SETTLEMENT AND METAMORPHOSIS IN THE ECHIURA: A REVIEW

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Two types of settlement and metamorphosis are distinguished. Males of the family Bonellidae have specialized attachment structures used during settlement; they undergo an abbreviated, neotenic metamorphosis. Information on settlement of echiurans other than bonellids is limited. Metamorphosis of the trochophore proceeds through the loss of trochal bands and protonephridia, as well as the transformation of the gastrointestinal valve and the pre- and posttrochal lobes into adult structures. The phenomenon of sex determination in the Bonellidae is reviewed.

INTRODUCTION

Echiurans occur in benthic habitats in shallow subtidal to hadal ocean depths[1] and in some areas, they represent a significant component of the benthic community.[2,3] Filter feeding species such as *Urechis caupo* are important in their ability to direct planktonic energy to the marine benthos.[4] Most echiurans feed on the nutrients in deposited sediments and thus play a major role in recycling energy in benthic communities.[5]

The Echiura are suitable subjects for the study of reproduction and development.[6] Early workers described the development of species in the genera *Echiurus*,[7,8] *Bonellia*[9,10,11] and *Lissomyema* (as *Thalassema*).[12] The detailed work of Newby[13,14] on *Urechis caupo* added to the knowledge of echiuran development. Recent investigations have dealt primarily with the cytological, ultrastructural, and biochemical aspects of gametogenesis (see Gould-Somero[15] for a review). Information on the settlement and metamorphosis of echiuran larvae is limited almost exclusively to the early studies. Neither electron microscopy nor advanced methods of light microtechnique have been employed in the investigation of echiuran larval development and metamorphosis.

The metamorphosis of an echiuran trochophore is a gradual transformation during which the structural and functional integrities of the individual are maintained. Metamorphoses of species in the genera *Echiurus, Lissomyema,* and *Urechis* are remarkably similar. *Bonellia*, on the other hand, represents a notable exception to the normal pattern of echiuran development.

The family Bonellidae is characterized by a striking sexual dimorphism in which the males are small neotenic forms living in the uterus of a female.[9] The larvae are sexually indifferent until settlement when their metamorphosis into a male or female form is determined either environmentally[16] or genetically.[17] Because settlement and metamorphosis of bonellid larvae are distinct among the Echiura, they will be considered separately.

SETTLEMENT OF ECHIURANS (EXCLUDING BONELLIDAE)

Within 24 hours after fertilization the gastrulae of *Lissomyema*,[12] *Urechis caupo*,[14] and *Ikedosoma gogoshimense*[18] begin to swim. Shortly afterward the gastrulae develop into planktotrophic trochophores. These larvae are equipped with two major ciliary bands. The largest and most powerful of these is the prototroch. This may be a single preoral band as in *Urechis*,[14] or it may be subdivided ventrally into two or three smaller bands as in *Echiurus*[8,19] and *Lissomyema*.[12] The second preoral band, the telotroch, is located just anterior to the anus. In *Urechis* this is subdivided into two distinct bands. Short cilia commonly cover the preoral lobe, and in some species a neurotroch is present extending from the mouth to the anus along the ventral midline.

The reaction of echiuran larvae to environmental cues is not fully understood. *Echiurus abyssalis* larvae are known to rise to the surface from depths as great as 1900 m.[19] This response probably is a negative geotropism since light levels at that depth are negligible and the larvae do not have eyes.

The larvae live for 2 to 3 months in the plankton before settling.[5,12,14,18] Impending settlement can often be recognized by contraction or flexing of the larval body.[8,12,21] This indicates that the body musculature is developed and that the crawling ability is imminent.

It has been shown that the larvae of *Listriolobus pelodes* settle in mid- to late spring, but no other information on seasonal settlement of echiuran larvae is available for comparison.[5]

It is generally believed that reliance upon chance encounters at the time of larval settlement is highly uneconomical.[22] An increasing number of investigations illustrate that invertebrate larvae are well equipped with sensory organs which might be used in habitat selection.[23] Two lines of evidence suggest that echiuran larvae conform to this generalization.

Indirect evidence that echiuran larvae may select appropriate substrates is found in the distribution of adults of *Listriolobus pelodes* near Santa Barbara, California.[2] Occurring in a large aggregation, they are distributed in a pattern which corresponds to a geographical area characterized by organically rich sediments of the finest particle size found anywhere on the Southern California mainland shelf.[24] Because the adults of this species cannot move over significant distances[5] and therefore cannot be considered effective in the formation of the aggregation, it is assumed that the larvae select the appropriate substrate.

