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Persistent Ancestral Feeding Structures in Nonfeeding Annelid Larvae

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Abstract. Evolutionary loss of the requirement for feeding in larvae of marine invertebrates is often followed by loss of structures involved in capturing and digesting food. Studies of echinoderms suggest that larval form evolves rapidly in response to loss of the requirement for feeding, but a lack of data from other taxa makes it difficult to assess the generality of this result. I show that many members of a large clade of annelids, the Sabellidae, retain ancestral systems for particle capture despite loss of the need and ability to feed. In at least one species, *Schizobranchia insignis*, an opposed-band system of prototrochal, food-groove, and metatrochal ciliary bands can concentrate suspended particles and transport them to the mouth, but captured particles are invariably rejected because larvae lack a functional gut. The persistence of particle capture systems in larvae of sabellids suggests that they have lost larval feeding very recently, that opposed bands are inexpensive to construct and operate, or that opposed bands have some alternative function. These observations also suggest a hypothesis on how the ability to feed is lost in larvae of annelids and other spiralians following increases in egg size.

Introduction

Larvae of some marine invertebrates require particulate food to complete development to the juvenile stage, but others cannot feed and instead rely on materials stored in the egg. These alternative nutritional modes are associated with differences in many other traits, including embryonic development (Wray and Bely, 1994), dispersal and population genetic structure (Palumbi, 1995), species duration (Jablonski, 1986), and perhaps most obviously, larval form. Feeding larvae bear structures that function in particle capture, ingestion, and assimilation, while nonfeeding larvae tend to lack such structures and are relatively simple in external form (Emlet, 1991). Many species with nonfeeding larval development evolved independently from ancestors that had feeding larvae (Strathmann, 1978). This suggests a strong evolutionary association between the loss of the requirement for feeding and the loss of larval feeding structures (Wray, 1996).

How does this association arise? One model suggests that the first step in the evolution of nonfeeding development is an increase in the energy content of the egg (Jagersten, 1972; Strathmann, 1975; Raff, 1987; Kempf and Todd, 1989; Hart, 1996; Wray, 1996; McEdward and Janies, 1997). Increased egg energy content permits larvae to complete development without particulate food. These derived larvae may feed facultatively. However, once larvae have lost the requirement for food, stabilizing selection on feeding performance is weakened, and mutations that affect the form or function of feeding structures may accumulate (Strathmann, 1975). Selection on other larval functions like swimming or developing rapidly and efficiently may also lead to changes in larval form (Wray and Raff, 1991; Emlet, 1994). Under these conditions, feeding structures eventually become nonfunctional, and the resulting larvae are obligately nonfeeding. Loss of the ability to feed may occur by any of many different changes in morphology, physiology, or behavior, but details of this process are mostly unknown (Kempf and Todd, 1989; Wray, 1996). Further reduction of ancestral feeding structures, which may occur rapidly after loss of the requirement for particulate food (Wray and Raff, 1991; Hart, 1996; Wray, 1996), then leads to simplification of external form in nonfeeding larvae (Emlet, 1991; Byrne *et al.*, 2001). This scenario is a specific example of a more general model of the reduction and loss of nonfunctional characters (Fong *et al.*, 1995).

Though larval feeding has been lost in many lineages of marine invertebrates (Strathmann, 1978), subsequent patterns of change in larval form have been studied primarily in members of only one phylum, the Echinodermata.

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Examples from other phyla would be useful in assessing the generality of these results. Here I provide such an example from a phylum only distantly related to the echinoderms, the Annelida. Sabellid annelids are sessile, tube-dwelling worms often known as "feather-duster worms" because of the crown of tentacles they extend from their organic, unmineralized tubes for suspension feeding. The family includes about 490 species that fall into two clades, the subfamilies Fabriciinae and Sabellinae (Rouse and Pleijel, 2001). All fabriciins whose reproduction has been studied (about 15 of 75 species) brood embryos that undergo direct development. Sabellins are more variable in reproductive biology, with some species brooding embryos and larvae through the juvenile stage; others brooding embryos and larvae for most of their development, releasing larvae for a brief planktonic phase before settlement; and still others releasing gametes directly into the sea where they are fertilized and develop into planktonic larvae. Though reproduction and development has been studied in few sabellins (about 30 of 415 species), these are broadly distributed in phylogenies of the family (Rouse and Fitzhugh, 1994). No sabellid larvae are known to feed (Rouse and Fitzhugh, 1994).

A look at the relationships of annelid worms suggests that nonfeeding development in sabellids represents a loss of larval feeding (Fig. 1). Sister clade to the Sabellidae is the Serpulidae, which includes both species with feeding larvae and species with nonfeeding larval development. Sister to the clade [Serpulidae, Sabellidae] is the Sabellariidae. All sabellariids whose development has been described have feeding larvae. These character states are shown in Figure 1, with inferences on larval nutritional mode in ancestral forms. As no extant sabellid is known to have feeding larvae, nonfeeding development is likely plesiomorphic in the family (Rouse and Fitzhugh, 1994). The inference that the common ancestor of serpulids and sabellids had a feeding larval stage relies primarily on the observation that feeding larvae of sabellariids and serpulids capture particles using similar systems of three parallel ciliary bands, the prototroch, food groove, and metatroch (Fig. 2: Strathmann *et al.*, 1972; Strathmann, 1987; Strathmann and Pernet, unpubl. data). The cilia of the prototroch, which form a transverse band anterior to the mouth, beat from anterior to posterior. These cilia create a swimming current as well as being involved in feeding. Suspended particles passing near them are overtaken and moved towards the surface of the larval body. Cilia of the metatroch, located posterior to the mouth, beat from posterior to anterior. Particles caught between these two ciliary bands are transported towards the mouth by cilia of the food groove. This is usually referred to as "opposed band" feeding. (Note that Rouse [1999, 2000a] argues that sabellariid larvae do not feed with opposed bands of cilia. However, larvae of the only sabellariids in which larval feeding has been studied, *Sabellaria alveolata* and *S. cementarium*, do feed in this way [Strathmann, 1987; Strathmann and Pernet, unpubl. data].) The presence of similar feeding structures in sabellariids and serpulids suggests that ancestors of the clades [Sabellariidae [Serpulidae, Sabellidae]] and [Serpulidae, Sabellidae] had larvae that fed with opposed bands of cilia. Nonfeeding development in the sabellids thus represents a loss of larval feeding. Rouse (2000a) also concluded that sabellids were derived from ancestors that had feeding larvae.

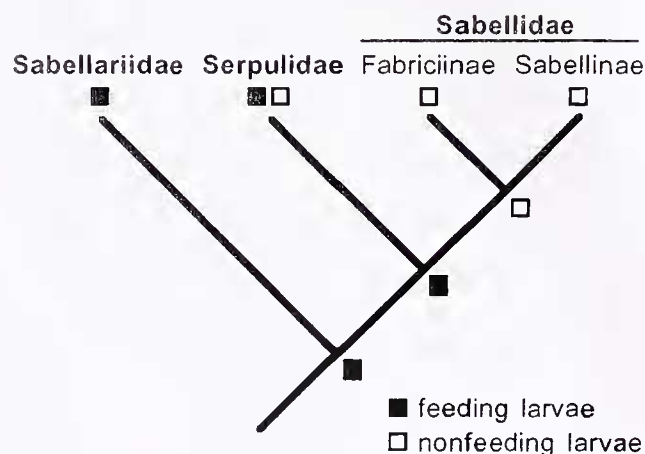

Figure 1. Relationships of sabellariid, serpulid, and sabellid annelids, as indicated by cladistic analyses of morphological and reproductive characters by Fitzhugh (1989) and Rouse and Fitzhugh (1994). This topology is also supported by analyses of DNA sequence data from the nuclear gene elongation factor-1alpha (D. McHugh, Colgate University, pers. comm.). Inferences on ancestral character states are discussed in the text.

