that lined the entire inner surface of the tube.

Time-lapse video sequences of the construction of these escape hatches showed that they were formed directly as the adult was laying down new tube material, not indirectly by the selective removal of material (by chemical dissolution or mechanical abrasion) from a previously built section of complete tube. It appeared that adults were building cylindrical tubes in the normal fashion, but not depositing calcareous material in the narrow gap between the disc and the rest of the tube. In many cases the edges of both the hole and disc were somewhat jagged (Fig. 3B, D). These jagged edges were formed because bouts of tube deposition, as noted above, were frequently interrupted by retraction into the tube for short periods. When tube deposition was resumed, worms frequently failed to lay down new material that was continuous with the last-deposited material, creating jagged edges.

Once the escape hatch was complete (12–18 h after it was started), the adult continued producing a normal

Figure 3. Formation of escape hatches in the tubes of *Salmacina amphidentata.* (A) This worm has finished building a peristome in the tube opening and is beginning to form an escape hatch. Scale bar = 250 μm. (B) Another worm at a later stage in formation of an escape hatch. Scale bar = 250 μm. (C) The tube opening of a worm that has just completed an escape hatch. Scale bar = 250 μm. (D) A recently finished escape hatch. Direction of growth to the left. Scale bar = 200 μm. (E) A recently finished escape hatch in section. Richardson's stain has marked a thin organic membrane that lines the entire tube and supports the disc in the hole (arrowheads). Direction of growth to the left. Scale bar = 250 μm. co = collar, di = calcareous disc, pe = peristome.
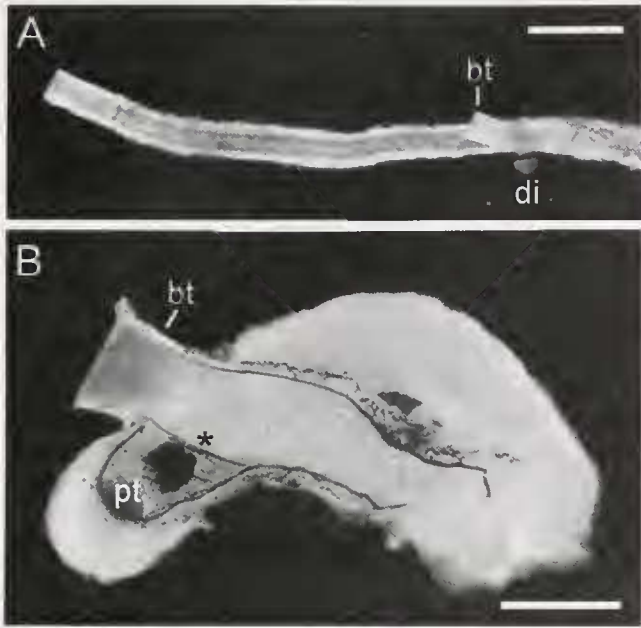
cylindrical tube (Fig. 3C–E). During this time, the bud continued to develop. It added additional thoracic segments by transformation of the anteriormost abdominal segments, and added abdominal segments by typical annelid teloblastic growth (Malaquin, 1895; Faulkner, 1930; Cresp, 1964). The radioles elongated, developed pinnules, and became ciliated. In 18 budding events whose timing I followed carefully, the bud detached from the parent worm from 2 to 10 days (mean = 3.3) after formation of the escape hatch. The detached bud could move forward and backward in the parent's tube, but it was unable to approach the main tube opening, which was blocked by the parent's body. By this time, however, the parent had typically added 3–8 mm of new tube beyond the escape hatch, so this structure was accessible to the bud. From 1 to 7 days (mean = 2.5) after the bud had detached from the parent, the calcareous disc was pushed out of the escape hatch. I did not observe this

process as it happened, so I do not know which worm (parent or bud) actually dislodged the disc. Soon after the disc was dislodged, the bud began to feed from the resulting opening. The disc often remained precariously attached to the hole for several weeks, presumably by the organic membrane that had originally held it in place (Fig. 4A).

On two occasions, during routine handling, I accidentally partially crushed the escape hatches prior to escape of the buds. In both cases, the buds were unable to exit through the damaged structures. The buds gradually got smaller over the course of 2 weeks trapped in the parental tubes, and they eventually died.