Until recently, direct evidence for substrate selection by echiuran larvae was lacking. However, unpublished observations by Suer,[25] working with *Urechis caupo* larvae, support the widely held view of Wilson[26,27] that the nutrient properties of sediments influence the settlement of benthic marine invertebrate larvae. Suer[25] has found that *Urechis* larvae settle preferentially on sediment having a high organic content.

After settlement, the larvae of *Lissomyema*[12] and *Urechis*[14] are able to swim for 4 or 5 days. Undoubtedly, this is the result of a gradual reduction of the prototroch and telotroch, as swimming becomes progressively more feeble. Ultimately, when most, if not all trochal cilia are lost, the larvae become confined to a benthic existence and crawl using the body musculature and ventral cilia.[8,12,14,21] The burrowing behavior of the juvenile has not been described, but since the body muscles are functional by this time, burrowing is assumed to be similar to the peristaltic method used by the adults.[5,28]

METAMORPHOSIS OF ECHIURANS (EXCLUDING BONELLIDAE)

Metamorphosis of an echiuran trochophore into the juvenile form is not a radical event in the animal's life history but rather it is a gradual process during which the structural and functional integrities of the animal are maintained. As a result, this stage of echiuran development has been given different interpretations in the literature. Newby[14] broadly interpreted metamorphosis as predating settlement and including the development of all of the adult organs. Hatschek[8] and Conn,[12] on the other hand, considered metamorphosis to be the loss and transformation of larval structures. Although the details vary between and within animal groups, I believe that there is an underlying definition which may be applied to all animals. Metamorphosis should first be defined as a time-frame that brackets the end of larval life and the beginning of a juvenile existence. Within this frame, metamorphosis involves the loss or transformation of one or more larval characters or structures. Thus, metamorphosis is a component of the developmental process, but is distinguishable from organogenesis. In this paper, metamorphosis of the Echiura is reviewed on the basis of the preceding definition.

Settlement of the trochophore larva marks the beginning of echiuran metamorphosis. At this time, the larva undergoes structural and functional changes which result in the juvenile form and adult habits. These changes include the loss of the trochal bands and protonephridia as well

as the transformation of the gastrointestinal valve, and the pre- and posttrochal lobes into adult structures.

The reduction of the prototroch and telotroch is the salient event in the change from a pelagic to a benthic existence. Apparently, the loss of the cilia is not rapid since *Urechis*[14] and *Lissomyema*[12] are able to swim for 4 to 5 days after settling. However, the body muscles already have differentiated, and the newly settled larva is well equipped for crawling and burrowing.

The larvae of *Echiurus*[8] and *Listriolobus pelodes*[29] possess a pair of protonephridia near the ventral midline posterior to the mouth. During metamorphosis, these structures retrogress and are replaced in the juvenile by metanephridia. The larvae of *Urechis* and *Lissomyema* do not have protonephridia at any time during their development.