Here I show that despite loss of the requirement and ability to feed as larvae, members of at least eight genera of sabellids retain opposed bands of cilia. In larvae of at least one species, *Schizobranchia insignis*, these structures can concentrate particles from suspension and transport them to the mouth, where they are invariably rejected because the larvae lack functional digestive systems. The persistence of ancestral systems for particle capture in nonfeeding sabellid larvae is unexpected in light of data from echinoderms, which suggest that once the requirement for larval feeding is lost, feeding structures are rapidly reduced or lost. These observations suggest that sabellids lost larval feeding very recently, that opposed bands are inexpensive to construct and operate, or that opposed bands have alternative functions in these larvae. These observations also suggest a hypothesis on how development mediates the loss of feeding ability in larvae of annelids and related phyla after increases in egg size.

Materials and Methods

Collection, spawning, and larval culture

I studied larvae of the sabellin sabellid *Schizobranchia insignis* Bush, 1905, intensively, and made additional observations on larvae of three other sabellins, *Demonax me-*

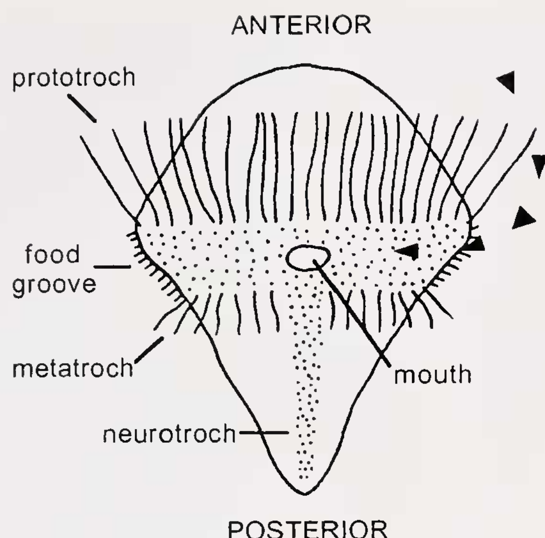

Figure 2. Diagrammatic ventral view of a larva of a serpulid annelid, showing structures used in the opposed-band feeding system and the path of a particle (arrowhead) captured from suspension. Cilia of a preoral band, the prototroch, beat from anterior to posterior. As they beat, particles that pass within their reach may be moved into a perioral band of cilia, the food groove. Metatrochal cilia beat from posterior to anterior and may help trap particles in the food groove. Once in the food groove, particles are transported ventrally to the mouth. Rejected particles are moved posteriorly by cilia of the neurotroch.

dius (Bush, 1905), *Myxicola aesthetica* (Claparède, 1870), and *Pseudopotamilla occelata* (Moore, 1905). All of these species except *D. medius* are broadcast spawners; adults of *D. medius* brood embryos and larvae in a gelatinous mass around the opening of the adult tube, and larvae emerge from masses for a brief (~1 day) planktonic period (McEuen *et al.*, 1983). Adult *S. insignis* and *P. occelata* were collected from floating docks on San Juan Island, Washington, from January to April in 2002 and 2003. The tube of each animal was cleaned of fouling organisms and trimmed to the length of the worm it contained. To induce spawning, 10–20 conspecific individuals were placed in a large container through which fresh seawater flowed. About half of the worms so treated spawned within 48 h, usually at night. I collected fertilized eggs from the bottom of the container the following morning, rinsed them several times in coarsely filtered seawater (FSW, mesh size ~5 μm), and cultured them in 1-liter beakers of FSW at densities of ~5 per milliliter. Beakers were partially submerged in flowing seawater at temperatures of 8–10 °C (near field temperatures) and stirred with paddles (M. Strathmann, 1987). Water in larval cultures was changed every 3–6 days. No food was added, but cultures certainly contained bacteria and unicellular protists. Larvae of *S. insignis* were followed through metamorphosis. These larvae settled on small pieces of adult tube that were scored with a razor blade to provide crevices. Juveniles were fed the unicellular algae *Isochrysis* sp. and *Rhodomonas* sp. at high concentrations.

I collected adult *Myxicola aesthetica* from floating docks in Anacortes, Washington, in July 2002. Adults spawned when removed from their tubes and subjected to gradual warming (to room temperature) and rapid cooling of the seawater they were incubated in. Embryos and larvae were raised as described above.

I obtained two egg masses of *Demonax medius* from the intertidal zone on the west side of San Juan Island in February and April 2002, and incubated them in FSW at 8–10 °C in the laboratory. I examined larvae that had been removed from masses, as well as planktonic larvae that had emerged from masses naturally. Planktonic larvae were raised as described above.

Larval morphology

Developmental stages were examined with a light microscope equipped with differential interference contrast (DIC) optics. Larvae were relaxed in a 1:1 solution of 7.5% $MgCl_2$ and seawater before examination. Body dimensions were estimated with a calibrated ocular micrometer. For scanning electron microscopy, relaxed larvae were killed by gradual addition of dilute formalin. They were then rinsed in Millipore-filtered seawater (MPFSW, mesh size 0.45 μm) and fixed for 2 h in 2% OsO_4 in 1.25% sodium bicarbonate buffer (pH 7.2). Fixed larvae were rinsed in distilled water, then dehydrated in ascending concentrations of ethanol. They were critical-point-dried, mounted on stubs with carbon adhesive disks, and sputter-coated with gold-palladium before being examined and photographed with a JEOL JSM-35 scanning electron microscope. Larvae of *Schizobranchia insignis* were also sectioned for study of internal anatomy. Relaxed larvae were fixed in 2.5% glutaraldehyde in 0.2 *M* Millonig's phosphate buffer for 1–3 h, rinsed in buffer, then post-fixed in 1% OsO_4 in buffer for 1 h. After dehydration in ethanol, larvae were embedded in EMBed-812 (Electron Microscopy Sciences, Inc.) with propylene oxide as an infiltration solvent. Sections ~1 μm thick were cut with glass knives and stained with Richardson's stain for light microscopy (Richardson *et al.*, 1960).

Functional morphology of ciliary bands

Beat patterns of cilia were recorded with a high-speed video camera (Motionscope 1000-S, RedLake Imaging Inc.) mounted on a compound microscope with DIC optics. Larvae were restrained under a coverslip supported with plasticene modeling clay. Images were collected at 250 frames per second and replayed at 10 frames per second. Sequences were routed from the camera through a Sony DVMC-DA2 analog-digital converter to a Macintosh iBook and saved as iMovie files (Apple Computer, Inc.).

Capture and transport of particles by larvae of *Schizobranchia insignis* were visualized by videotaping larvae in a suspension of particles. Larvae were mounted on a slide under a coverslip supported with plasticene modeling clay.