Once a bud had gained access to the exterior *via* the escape hatch, it constructed its own tube using the parental tube as a starting point (Fig. 4A, B). As the new tube emerged from the hole, it was initially directed upward at an angle of about 45 degrees from the parent's tube, continuous

Figure 4. Growth beyond the escape hatch in tubes of *Salmacina amphidentata.* (A) A recently opened hatch, with the calcareous disc still precariously attached to the parent's tube. The bud that opened the hatch has started to build its own tube from the opening. Scale bar = 750 μm. (B) A recently opened escape hatch in section. The parent's tube is growing towards the lower left. Note that the bud has completely blocked off the parent's tube with its own tube material (asterisk) and that the bud's tube is continuous with the peristome secreted by the parent prior to the formation of the escape hatch. The black material in the parent's tube is organic debris. Scale bar = 250 μm. bt = new tube formed by bud, di = calcareous disc, pt = parental tube.

with the peristome formed by the parent immediately prior to constructing the escape hatch (Fig. 4B), but it usually curved back down to the substratum rapidly. A new bud would often build a peristome and escape hatch in its new tube just a few hundred micrometers beyond its point of emergence from the parent's tube. This new escape hatch was eventually used by the bud's own asexually produced offspring 1 to 2 weeks later.

This growth pattern was seen several hundred times in laboratory-raised aggregations. In field-collected aggregations, events of asexual reproduction and tube growth were more difficult to follow, because the substrate was opaque, the aggregations were composed of dense masses of tubes, and tubes were partly covered with other encrusting organisms. Nevertheless, all stages of skeletal growth described above were seen regularly in aggregations collected from the field. Peristomes in tubes were almost always immediately followed by either escape hatches or tubes branching off. The junction between a parental tube and the tube of a bud was always located immediately distal to a peristome in the parental tube. On a few occasions, recently dislodged calcareous discs were seen at the junction between bud and parental tubes.

Tube growth in Filograna *and* Salmacina *spp. from other regions*

I examined aggregations of *Filograna* and *Salmacina* spp. from the Atlantic, Indian, and Pacific oceans in the collections of the U.S. National Museum of Natural History, and in my own collections (Table 1). In all of the aggregations I examined that had clearly visible branching tubes, branching patterns were as described above for *Salmacina amphidentata.* Side tubes always branched from parental tubes immediately distal to peristomes in the parental tube. Further, in three lots of preserved material from the Pacific ocean, I observed escape hatches identical in form to those described above.

Discussion

These observations bear on two questions about asexual reproduction in *Filograna* and *Salmacina* spp.: how do clonal offspring gain access to the exterior of the parental tube, and how do they begin to form their own tubes? The answers to these questions center on the escape hatches built into tubes by parent worms. Buds or parents open these escape hatches, which allows buds to reach the outside of the parental tubes. Buds also use the resulting openings as starting points for their own new tubes. This has been most carefully documented in *Salmacina amphidentata* cultured in the laboratory, but clear evidence of escape hatch formation can also be seen regularly in tubes of that species collected from the field, and in preserved material of other species of *Filograna* and *Salmacina* from geographically widespread localities (Table 1). Alternative hypotheses on how buds escape parental tubes (*e.g.,* briefly sharing the main opening of the tube with the parent, or boring a hole through the parent's tube: Benham, 1927; Hanson, 1948) are not supported by the results of this study, nor by any other data that I am aware of.

It is remarkable that escape hatches in the tubes of *Filograna* and *Salmacina* spp. have not been described before, since asexual reproduction in these two genera has been the focus of detailed studies for well over a century (*e.g.,* Huxley, 1855; Malaquin, 1895; Faulkner, 1930; Vannini and Ranzoli, 1954; Cresp, 1964; Nishi and Nishihira, 1994). In part, this may be because most of these workers focused their attention specifically on the morphology of developing buds, and not on the consequences of posterior budding for clonal offspring. Still, the questions of how buds might escape parental tubes and subsequently form their own tubes have been asked regularly over the past 75 years (Benham, 1927; Hanson, 1948; ten Hove, 1979; Nishi, 1992; ten Hove and van den Hurk, 1993).