Located in the gut of the *Urechis* trochophore is a large gastrointestinal valve, separating the stomach from the intestine[14] (Fig. 1A). This structure forms a nearly complete barrier between the two areas, the only opening being a ciliated hole at the ventral midline. A ciliated furrow in the intestine runs from the gastrointestinal valve opening to the anus. During premetamorphosis, the dorsal and lateral margins of the gastrointestinal valve move posteriorly and ventrally. This enlarges the larval stomach and forms a caecum (Fig. 1B). The posterior wall of the caecum ruptures during metamorphosis so that the stomach opens directly into the intestine (Fig. 1C). The former gastrointetstinal valve lies on the ventral floor of the midgut and forms the dorsal wall of the ciliated groove. The groove is thus converted into a ciliated tube which opens to the midgut at both ends. Although the tube eventually closes, Newby[14] argued that it later reopens, and therefore is the primordium of the midgut siphon (Fig. 1D). Conn[12] also considered the ciliated intestinal groove to be the primordium of the siphon in *Lissomyema*. On the other hand, Baltzer, studying *Echiurus*, stated that the ciliated intestinal groove and the siphon are not related. Newby[14] dismissed Baltzer's observation as incorrect because he had worked only with whole mounts.
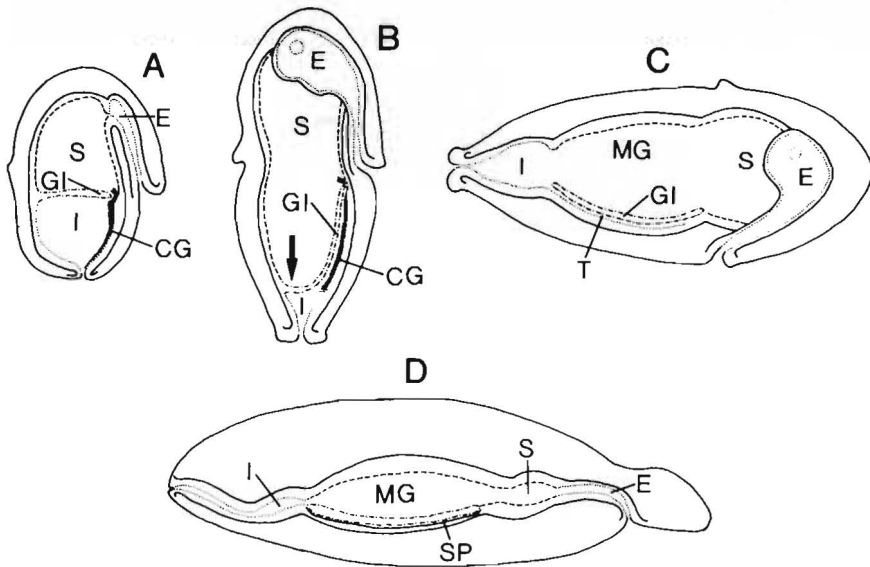

Fig. 1. The transformation of the larval gastrointestinal valve into the primordium of the adult midgut siphon in *Urechis caupo*. A. 4-day old larva, the gastrointestinal valve separates the larval stomach from the intestine. B. 35-day old larva, the dorsal and lateral margins of the gastrointestinal valve are shifted posteriorly. C. 50-day old larva, the gastrointestinal valve has ruptured dorsally making a linear pathway from the midgut to the intestine. The valve lies on the floor of the midgut transforming the cilitated groove into a ciliated tube. D. 60-day old larva, the siphon primordium lies along the ventral side of the midgut. I, intestine; MG, midgut; S, larval stomach; SP, siphon primordium; T, ciliated tube. Arrow indicates the region of the caecum where the gastrointestinal valve will rupture (based on Newby[14]).

One of the most noticeable changes during metamorphosis is the formation of the adult body shape. This is accomplished, anteriorly, by the lengthening of the preoral lobe to form the proboscis. During elongation, the muscle mass of the preoral lobe increases dramatically and reduces the blastocoel to a network of interconnecting cavities beneath the ciliated ventral epithelium (Fig. 2).

In the larva the blood vessels, circumesophageal nerve ring, and part of the esophagus extend into the preoral lobe. As the lobe elongates the nerve ring and blood vessels are drawn out with it and can be identified in the adult proboscis (Fig. 2). The esophagus does not lengthen; instead it is excluded from the pretrochal region.[12] Although proboscides of filter feeding echiurans such as *Urechis* are very small, they are derived from the larval preoral lobe in essentially the same manner as those of deposit feeders.[14]

The short cilia covering the preoral lobe of the larva become restricted to the ventral surface of the proboscis and are used in proboscis extension and food gathering.[5,30,31]

Concurrent with the formation of the proboscis, the postoral region expands to form the body of the adult. Conn[12] believed that in *Lissomyema*, the expansion was due to a sudden influx of water through the anal vesicles. Newby[14] reported that an increase of the coelomic fluid is responsible for the postoral expansion of *Urechis*, but he did not speculate on the source of the fluid.

The metamorphosed juvenile looks essentially like a small adult. Its body is transparent and characteristically undergoes peristaltic contractions. The proboscis actively explores the surrounding sediment for food.