The coverslip was pressed down just enough to prevent larvae from swimming rapidly. High concentrations of polystyrene divinylbenzene beads (3-μm diameter, blue-dyed, Polysciences, Inc.) were added and larvae were observed with a compound microscope. Beads had previously been incubated in a 2.5% solution of bovine serum albumin (BSA) in distilled water for 1–2 h, then rinsed and resuspended in MPFSW. Such beads are readily captured and ingested by free-swimming or restrained feeding larvae of sabellariids and serpulids (Strathmann and Pernet, unpubl. data). Images were collected with a video camera (Sony CCD SSC-S20) and recorded on VHS tape, with a date-time generator providing a time signal. Tapes were played frame by frame for analysis.

Possible functions of opposed ciliary bands in sabellid larvae

I tested two hypotheses on functions of opposed ciliary bands in larvae of sabellids.

Opposed ciliary bands are used for capturing particulate food during the planktonic larval stage. I verified that larvae of *Schizobranchia insignis* and *Demonax medius* are non-feeding by offering them particulate "food" through development and later examining their guts for ingested particles. For each assay, 20 larvae were placed in 20 ml MPFSW in a vial. Two types of particles were added to each vial: blue-dyed polystyrene divinylbenzene beads 3 and 6 μm in diameter, previously incubated in 2.5% BSA as described above, and single-celled algae (*Dunaliella* sp.). Final concentrations of beads were 5–10 per microliter of each diameter; I did not measure algal concentrations. I included several feeding larvae of the echinoid echinoderm *Strongylocentrotus droebachiensis* or the serpulid annelid *Serpula columbiana* in each vial as positive controls. Vials were slowly rotated on a plankton wheel in the dark at 10 °C for 1–2 h. Larvae of sabellariids and serpulids feed at high rates under these conditions (Pernet, unpubl. data). Larvae were preserved at the end of feeding assays by addition of buffered formalin. They were later cleared and mounted in glycerin, and examined with a compound microscope to determine whether they had ingested particles. Through processing, beads retained blue dye, and algae retained pigment; both types of particles were easily visible when present in larval guts. Ten sabellid larvae, as well as several larvae known to feed, were examined in each assay. For *S. insignis*, assays were conducted every 5 days, from the 5th day after fertilization until larvae were 30 days old. For *D. medius*, feeding assays were carried out with swimming larvae that had left egg masses on their own.

Opposed ciliary bands are sites of uptake of dissolved proteins. Moran (1999) found that encapsulated larvae of several marine gastropods use velar cells to take up dissolved proteins from the capsular fluid. In related species with planktonic feeding larvae, these cells bear cilia that function in an opposed-band feeding system. Using the methods of Rivest (1981) and Moran (1999), I tested the hypothesis that larvae of *Schizobranchia insignis* or *Demonax medius* take up proteins with the cells that bear the opposed ciliary bands. Ten larvae were placed in 1 ml of fluoresceinisothiocyanate-conjugated bovine serum albumin (FITC-BSA) dissolved in MPFSW (1 mg/ml). Negative controls were incubated in MPFSW only. Larvae of species known to take up large proteins (veligers of *Littorina sitkana* [Moran, 1999] or *Crepidula adunca* [Rivest, 1981]) were included in all vials as positive controls. Larvae were incubated in test solutions for 6 h at 10 °C in the dark. After incubation, they were rinsed in MPFSW and observed by epifluorescence microscopy. At least five sabellid larvae, as well as several positive controls, were examined from each treatment. For *S. insignis*, assays were conducted every 5 days, from the 5th day after fertilization until larvae were 30 days old. For *D. medius*, assays were carried out with larvae that had hatched from their individual capsules but had not yet left egg masses on their own (these larvae were about 5–15 days old, and were removed from egg masses by agitation).

Results

Larval development and form

Schizobranchia insignis. Except for notes on egg size and developmental mode (Lee, 1970, 1975; McEuen *et al.*, 1983), development of this species has not previously been described. A summary of its development can be found in Table 1.

In the spawning events I observed, males always spawned before females. Fertilized eggs were spherical, opaque, negatively buoyant, gray in reflected light, and surrounded by an elevated envelope (Fig. 3A). Mean diameters (± one standard deviation) of 20 eggs from each of three separate females were 154 (3.4), 156 (2.8), and 157 (2.8) μm; the fertilization envelope brought the total diameter to 180–190 μm (Fig. 3A). First and second cleavages were both markedly unequal (without the formation of polar lobes), leading to a four-cell stage with one cell that was much larger than the others (Fig. 3B).

Two days after fertilization, embryos had become pear-shaped trochophore larvae (Fig. 3C). The widest part of the body bore a transverse, preoral band of cilia, the prototroch. The prototroch was composed of three parallel tiers of cilia—an anterior tier of short cilia, a middle tier of long compound cilia, and a posterior tier of short compound cilia. Cilia of all three tiers protruded through the fertilization envelope. They could be identified as preoral because a slight depression had appeared just behind them, midventrally at the site of the developing mouth.

By the 3rd day after fertilization, two additional bands of transverse cilia had appeared (Fig. 3D). Immediately posterior to the prototroch, at the level of the larval mouth, was

Table 1

Schedule of development in Schizobranchia insignis *raised at temperatures of 8–10 °C*

Time	Stage or event
Planktonic larval development	
0	Fertilization
1 d	Prototrochal cilia protrude through egg envelope; weakly swimming
2 d	Pear-shaped trochophores; prototroch well developed; ocelli, head and anal vesicles present
3 d	Metatroch and food-groove cilia present; neurotroch present; competent to settle
4 d	Notochaetae in chaetiger 1
5 d	Notochaetae in chaetiger 2
11 d	Notochaetae in chaetiger 3; neurochaetae (uncini) in chaetigers 2 and 3
12–15 d	Neurochaetae (uncini) in chaetiger 4
Post-settlement development	
0	Settlement; mucus tube formed around body
1 d	Neurotroch between peristomium and pygidium lost in some individuals; collar lobes present on peristomium
2 d	Neurotroch between peristomium and pygidium lost in all individuals; prototroch lost middorsally; radiole buds present on prostomium
3 d	Prostomial snout present; radiole buds each divided into 2 lobes; anus present
4 d	Radiole buds each divided into 3 lobes, ciliated; all long cilia of larval prototroch gone; ingestion of snout begins?
5 d	Radiole buds each divided into 4 lobes
6–7 d	Juvenile feeding begins

Timing data are summarized from observations of cohorts of offspring from six separate females. Though competent to settle 3 days after fertilization, larvae, if not offered a suitable settlement substrate, continue swimming and remain competent to settle until at least 30 days post-fertilization. For the schedule of post-settlement development, larvae were allowed to settle after a 15-d planktonic period. The timing of events in post-settlement development was similar in larvae that had 9-d and 30-d planktonic periods.

a narrow band of short, simple cilia. The width of this perioral band was about 4–5 μm in five 10-day-old larvae. Just behind this perioral band was a postoral band of simple cilia. These ciliary bands are shown in greater detail in an illustration of an older larva (Fig. 3E). Positionally, these bands of cilia are identical to the perioral food groove and postoral metatroch known from annelid larvae that feed with opposed bands of cilia, and I will refer to them as food groove and metatroch in the rest of this paper. Both food groove and metatroch were incomplete dorsally; the dorsal gap in the food groove was substantially wider than that in the metatroch. Three-day-old larvae had also developed a midventral band of short cilia, the neurotroch, which stretched from the metatroch to the posterior end of the body.