The observations reported here also show clearly how the clonal offspring of *Salmacina amphidentata,* and at least some other *Filograna* and *Salmacina* spp., are retained near parent worms and contribute to the growth of aggregations

Evidence of the formation of escape hatches for clonal offspring in tubes of Filograna *and* Salmacina *spp.*

Species*	Collection locality	Evidence of escape hatch formation	Year collected
Filograna implexa (NMNH #4462)	Atlantic Ocean: Martha's Vineyard, Massachusetts, USA, 55 m	Tubes branch distal to peristomes	1893
Filograna sp. (NMNH #79634)	Atlantic Ocean: Tangier Harbor, Morocco, 5 m	Tubes branch distal to peristomes	1982
Filograna sp. (NMNH #79633)	Indian Ocean: Mauritius, 18 m	Tubes branch distal to peristomes	1974
Filograna sp. (NMNH #81507)	Pacific Ocean: South Pacific (43°48 S, 147°34 E), 146 m	Escape hatches present; tubes branch distal to peristomes	1968
Filograna sp. (NMNH #98500)	Pacific Ocean: Galapagos Islands, 0–15 m	Tubes branch distal to peristomes	1966
Salmacina amphidentata (author's collection)	Atlantic Ocean: Fort Pierce, Florida, USA, 0–1 m	Escape hatches present; tubes branch distal to peristomes	2000
Salmacina dysteri (NMNH #67631)	Atlantic Ocean: Charleston, South Carolina, USA, 32 m	Tubes branch distal to peristomes	1978
Salmacina dysteri (author's collection)	Pacific Ocean: Pearl Harbor, Hawai'i, USA, 0–1 m	Escape hatches present; tubes branch distal to peristomes	2000
Salmacina incrustans (NMNH #50436)	Atlantic Ocean: La Parguera, Puerto Rico, 1 m	Tubes branch distal to peristomes	1963
Salmacina tribranchiata (author's collection)	Pacific Ocean: Santa Cruz, California, USA, 0–1 m	Escape hatches present; tubes branch distal to peristomes	2000

* Names listed are those used in the collections of the U.S. National Museum of Natural History (NMNH), or, for specimens in the author's collection, those used in regional guides (California: Blake, 1975; Hawai'i: Bailey-Brock and Hartman, 1987). Both generic and specific identifications should be viewed with skepticism. See Materials and Methods for additional comments on systematics.

of branching tubes. It should be noted that aggregations of *Filograna* and *Salmacina* spp. are not necessarily composed only of the genetically identical offspring of a single founder individual. Recruitment of planktonic larvae may contribute substantially to their growth (Nishi *et al.*, 1996; Nishi and Nishihira, 1997). The branching tubes characteristic of aggregations of *Filograna* and *Salmacina* spp., however, are undoubtedly the consequence of repeated bouts of asexual reproduction. Many species of serpulids that do not reproduce asexually also form aggregations (ten Hove and van den Hurk, 1993), but these do not contain branching tubes.

Mechanisms and timing of escape hatch formation in Salmacina amphidentata

Though the escape hatches formed by *Salmacina amphidentata* are among the most complex tube structures known from the serpulids, the actual mechanism of their formation does not seem to have required great modifications of the routine processes of tube growth. The ability to carefully control where calcareous tube material is deposited around the margin of the tube, which permits *S. amphidentata* to build escape hatches, is widespread in serpulids. Many species, for example, deposit more material at some locations around the tube margin than at others, resulting in the formation of sculptural elements like keels or spines (*e.g.*,

Pomatoceros triqueter: Hedley, 1958; *Filogranula gracilis* (as *Omphalopoma*): Zibrowius, 1968; *Spiraserpula* spp.: Pillai and ten Hove, 1994). A few species form even more complex structures such as brood chambers for developing embryos (Ben-Eliahu and ten Hove, 1989; Nishi, 1993). Adults of *S. amphidentata* avoid depositing any calcareous tube material at all in the narrow gaps between the calcareous disc and the rest of the tube, while depositing it elsewhere along the tube margin. Such precise control over the location of deposition may be mediated by two processes. First, the slurry of calcareous crystals in a fluid organic matrix may be deposited directly at the correct sites on the tube margin by the pair of calcium-secreting glands on the ventral lobe of the collar, with the adult rotating in the tube to position the gland openings correctly. However, in each of the two episodes of escape hatch formation I observed in detail by time-lapse video, the adult worm rotated in its tube infrequently. Further, for most of the time spent building hatches, the openings of calcium-secreting glands were approximately adjacent to the gaps between the calcareous disc and the rest of the tube, exactly where no calcareous tube material was deposited. A more plausible possibility is that muscular movements of the collar mold the slurry of tube material into the correct positions on the tube margin. I did not observe such movements, but would expect them to be subtle and difficult to see at the low

magnifications used in this study. The slurry of tube material is not molded onto the tube margin by the action of cilia on the collar, as its relevant surfaces lack cilia (not shown).