SETTLEMENT OF BONELLIDAE

In contrast to other echiurans, bonellids produce large yolky eggs. These are deposited in masses of gelatinous strings after fertilization by dwarf males living in the female's uterus.[9]

The larvae hatch after two days and begin crawling about using the short cilia which cover the body. By the end of the third or fourth day the prototroch and telotroch have developed and a small green-pigmented swimming trochophore has formed. Bonellid trochophores are lecithotrophic and have a midgut without a mouth or anus.[9] Eyes are present in *Bonellia viridis* but are lacking in *B. fuliginosa*. Further, bonellid larvae differ from those of other echiurans by having a specialized attachment structure which is used at the time of settlement.
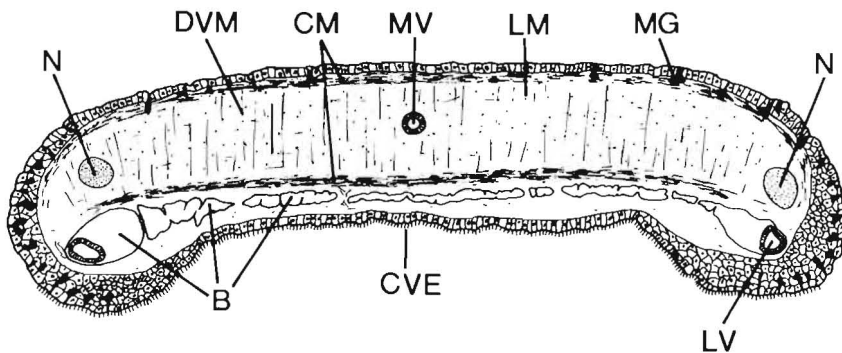

Fig. 2. Transverse section through the proboscis of an adult *Listriolobus pelodes*. The blastocoel from the larval preoral lobe is retained as a string of small cavities beneath the ventral epithelium. B, blastocoel remnants; CM, circular muscles; CVE, ciliated ventral epithelium; DVM, dorsoventral muscles; LM, longitudinal muscles; LV, lateral blood vessel; MG, mucus glands; MV, median blood vessel; N, nerve loop.

Baltzer[16] observed that, prior to settlement, the larvae of *B. viridis* exhibit a strong but short positive phototaxis. The egg masses of this species usually are deposited in rock crevices; hence the photoresponse may be important for the emergence of the larvae and their subsequent dispersal.

Time is an important factor in bonellid settlement relative to sexual development. The larvae may settle at any time from the third day to 3 or 4 weeks after fertilization.[11] However, as shown by Baltzer[32] and Leutert,[33] larvae settling on an adult female before their sixteenth day will tend to become males while those settling later or on a substrate free of female influence tend to become females.

As mentioned previously, bonellid larvae are equipped with specialized attachment structures which are used at the time of settlement. However, the role played by these structures in settlement is known only in those larvae which settle on an adult female and are destined to become males.

The attachment structure of *Bonellia viridis* consists of a large bilobed gland complex located on the ventral surface just behind the prototroch.[34] At the time of settlement, the larva makes contact with the proboscis of an adult femlae and becomes attached by secretions from the anterior region of the complex. After the initial bond is formed, the region of secretory attachment enlarges posteriorly to the midbody area. The pretrochal lobe and the posterior section of the body do not adhere (Fig. 3A). Metamorphosis begins shortly after attachment.

A different type of attachment structure has been reported in the trochophore of *B. fuliginosa*.[35] In this larva a muscular sucker is present midventrally immediately behind the prototroch. Michel[35] found the sucker to be very similar in construction to the acetabulum of the trematodes. When the body of a female *B. fuliginosa* is encountered, strong muscular contractions of the sucker securely fasten the larva to the adult (Fig. 3B).

METAMORPHOSIS OF BONELLIDAE

The extreme sexual dimorphism of bonellid echiurans was first reported in 1868 by Kowalevski[36] and by 1879 the development of the male and female was described.[9] Baltzer[37] noted that a small sexually indifferent trochophore is produced which, depending on the substrate it settles on, will metamorphose into a male or female (Fig. 4). Because male and female metamorphoses are entirely different processes, the larvae remain in a generalized condition until settlement. The adult organs and characters are present in a rudimentary stage in the larvae, that is, at the point where male and female developmental patterns diverge. Thus, most of organogenesis is delayed until the adult sex is determined at settlement.