Three-day-old larvae were competent to settle and metamorphose. However, if larvae were not offered settlement substrates and were maintained in stirred cultures in clean glass beakers, most remained planktonic until at least 30 days after fertilization. Changes in form in larvae from 3–30 days of age—mostly involving the appearance of chaetae in developing segments (*e.g.*, Fig. 3F)—are summarized in Table 1. Between the 25th and 30th day of development, many larvae began to show signs of metamorphosis while still in the plankton. In particular, they developed a pair of dorsolateral bulges on the prostomium. In normally metamorphosing worms, these bulges go on to form the adult feeding tentacles, or radioles. Mortality of larvae appeared to increase greatly at aronnd 30 days.

Sections of larvae fixed at various ages allowed description of changes in gut morphology during development. Transverse sections of 8-day-old larvae showed that the mouth was represented only by a shallow, ciliated midventral depression (Fig. 3G). This depression was not connected to the midgut. The midgut wall was composed of endodermal cells that were so large that they completely occluded its lumen. Eight-day-old larvae had neither an intestine nor an anus. In 20-day-old larvae, the mouth had increased in depth and led to a very narrow ciliated stomodaeum (Fig. 3H). In serial sections of five 20-day-old larvae, I was unable to find a connection between the stomodaeum and midgut. The cells lining the midgut had shrunk slightly, and it now had a narrow central lumen at the level of the stomodaeum. This lumen disappeared posteriorly. I observed no intestine or anus in these larvae.

Larvae of *Schizobranchia insignis* were competent to settle from 3 to at least 30 days after fertilization. When offered pieces of adult tube in still water, 20%–50% of larvae settled within 24 h. Larvae usually settled in crevices in the tubes, or on the glass bowl beneath pieces of tube. The events following settlement of 15-day-old larvae are summarized in Table 1. Note that prototrochal cilia had been resorbed or shed by 4 days after settlement, before juveniles began feeding.

Demonax medius. All larvae of *D. medius* that I examined—larvae extracted from gelatinous brood masses, and larvae that had escaped from masses for a brief planktonic period—had prototroch, food groove, and metatroch cilia similar to those seen in *Schizobranchia insignis* (Fig. 4A). The width of the food groove ranged from about 6–8 μm in five larvae that had left egg masses on their own. The metatrochal band was slightly broader than that of *S. insignis.* In most other respects my observations of development of two broods of *D. medius* are in accord with those of McEuen *et al.* (1983). One addition to their description is that embryos and early larval stages in gelatinous egg masses were individually encapsulated in spherical capsules only slightly larger in diameter than the developing worms themselves. Capsules are present in addition to fertilization envelopes like those described above for *S. insignis.* In the broods I observed, larvae hatched from capsules about 5–7

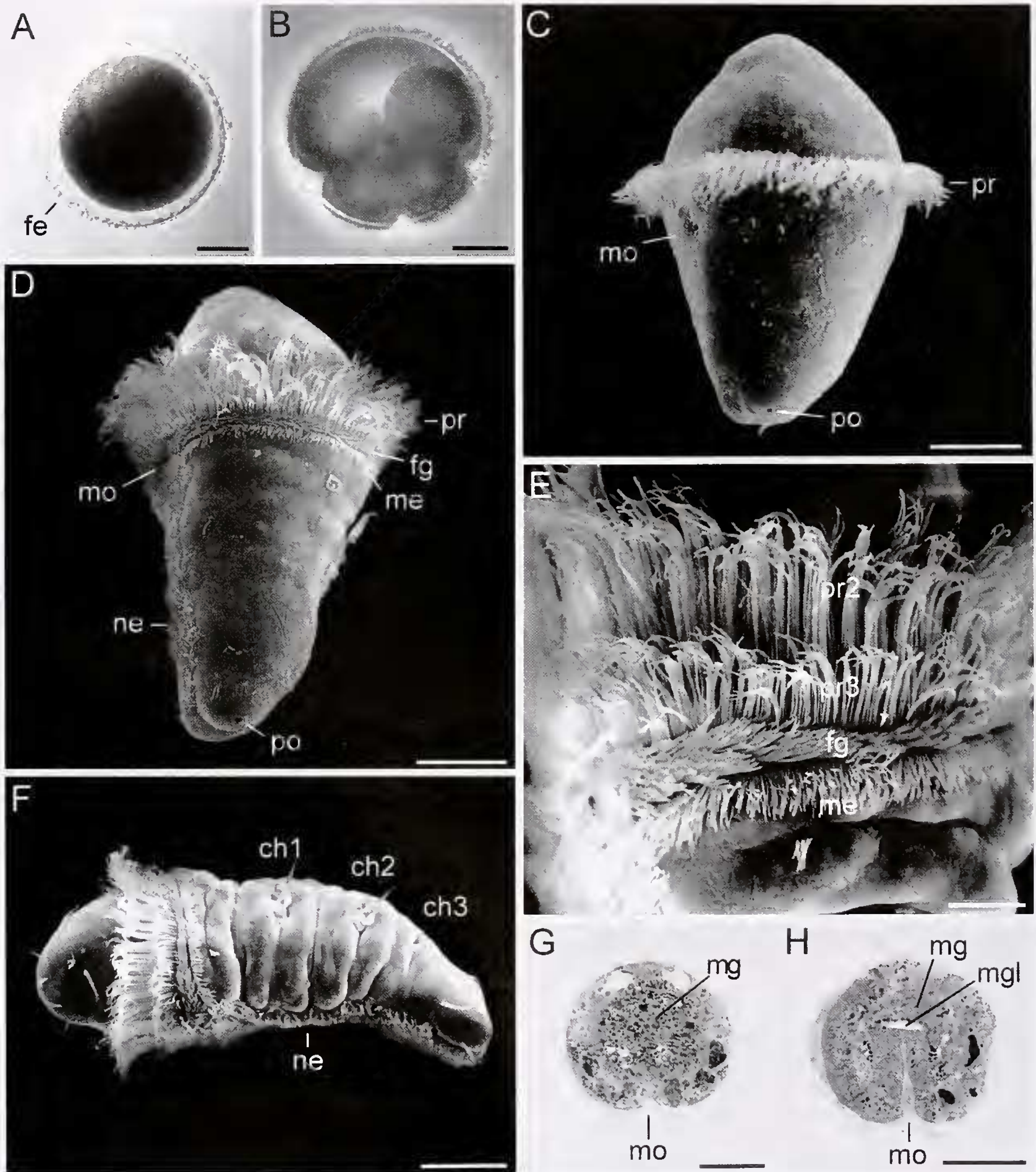

Figure 3. Embryonic and larval stages of *Schizobranchia insignis.* (A) Fertilized egg. (B) Four-cell embryo. (C) Two-day-old larva. (D) Three-day-old larva. (E) Detail of opposed ciliary bands of fourteen-day-old larva. (F) Fourteen-day-old larva. (G) Transverse section of eight-day-old larva at the level of the mouth. (H) Transverse section of twenty-day-old larva at the level of the mouth. Scale bars = 50 μm, except for (E) where the scale bar = 15 μm. ch1 = first chaetiger, ch2 = second chaetiger, ch3 = third chaetiger, fe = fertilization envelope, fg = food groove, me = metatroch, mg = midgut wall, mgl = midgut lumen, mo = mouth, ne = neurotroch, po = pore of one of the paired anal vesicles, pr = prototroch, pr2 = second tier of prototrochal cilia, pr3 = third tier of prototrochal cilia.

days after egg deposition, and they emerged from egg masses about 7–15 days after deposition.