In *Salmacina amphidentata*, the calcareous discs of the escape hatches were supported in place by a thin organic membrane (Fig. 3E). This organic layer lined the inner surface of the entire tube. Though it is rarely mentioned in accounts of tube structure or growth, a similar "coating membrane" has been found lining the tubes of at least five other species of serpulids (Muzii, 1968; Pernet, pers. obs.), and is probably an integral component of the tube in all species of serpulids. This is presumably the material that the chaetae of serpulids interact with during routine movement up and down the tube, and also during anchoring when a predator attempts to pull the worm from the tube (*e.g.*, Merz and Woodin, 2000). Two observations suggest that the coating membrane is deposited prior to or at the same time as the calcareous tube material. First, during the process of escape hatch formation, *Filograna* and *Salmacina* spp. construct a calcareous disc that is discontinuous with the rest of the calcareous tube. As noted earlier, however, it appears that the growth of calcareous tubes in all serpulids requires a pre-existing tube margin onto which new calcareous material can be added. During the deposition of calcareous discs, it seems likely that such a pre-existing tube margin is present in the form of a coating membrane. Second, the calcareous disc is supported in place by the coating membrane; it seems likely that this is true throughout its construction. That the coating membrane has been found in all serpulids that have been carefully examined (Muzii, 1968; Pernet, pers. obs.) and that it appears to be deposited prior to or simultaneously with the calcareous portion of the tube suggest that it plays an important and rarely considered role in tube formation. It may also be important in considerations of the evolution of the calcareous tube, a defining feature of the serpulids (Rouse, 2000).

The close temporal coordination of events in asexual reproduction and tube growth in *Salmacina amphidentata* suggests the possibility of signaling pathways between buds and parents. Only after a developing bud had reached a certain stage (with unbranched radioles, 0–3 thoracic segments, and rudimentary thoracic membranes) did the parent worm begin to form an escape hatch. Adults never built escape hatches unless they were in the midst of a budding cycle. What stimulates the parent worm to alter its pattern of tube deposition at this particular stage in bud development? It is possible that bud and escape hatch formation are part of a single developmental pathway in adult worms, such that once asexual reproduction is initiated, escape hatch formation several days later is inevitable. Alternatively, once a bud reaches a certain stage, it may somehow signal to the adult that it is an appropriate time to form an escape hatch. For example, buds at the stage shown in Figure 2 may already be producing their own hormonal products, perhaps

involved in controlling such processes as the transformation of former adult abdominal segments into thoracic segments for the bud. Adult worms, which are still attached to the buds at this stage, might use such signals as a cue to alter their patterns of tube deposition. Endocrine substances produced in the prostomium (or in other parts of the anterior end) are well known as signals controlling the timing of asexual reproduction and changes in chaetal morphology in other polychaetes (*e.g.*, Schroeder, 1967; Franke and Pfannenstiel, 1984). A test of this hypothesis might involve implanting the prostomium of a developing bud into the body of a non-budding adult, and examining the effects on tube growth.

Once buds have detached from the parent, they are free to move forward and backward in the parental tube. Within a few days, however, they must gain access to the outside *via* the escape hatch. I did not determine which worm actually dislodged the calcareous disc from the escape hatch, though it seems likely that the bud does this rather than the parent, which has usually grown well beyond the escape hatch by this time. Buds may identify the site of the escape hatch by simply moving around and testing the tube walls until they find a weak spot. Dislodging the calcareous disc presumably does not require much force, as it is held in place only by a very thin coating membrane.