Female bonellid trochophores metamorphose into juveniles in essentially the same manner as that described for other echiurans.[9,11] Shortly after settlement, the prototroch and telotroch are lost and the short cilia covering the body become restricted to the ventral surface of the preoral lobe (Fig. 4B). This lobe elongates and flattens to form the proboscis. As the proboscis lengthens, the anterior nerve loop is drawn out with it (Fig. 4C). The two larval eyes in *B. viridis* remain associated with the anterior part of the nerve loop, and are displaced to the proboscis tip.[11] Adult *Bonellia viridis* will respond to strong illumination on the proboscis.[31]

The post-trochal region becomes the body of the adult by expansion and increased muscularization. A gastrointestinal valve has not been reported in the larval gut,[9] although a midgut siphon is present in the adult.[38] Female *B. viridis* retain their green pigment over the entire body through metamorphosis and into adulthood.[39]

Male metamorphosis and development begin a few hours after settlement on a mature female.[34] The first change is the loss of the ciliary bands. Loosli[34] reported that in *B. viridis* the prototroch is lost first, followed shortly afterward by the telotroch. Within 13½ hours after settlement both bands are gone but the body remains covered by short cilia (Fig. 4D). Similarly, Michel[35] reported that the trochal cilia of *B. fuliginosa* are lost shortly after settlement.
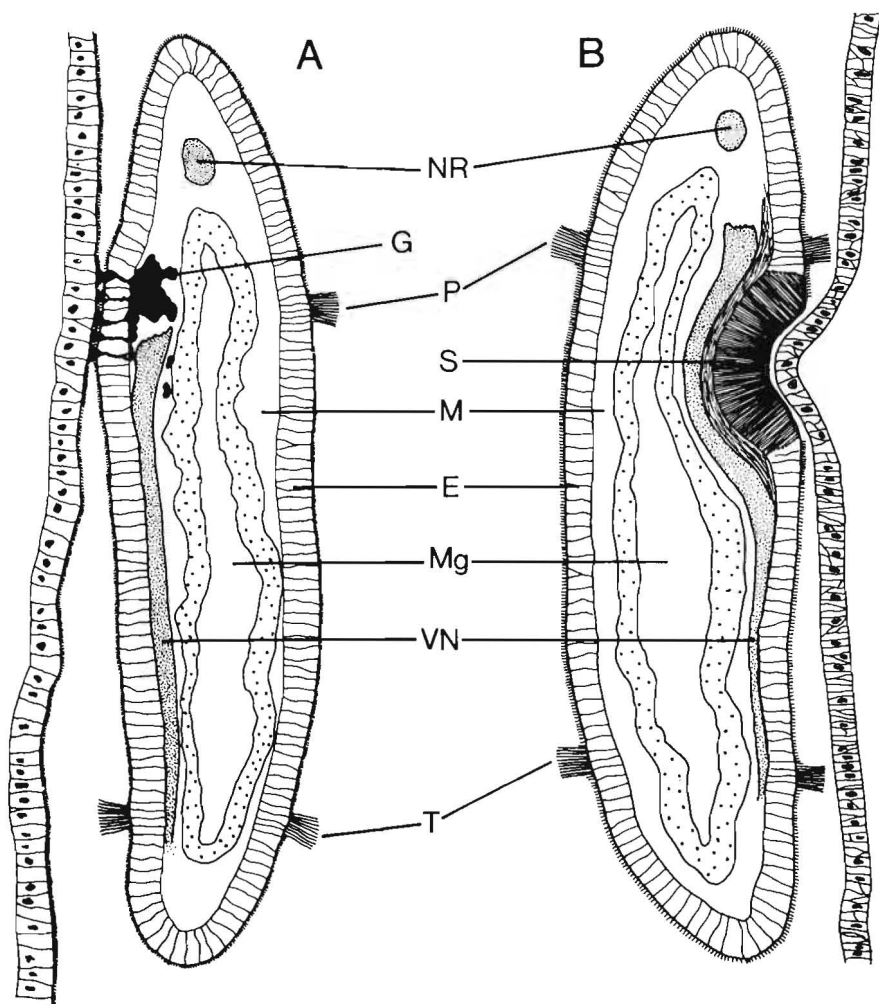
Fig. 3. Sagittal section representations of *Bonellia* trochophores at the time of settlement on an adult female. A. *B. viridis*, secretions from the gland complex firmly attaches the larva to the adult proboscis. B. *B. fuliginosa,* the larva attaches to the body wall of an adult female with a muscular sucker. E, epidermis; G, gland complex and secretions; M, mesoderm; Mg, midgut; NR, anterior nerve ring; P, prototroch; S, sucker; VN, ventral nerve. (A. based on Loosli,[34] B. based on Michel[35]).