Myxicola aesthetica. Fertilized eggs of *M. aesthetica* were spherical, opaque, negatively buoyant, rose-colored in reflected light, and surrounded by an elevated envelope. The mean diameter (± one standard deviation) of 10 eggs from

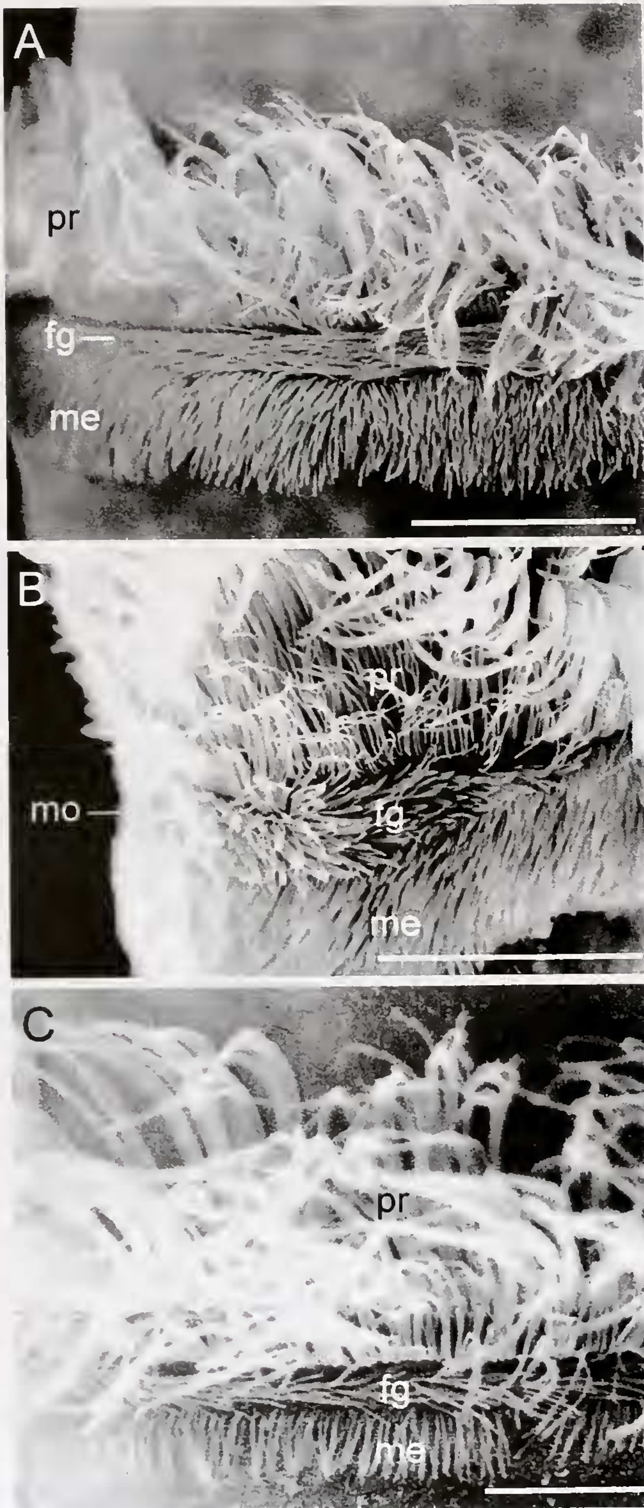

Figure 4. Opposed ciliary bands of *Demonax medius*, *Myxicola aesthetica*, and *Pseudopotamilla occelata*. (A) Ciliary bands in the right ventral region of larva of *D. medius*. (B) Left lateral view of ciliary bands of 11-day-old larva of *M. aesthetica*. (C) Left dorsal view of ciliary bands of 3-day-old larva of *P. occelata*. Scale bars = 25 μm. fg, food groove; me, metatroch; mo, mouth; pr, prototroch.

a single female was 130 (4.9) μm. First and second cleavages were distinctly unequal. At incubation temperatures of 12–13 °C, larvae began swimming by about 18 h after fertilization. By 2 days after fertilization, larvae bore prototrochal, food-groove, and metatrochal cilia similar to those seen in *Schizobranchia insignis* (Fig. 4B). The width of the food groove was about 6 μm in five larvae. As in *Demonax medius*, the metatrochal band of *M. aesthetica* was broader than that of *S. insignis*.

Pseudopotamilla occelata. Fertilized eggs of *P. occelata* were spherical, opaque, negatively buoyant, gray in reflected light, and surrounded by an elevated envelope. The mean diameter (± one standard deviation) of 15 eggs from a single female was 142 (2.9) μm. First and second cleavages were distinctly unequal. By 3 days after fertilization, larvae bore prototroch, food-groove, and metatroch cilia similar to those seen in *Schizobranchia insignis* (Fig. 4C). The width of the food groove was about 6 μm in five larvae.

Functional morphology of ciliary bands

Food-groove and metatrochal ciliary bands in larvae of all four species behaved like similar ciliary bands in larvae that feed with opposed bands of cilia (*e.g.*, Strathmann *et al.*, 1972). The directions of effective strokes of cilia were anterior to posterior (prototroch), posterior to anterior (metatroch), and laterally towards the mouth (food groove). All three bands of cilia could arrest their beat, apparently independently of the others. When metatrochal cilia were not beating, they lay flat along the larval body, pointed posteriorly. These ciliary beat patterns were confirmed by analyses of high-speed video footage, but were also clearly visible by inspection of living larvae at 400× final magnification.

I examined the ability of larvae of *Schizobranchia insignis* to concentrate particles from suspension and transport them to the mouth using opposed bands of cilia. I videotaped four 9-day-old larvae in suspensions of 3-μm beads for a total of 22 min. This footage included at least 30 particle captures. Analysis of these video sequences indicated that beads were caught in the current generated by the prototrochal cilia and moved into the food groove. Resolution of images was not sufficient to determine if metatrochal cilia were actively beating during captures. Captured beads were transported in the food groove around the body towards the larval mouth (Fig. 5). Once at the mouth, beads were moved along the neurotroch until they fell off the posterior end of the body.

Possible functions of opposed ciliary bands in sabellid larvae

I tested the hypothesis that planktonic larvae of *Schizobranchia insignis* or *Demonax medius* feed on particulate food by offering larvae particles that are readily ingested by