I did not measure rates of asexual reproduction, or how these might be related to rates of tube growth. In laboratory cultures, both the frequency of asexual reproduction and the rate of tube growth appeared to vary within and among clones, resulting in great variation in the distance between side branches from parental tubes. Many factors, both environmental (food levels, temperature, water flow) and genetic, might affect these parameters, as has been described in other clonal organisms (*e.g.*, Sebens, 1980; Keen and Gong, 1989). Understanding the determinants of rates of asexual reproduction and tube growth in *Filograna* and *Salmacina* spp. is of particular interest since these parameters should strongly affect the eventual form of clonal aggregations. In nature, aggregations are quite variable in form.

Distribution and evolution of escape hatches in asexually reproducing serpulids

Some *Filograna* and *Salmacina* spp. from the Atlantic, Indian, and Pacific oceans form escape hatches for their clonal offspring (Table 1). In three lots of relatively recently preserved material, escape hatches identical in form to those described above in *Salmacina amphidentata* were present. In the remaining six lots of material I examined, which had been in preservative for 18–107 years, branching patterns of tubes were identical to those described above, but no intact escape hatches were found. The absence of intact escape hatches in this older material is not too surprising,

even if escape hatches were present at the time of collection. The tubes of these species are delicate and are often damaged during collection and handling. It is also likely that they are gradually dissolved during storage, unless preservatives are well buffered. Escape hatches in particular are prone to damage or dislodgement, as the organic membrane that supports calcareous discs in the tube is extremely thin (Fig. 3E). These comparative data suggest that the formation of escape hatches for clonal offspring is widespread in members of *Filograna* and *Salmacina*.

Asexual reproduction is also known from several other genera of serpulids (*Filogranella, Filogranula, Josephella, Rhodopsis,* and *Spiraserpula*). Members of these genera that reproduce asexually do so by posterior budding, as in *Filograna* and *Salmacina* spp. (ten Hove, 1979; Ben-Eliahu and ten Hove, 1989; Nishi, 1992; Pillai and ten Hove, 1994). Their clonal offspring should also face the dual problems of escaping from the parental tubes and starting to build their own tubes. How they solve these problems is not known. Aggregations of branching tubes similar to those of *Filograna* and *Salmacina* spp., suggesting some mechanism of reaching the exterior of the parental tube but not dispersing from it, are reported in members of three of these genera (*Filogranella, Josephella,* and *Spiraserpula:* ten Hove, pers. comm.; George, 1974; Pillai and ten Hove, 1994).

A few members of a closely related family of tube-dwelling polychaetes, the Sabellidae, also reproduce asexually by posterior budding (Knight-Jones and Bowden, 1984). Sabellid tubes are typically constructed of secreted organic compounds and sediment particles, and are not calcified. How the clonal offspring of sabellids escape the parental tubes is not known. Adults of several species are apparently capable of boring holes in their own tubes (Fitzsimmons, 1965), and it is possible that buds are able to do this as well. Once buds have gained access to the exterior of parental tubes, they may be free to disperse far from their parents before building new tubes, as many sabellids are able to build tubes *de novo* throughout their lives (Fitzsimmons, 1965; Lewis, 1968).

In serpulids, asexual reproduction by posterior budding is only profitable if buds have some way of gaining access to the exterior of the parent's tube (such as escape hatches), but it is difficult to imagine how escape hatches might have evolved in the absence of asexual reproduction. One scenario for the evolution of both of these traits (which seem to be tightly correlated in extant species of *Filograna* and *Salmacina,* at least) involves the initial evolution of posterior budding. Tubes of serpulids—especially the very thin tubes characteristic of species that have small maximum body sizes (Nishi, 1993)—are easily damaged by chance biological or physical insults, and buds may have originally used such fortuitous openings to gain access to the exterior of parental tubes. Adult worms that secreted generally weaker skeletons, or skeletons with weak points, would

have had more consistent success in fledging clonal offspring, though this might have been balanced by greater vulnerability to predators. Selection might then eventually lead to adults that built extremely weak points—escape hatches—into their tubes to ensure that buds would find exits. This is obviously speculative. However, this scenario might be tested with comparative data on living clonal serpulids examined in the context of a well-supported phylogeny. Neither sort of data is yet available. Such an analysis might reveal persistent and informative variation in the mechanisms buds use to gain access to the exterior of parental tubes.

Acknowledgments

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