The muscular sucker used by *B. fuliginosa* larvae to attach to the body of the female degenerates shortly after settlement.[35] In spite of the loss, the larva remains in firm contact with the adult throughout metamorphosis. Loosli[34] reported that in *B. viridis* the region of glandular attachment expands posteriorly, but the anterior and posterior ends do not adhere (Fig. 3A). The gland complex is retained during metamorphosis, and presumably into adulthood.

In direct contrast to other echiurans, the pretrochal lobe of the metamorphosing bonellid male undergoes a striking reduction. In the larva, the lobe represents approximately one third of the overall body length, but during metamorphosis it becomes virtually nonexistent[34,35] (Fig. 4E). The nerve ring becomes deflected posteriorly as a consequence of this shrinkage. Concurrent with the reduction of the anterior lobe, the posttrochal region elongates, but does not expand.[11] It has been suggested that this elongation may be due to a displacement of tissue from the pretrochal lobe during its reduction.[34] Michel[35] and Loosli[34] reported that the green

pigment which covered the anterior lobe of the larva is lost during metamorphosis. In *B. viridis,* some of the pigment becomes incorporated into the subepidermal tissue and migrates to the posttrochal body region, leaving the head as a small white cap.[34]

The pair of eyes of *B. viridis* larvae is lost at some time during male metamorphosis.[11] No eyes are present in the larva or adult of *B. fuliginosa.*[35]

Organogenesis occurs concurrently with metamorphosis. The result is a dwarf male designed solely for reproduction. The small ciliated body consists of a seminal vesicle, a gut without a mouth or anus, and a pair of metanephridia (Fig. 4E). The suppression of adult structures and retention of larval features led Baltzer[11] to consider the bonellid male to be a neotenic form.

FACTORS INFLUENCING THE METAMORPHOSIS OF BONELLIDAE

A discussion of echiuran metamorphosis would be incomplete without considering the problem of sex determination in *Bonellia.* Differentiation into a male or female, and hence male or female metamorphosis, is determined, according to Baltzer,[11,16] by the substrate on which a sexually indifferent larva settles. His experiments showed that up to 92% of the larvae which settled on a clean substrate became females, while the remainder became intersexes and sterile males. In addition, when provided with an opportunity to settle on the proboscis of a female, approximately 70% did so and metamorphosed into males. Those not attaching to the proboscis became females.[37,39,40,41,42] On the basis of these data, Baltzer[11] inferred that male differentiation may be the result of inhibited female development.

Baltzer reasoned that if complete male development requires association with the proboscis, then development possibly is affected by a determining substance which diffuses from the proboscis to the larva. This theory was supported by experiments which showed that aqueous extracts of female proboscides produced males in the majority of larvae tested.[43] Further, heat-stable aqueous extracts of the intestine also were found to be effective in inducing male development if used at a concentration of one part (by weight) dried tissue to 6000 to 9000 parts
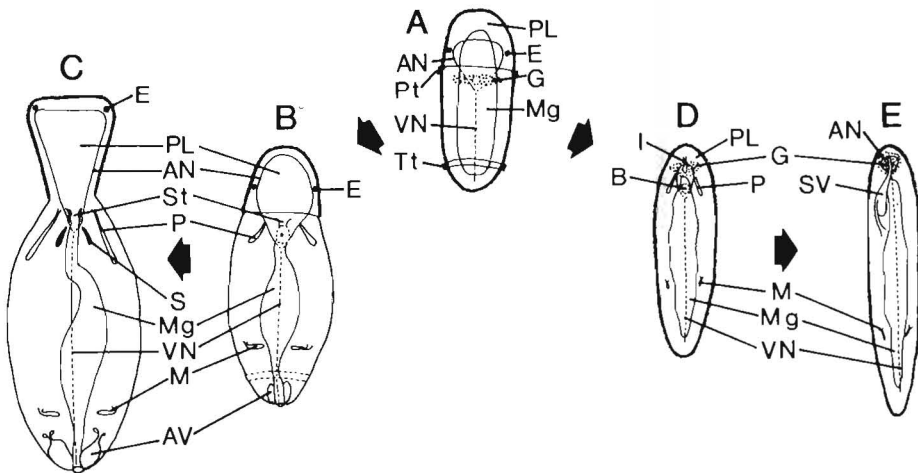