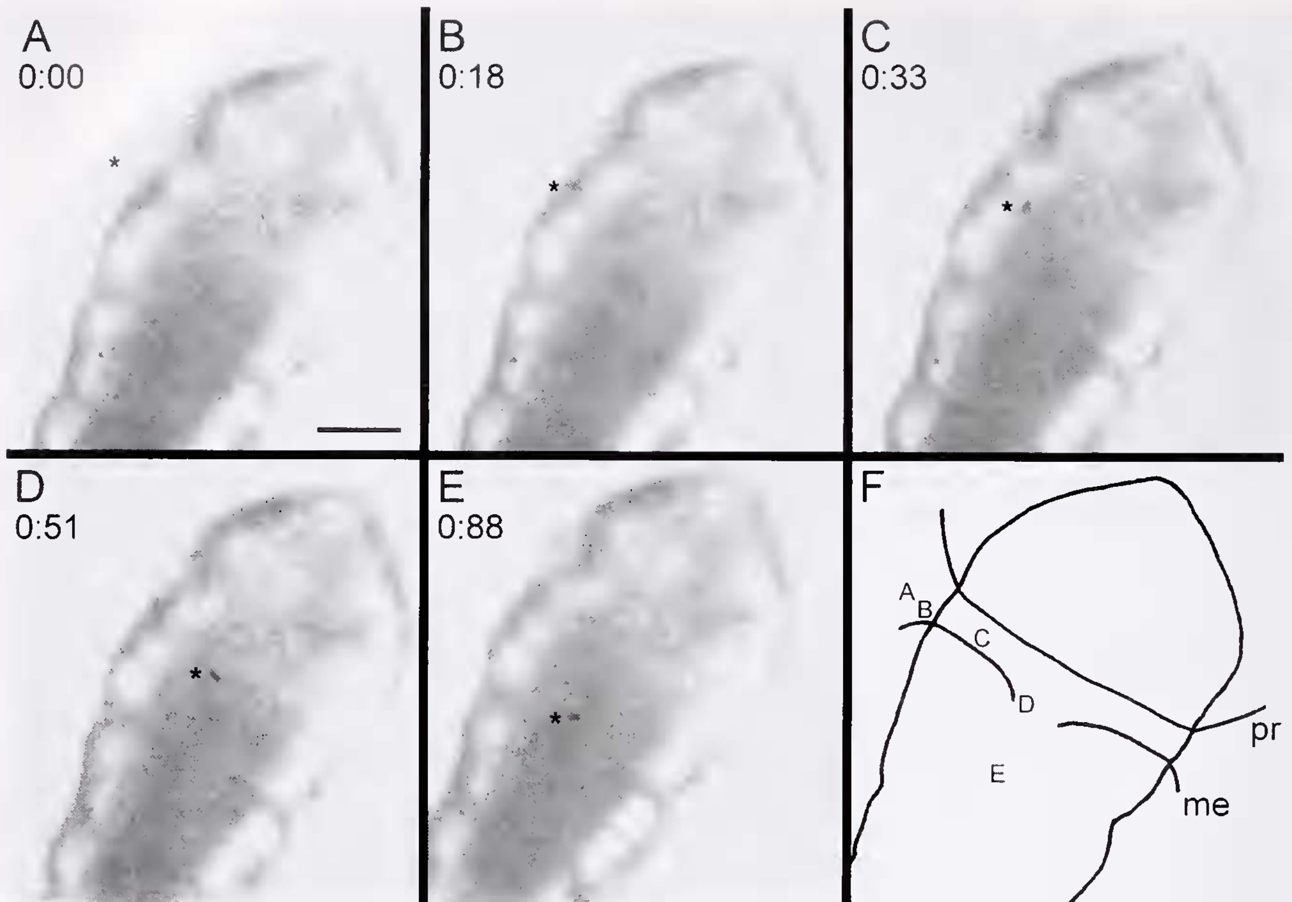

Figure 5. Capture and transport of a particle by a 9-day-old larva of *Schizobranchia insignis*, in ventral view. (A, B) Capture of a particle. (C, D) Transport to the mouth in the food groove. (E) Transport posteriorly on the neurotroch. Time (s) is marked on each frame. Scale bar = 50 μm; the asterisk is adjacent to the particle. (F) Composite diagram of particle positions in (A–E): me = metatroch, pr = prototroch. For clarity, only positions of the prototroch and metatroch are shown. Food-groove cilia lie between them, and the neurotrochal cilia run from the gap in the metatroch to the posterior tip of the body. The mouth is slightly anterior to the position of the particle shown in (D).

related feeding larvae. Particulate food was never observed in the guts of larvae of *S. insignis* ranging from 5 to 30 days old, or in the guts of larvae of *D. medius* that had hatched naturally from egg masses. Feeding larvae of echinoid echinoderms or serpulid annelids included as positive controls always ingested many of both types of particles.

I also tested the hypothesis that ciliated cells of the prototroch, food groove, or metatroch are involved in the uptake of dissolved proteins by endocytosis. Larvae of *Schizobranchia insignis* and *Demonax medius* exposed to FITC-BSA never showed any differences in fluorescence relative to larvae incubated only in MPFSW. In both treatments, larval chaetae autofluoresced when excited with ultraviolet light. Larvae known to be able to take up large dissolved proteins always showed fluorescence indicating uptake of FITC-BSA in velar cells (*Littorina sitkana:* Moran, 1999) or cells of the "larval kidney" (*Crepidula adunca:* Rivest, 1981) when incubated in FITC-BSA, but not when incubated in MPFSW.

Discussion

Evolutionary loss of the requirement for larval feeding has occurred repeatedly in many phyla, and is typically followed by the loss of structures formerly involved in feeding (Strathmann, 1978; Emlet, 1991; Wray, 1996). Detailed hypotheses on how this association arises, however, have been tested mainly in the context of comparative data on members of only one phylum, the Echinodermata (Hart, 1996; Wray, 1996). The observations reported here on larvae of annelids permit an initial assessment of their generality. These data are also useful in generating hypotheses on other topics, including the specific sequence of events that lead to loss of feeding ability after increases in egg size and energy content in animals that develop *via* spiral cleavage.

Ancestral feeding structures in nonfeeding annelid larvae

I interpret the transverse ciliary bands of the sabellid larvae described here as homologs of the prototroch, food

groove, and metatroch of closely related feeding larvae (*i.e.*, those of serpulids and sabellariids). Almost all annelid larvae bear a prototroch, and in all cases where detailed observations have been made, these are homologous by developmental criteria (Rouse, 1999). The lineages of the cells that bear food-groove and metatrochal cilia are not known in any detail, so other criteria must be used to infer homology of these ciliary bands. Two criteria that support my interpretation are those of position and behavior. The perioral and postoral cilia of larvae of *Schizobranchia insignis, Demonax medius, Myxicola aesthetica,* and *Pseudopotamilla occelata* are located in the same positions as the food-groove and metatrochal cilia, respectively, of feeding sabellariid and serpulid larvae, and they show similar beat patterns (perioral cilia beat laterally towards the mouth, and postoral cilia beat from posterior to anterior). Together, the preoral, perioral, and postoral ciliary bands of *S. insignis* are capable of capturing particles from suspension and transporting them to the mouth, like the opposed bands of related feeding larvae. Given current hypotheses on the relationships of sabellariids, serpulids, and sabellids, phylogenetic criteria for homology are also met (Fig. 1; Lauder, 1994). Under this interpretation, the food-groove and metatrochal ciliary bands of sabellid larvae can be identified as ancestral structures retained after loss of larval feeding in the family.

The hypothesis that the opposed ciliary bands of these sabellid larvae are homologous to those of related feeding larvae might be falsified with new phylogenetic, developmental, anatomical, or physiological data, but given current knowledge it appears likely that it is correct. An alternative hypothesis is that the opposed ciliary bands of sabellids evolved in parallel with those of sabellariids and serpulids. This seems unlikely on grounds of parsimony. Further, the opposed ciliary bands of larvae of *Schizobranchia insignis* lack obvious functions, which makes it difficult to understand how they might have evolved independently.

Opposed ciliary bands may be widely distributed among the nonfeeding larvae of sabellin sabellids. My observations show that food-groove and metatrochal ciliary bands are present in larvae of members of at least four genera of sabellins. I examined published descriptions of nonfeeding larvae of sabellids for further evidence on the distribution of opposed bands of cilia in the family. This survey revealed probable opposed bands in nonfeeding larvae of four more genera of sabellins. Scanning electron micrographs provide evidence for the presence of food groove and metatroch in members of *Amphicorina* (Rouse and Fitzhugh, 1994) and *Laonome* (Hsieh, cited in Fig. 12.5D of Pernet *et al.*, 2002). A text description of the "prototrochal" cilia of *Megalomma* (as *Branchiomma*) *vesiculosum* (Wilson, 1936) is also suggestive of opposed bands. Early larvae of *M. vesiculosum* have three tiers of prototrochal cilia, but slightly later in development two more rows of cilia appear posterior to the prototroch. The most posterior row, when arrested, points posteriorly. This is precisely the pattern seen in *Schizobranchia insignis*, where the two new rows of cilia form the food groove and metatroch, and where the metatroch, when arrested, points posteriorly. Finally, a text description and drawings of larvae of *Chone teres* also suggest the presence of opposed bands. Okuda (1946) states that the "prototroch" of larvae of *C. teres* is composed of one band of long cilia followed posteriorly by two adjacent bands of short cilia. Unfortunately, he does not specify the spatial relationships of these cilia to the larval mouth. His drawings show ciliary bands similar to those observed here in four genera of sabellin sabellids. Published illustrations of larvae of several other sabellins (*e.g.*, *Amphiglena nathae* [Rouse and Gambi, 1998] and *Perkinsiana riwo* [Rouse, 1996]) may indicate the presence of only a single band of cilia behind the prototroch, but more observations of these larvae are needed. I found no descriptions of sabellin larvae that unequivocally indicate the absence of food groove and metatroch.

Thus, members of at least eight genera of sabellins likely possess opposed bands of cilia. According to recent phylogenies (Rouse and Fitzhugh, 1994; Fitzhugh and Rouse, 1999), these genera are not clustered in any particular subclade of the Sabellinae: some (*e.g.*, *Amphicorina, Myxicola*) arise from deep nodes in the tree, and others (*e.g., Laonome, Schizobranchia*) from shallow nodes. All known sabellin reproductive strategies (brooding to the juvenile stage, brooding with release of a planktonic larva, and freespawning: Rouse and Fitzhugh, 1994) are represented in the species for which there is evidence of opposed ciliary bands. I conclude that opposed ciliary bands are probably widespread in the sabellins, a clade that includes over 400 species. In contrast, opposed ciliary bands do not appear in the direct-developing embryos of fabriciin sabellids (Rouse and Fitzhugh, 1994).

Though metatrochal cilia have previously been identified by position in larvae of several species of sabellins (*e.g.*, Rouse and Fitzhugh, 1994), their presence has more usually gone unnoticed. That metatrochal cilia may act with prototrochal and food-groove cilia as opposed-band systems in these larvae has not previously been described. It is possible that opposed ciliary bands are more widely distributed in nonfeeding annelid larvae. Indeed, the nonfeeding larvae of some serpulids also have opposed ciliary bands, at least by the criterion of position (Kupriyanova *et al.*, 2001; Nishi and Yamasu, 1992). Other annelid clades that should be examined for the presence of opposed bands in nonfeeding larvae include those that contain both species whose larvae feed with opposed bands and species that have nonfeeding larval development. Phylogenetic hypotheses delimiting some of these clades (*e.g.*, those including the families Amphinomidae, Opheliidae, and Polygordiidae, all of which include species that feed with opposed bands of cilia: Pernet *et al.*, 2002) are illustrated by Rouse (2000b). In some of these clades, nonfeeding larvae probably evolved from ancestors that possessed opposed bands of cilia.

Knowledge of the distribution of opposed bands in larvae of these groups will provide additional insight into the evolution of larval form in annelids.

Why have ancestral feeding structures been retained by nonfeeding sabellid larvae?

One possible explanation for the retention of feeding structures is that sabellids lost the need and requirement for larval feeding only very recently. If nonfeeding development is plesiomorphic for the sabellids, a minimal estimate of the age of their loss of larval feeding is the time of the clade's origin. Unfortunately, scarce fossil and molecular data make it difficult to estimate the age of this clade. Sabellid tubes are usually unmineralized, so are not expected to be easily preserved. Though putative sabellid fossils have been described from strata ranging from the lower Miocene to the Devonian (*e.g.*, Howell, 1962; Plicka, 1968; Termier *et al.*, 1973; Hayward, 1977; Schweigert *et al.*, 1998), these identifications are quite tentative. Sabellids are as speciose and widely distributed as their sister taxon, the serpulids, which are known from the Devonian (Glasby, 2000; Rouse and Pleijel, 2001). Assuming that patterns of diversification were similar in the two clades, this can be interpreted as weak evidence that sabellids are also Paleozoic in origin. Accurate determination of the age of the Sabellidae clearly requires more fossil and molecular data.

A second possibility is that food groove and metatrochal cilia might be very inexpensive to construct and operate. If there is little cost to maintaining these structures, they may be relatively invisible to selection for economy during development. Reduction of these ciliary bands might still occur, but only as a result of the slow accumulation of mutations in genes specifying their development. Indeed, degeneration of the opposed-band system may well be occurring in sabellids, as indicated by their unusually narrow food grooves. In annelid and mollusc larvae that feed with opposed bands, the food groove is typically 20–30 μm wide (Phillips and Pernet, 1996; Pernet, unpubl. data), but in the sabellid larvae examined here, food grooves ranged in width from 5–8 μm. Width of the food groove may constrain the sizes of particles transported to the mouth (R. Strathmann, 1987; Hansen, 1993). Thus the opposed bands of sabellid larvae, though "functional" in the sense that they can capture and transport particles, likely cannot capture particles of as wide a size range as those of related feeding larvae.

Finally, one might expect ancestral feeding structures to be retained in nonfeeding larvae if they have functions other than feeding. Prototrochal cilia clearly function in swimming in both feeding and nonfeeding annelid larvae. However, functions other than particle capture have rarely been considered for food groove and metatrochal ciliary bands in free-swimming larvae. Opposed ciliary bands have been retained in the encapsulated, "non-feeding" larvae of numerous species of gastropods, but in these cases the opposed bands continue to have clear functions (*e.g.*, feeding on maternally provided particles within the capsule: Chaparro *et al.*, 2002). A variety of alternative functions of opposed ciliary bands in free-swimming sabellid larvae are possible. For example, opposed bands might serve as the site of uptake of dissolved organic material; might play a role in assessing potential settlement sites; might be involved in forming the initial juvenile tube; or might be used in feeding by recently settled juveniles while the definitive adult feeding structures develop. It is also possible that food groove and metatrochal ciliary bands play some role in development. Several of these hypotheses (uptake of dissolved organic material, feeding in juveniles) are likely false, as suggested by data reported in this paper. There is currently little evidence available with which to assess other hypotheses.

Retention of functional ancestral particle capture systems in nonfeeding larvae of sabellids contrasts sharply with data from echinoderms, where larval feeding structures appear to be lost very rapidly after loss of the requirement for particulate food during larval development (Wray and Raff, 1991; Hart, 1996; Wray, 1996). To make a more general assessment of how rapidly larval form responds to changes in larval nutritional mode, studies of more independent evolutionary events are clearly required. Since evolutionary losses of the requirement for larval feeding have been common in the history of marine invertebrates (Strathmann, 1978), as well-supported phylogenies become available for more taxa it should be possible to identify many more specific examples of the evolutionary loss of larval feeding, to estimate the timing of these events, and to assess how they are related to the evolution of larval form.