Fig. 4. Metamorphosis of male and female *Bonellia viridis* from a sexually indifferent trochophore. A. a sexually indifferent trochophore. B. an intermediate stage in female metamorphosis; note the loss of the trochal bands and the posttrochal body cilia. C. a juvenile female: the pre-trochal lobe has elongated to form the proboscis; the trunk has enlarged greatly. D. an intermediate stage in male metamorphosis: the trochal bands are lost and the pretrochal lobe has shortened considerably. E. a juvenile male: the pretrochal lobe is reduced to a mere cap; the short body cilia are retained. AN anterior nerve loop; AV, anal vesicle; B, blastopore; E, eye; G, gland complex; I, invagination; M, metanephridium; Mg, midgut; P, protonephridium; PL, pretrochal lobe; Pt, prototroch; S, setae; St, stomodaeum; SV, seminal vesicle; T, telotroch; VN, ventral nerve (based on Baltzer[11]).

sea water. Higher concentrations (1:1000 to 4000) proved inhibitory or fatal to the larvae.[43,44,45] Acetone extracts of the gut were ineffective in inducing male differentiation.[46]

Baltzer's theory proposing a diffusable sex determining substance also was supported by the observation that the completeness of male development is proportional to the amount of time the larva remains attached to the female.[10,42,47,48,49,50] Differentiation of each of the male organs requires a different threshhold of exposure to the proboscis for complete development. For example, reduction of the preoral lobe requires only a weak stimulus, while development of the seminal vesicle and viable sperm necessitates progressively longer exposures.[39,48]

Premature shortening of the length of time on the proboscis slows development and results in the formation of incomplete males, "intersexes."[37,51,52,53] Because the intersexes are most differentiated anteriorly, Baltzer[39] suggested that the secretions from the gland complex may act either as a bridge for the transport of the differentiating substance from the proboscis to the larva, or at least as a means of increasing the larval permeability to the substance.

Another line of investigation into the sex determination problem was pursued by Herbst.[54] He showed that by varying the ionic concentration of certain chemical elements in sea water, complete masculine development could be induced without exposure to a proboscis. For instance, increasing the concentration of copper or potassium resulted in the development of 70 to 80% males.[55,56] Decreasing the concentration of magnesium or sulphate had a similar effect in 90% of the larvae.[57,58] Increasing the acidity of the water also favored male development.[54] On the basis of his experiments he formulated the theory that hydration of the larva results in female development whereas dehydration favors male development.[59,60,61] Unfortunately, the relevance of these factors in nature is not known.

In summary, the studies of Baltzer and Herbst suggest that male development is a result of an interference or inhibition of female development by some unknown factor.

Wilczynski[17,62] took an opposing viewpoint and stated that sex determination is genetically determined during oogenesis. He was unable to discern the chromosomes in the oocytes, but he did claim to find two distinct egg types based on their differential reactivity to a stain which distinguishes male from female tissues. According to his studies the ultimate origin of the egg dimorphism lies with the incorporation by the oocyte of one of its two types of nurse cell nuclei early in oogenesis. Gould-Somero[15] questioned the presence of egg dimorphism as described by Wilczynski since the oocytes he worked with were not homogeneous and contained some obviously degenerate cells.

A recent publication by Leutert[33] re-examined the controversy between Baltzer and Wilczynski. Baltzer's original experiments were repeated using a sample size large enough to be treated statistically. Leutert's results support Baltzer's hypothesis by showing that sex is phenotypically determined in a significant number (43 to 83%) of the larvae. However, the remaining larvae are composed of some whose sex is genetically determined and others whose sex is unalterably indifferent. The fate and ecological significance of sexually indifferent juveniles in natural conditions is not known.

Leutert[33] also investigated oogenesis in *Bonellia viridis* using electron microscopy, and did not find any ultrastructural evidence of dimorphism among the nurse cells. Only a small amount of nurse cell material is passed to the oocyte during oogenesis, and this at an early stage. Further, no evidence of egg dimorphism was found at any stage of oogenesis.

Clearly, the problem of sex determination in *Bonellia* remains unsolved. The available evidence supports the earlier hypothesis that, for the majority of larvae, sex is determined phenotypically. However, a small percentage are unaffected by the determining factors and develop contrary to the phenotypic individuals. It is difficult to speculate whether this difference is due to genetic determination, or to an immunity of the larva to the influence of the substance. Finally, the identity of the determining substance and the nature of its action as a control of metamorphosis and development are unknown.

ACKNOWLEDGMENTS

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