Events in the evolutionary loss of larval feeding ability

The observations reported here are generally consistent with the model discussed above to account for the association between loss of the requirement for food in the larval stage and loss of larval feeding structures. I argued above that sabellids are derived from an ancestor that had a feeding larval stage. For purposes of comparison, I assume that this ancestor shared characteristics of extant sabellariid and serpulid species that have feeding larvae. The evolution of increased energy content in the egg is thought to be the first step in the evolution of nonfeeding development. Compared to the eggs of sabellariids and serpulids with feeding larvae, which range in diameter from about 45–90 μm (Giangrande, 1997 [her reference to 150-μm eggs in *Sabellaria spinulosa* is incorrect, according to Wilson (1929)]; Kupriyanova *et al.*, 2001), the eggs of sabellids are relatively large (>110 μm: Rouse and Fitzhugh, 1994) and energy-rich (Pernet and Jaeckle, unpubl. data). The second step in this sequence is loss of the ability to feed. All known sabellid larvae are obligately nonfeeding. However, they retain some ancestral feeding structures (in particular, cilia of the food

groove and metatroch). These larvae can thus be interpreted as intermediates in the evolutionary sequence discussed above. Such intermediates have previously been considered rare (Hart, 1996; Wray, 1996), but my observations suggest that they may be common in this clade of annelids.

These data may be useful in answering a question that has previously been poorly understood—that is, what specific events lead to the loss of larval feeding ability after an evolutionary increase in egg provisioning? Wray (1996) noted that loss of the ability to feed might be the result of any of many changes, such as loss of a particular digestive enzyme, loss of some aspect of ciliary coordination, or failure to complete morphogenesis of the larval mouth. He also noted that the rarity of nonfeeding larvae with only slightly derived morphology makes it difficult to identify the specific changes in morphology or behavior that render larvae unable to feed. Most putative intermediates (*e.g.*, the sea urchin *Phyllacanthus imperialis:* Olson *et al.*, 1993) have undergone changes in multiple feeding-related traits (*e.g.*, number and shape of arms, structure of the larval gut) since divergence from a feeding ancestor, and it is thus difficult to identify any one key change that resulted in a loss of feeding ability.

As putative intermediates, larvae of sabellins may provide insight into how larval feeding ability is lost after evolutionary increases in egg size. Larvae of *Schizobranchia insignis*, and probably many other sabellins, possess the ciliary bands needed to capture food particles from suspension. These ciliary bands remain capable of capturing particles and moving them to the mouth. The mouth, however, does not connect to the midgut, which in any case has no (or, later in development, a very small) lumen because the cells that make up its wall are swollen with energy reserves. Loss of feeding in larvae of *S. insignis* may thus be related primarily to a change in the digestive system.

This observation, along with a consideration of annelid development, suggests a specific hypothesis on the link between increased maternal provisioning of the egg and the loss of larval feeding ability. Embryos of annelids (and those of members of several other phyla, together known as spiralians) undergo spiral cleavage, a process in which the fates of some blastomeres are specified early in development. In spiralian embryos, the descendants of four cells—3A, 3B, 3C, and 4D—form the endoderm, the embryonic tissue that becomes the larval midgut. As a result of unequal cleavages from the third through the fifth cleavage cycles, these cells, known as macromeres, are usually far larger than the remaining embryonic cells, the micromeres. At gastrulation, the macromeres and their descendants are internalized, where they form the larval midgut (Kume and Dan, 1968; Anderson, 1973).

Annelids that have feeding larval stages typically have small eggs (Schroeder and Hermans, 1975). In these species, the descendants of 3A–C and 4D form the wall of a larval midgut that has a substantial lumen. In annelids with nonfeeding larvae, however, eggs are larger. Increased egg volume is presumably due mainly to the addition of material used to fuel development of the larvae or juveniles. During early development most of this additional material is shunted to the macromeres for storage and gradual mobilization. In annelid embryos that develop from large eggs, then, macromeres are proportionally larger (relative to the micromeres) than those of embryos that develop from small eggs (Schneider *et al.*, 1992). Construction of a functional midgut may be difficult when a large volume of endodermal cells must fit in the relatively small volume delimited by the remaining cells of the embryo. In this case, it may be impossible to assemble the endoderm into a midgut that has a substantial lumen, or any lumen at all. Indeed, nonfeeding larvae of annelids typically lack midgut lumens (Wilson, 1936; Anderson, 1973; Heimler, 1988). In addition to simple size constraints, in annelid species with large eggs, division of endodermal cells (and subsequent gut morphogenesis) may be delayed relative to species with small eggs (*e.g.*, Schneider *et al.*, 1992).

Thus, in annelids, a quantitative increase in egg volume, typically thought to be the initial step in the loss of larval feeding, may lead directly to a loss of larval digestive ability because of spatial constraints or delays in gut morphogenesis. These effects may persist through larval development, resulting in nonfeeding larval development; alternatively, they may last only through part of larval development, delaying the onset of larval feeding until cells of the midgut wall shrink as energy stores are consumed.

This hypothesis is attractive because of its simplicity and its vulnerability to test. One approach is the experimental reduction of endoderm volume by removal of one or more presumptive endodermal cells (*e.g.*, Boring, 1989; Clement, 1962; Martindale, 1986). Carrying out this manipulation in annelid embryos that normally develop into nonfeeding larvae with occluded midguts might yield larvae with open midguts. It is not clear how morphogenesis of the rest of the gut (*e.g.*, mouth and stomodaeum) might be affected by macromere ablation, but it is possible that a simple reduction of endodermal cell volume might permit the development of a complete larval gut. In species that retain ancestral particle capture systems, like the sabellids described here, this manipulation might result in conversion of a nonfeeding larva to a feeding larva.

Another approach is to use intraspecific variation in egg size and larval nutritional mode to examine the effects of egg size on midgut development. For example, some individuals of the annelid *Streblospio benedicti* (family Spionidae) produce small eggs (56–70 μm diameter) that develop into feeding larvae, while others produce large eggs (115–152 μm) that develop into nonfeeding larvae (Levin, 1984; Schulze *et al.*, 2000). If the hypothesis proposed above is correct, nonfeeding development in *S. benedicti* should be a result of reduction in size of the midgut lumen or delayed development of the midgut in larvae that develop

from large eggs. This prediction can be tested with comparative developmental data.

This idea might also apply to other spiralian taxa such as molluscs and entoprocts. As their development is similar, they should be subject to similar effects of endodermal volume on midgut morphogenesis. Both approaches to testing the hypothesis—deletions of endodermal blastomeres and comparative studies within species with variable egg size and developmental mode (*e.g.*, the ascoglossan *Alderia modesta:* Krug, 1998)—may be fruitful.

This hypothesis links changes in development (in this case, egg size and allocation to endodermal lineages) to changes in the form and function of later stages (loss of larval feeding ability *via* delayed midgut morphogenesis). Such connections have long been sought by developmental biologists (*e.g.*, Lillie, 1899; Freeman and Lundelius, 1992). In addition, it may be useful in explaining a peculiar observation—that correlated intraspecific variation in egg size and larval nutritional mode (a form of "poecilogony") appears to be limited in distribution to spiralians, in particular annelids and molluscs (Chia *et al.*, 1996). I propose that annelid and mollusc species with great intraspecific variation in egg size may show correlated variation in larval nutritional mode because of spatial constraints or heterochronic effects on midgut morphogenesis imposed by their conserved pattern of development.

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