Sequential developmental programmes for retractor muscles of a caenogastropod: reappraisal of evolutionary homologues

Louise R. Page

Department of Biology, University of Victoria, PO Box 3020 STN CSC, Victoria, British Columbia, Canada V8W 3N5
(lp@uvm.uvic.ca)

Evolutionary changes in the development of shell-attached retractor muscles in gastropods are of fundamental importance to theories about the early evolution and subsequent diversification of this molluscan class. Development of the shell-attached retractor muscle (columellar muscle) in a caenogastropod has been studied at the ultrastructural level to test the hypothesis of homology with the post-torsional left retractor muscle (larval velar retractor) in vetigastropod larvae. The vetigastropod muscle has been implicated in the generation of ontogenetic torsion, a morphogenetic twist between body regions that is important to theories about early gastropod evolution. Two shell-attached retractor muscles develop sequentially in the caenogastropod, *Polinices lewisii*, which is a pattern that has been also identified in previous ultrastructural studies on a vetigastropod and several nudibranch gastropods. The pattern may be a basal and conserved characteristic of gastropods. I found that the first-formed retractor in larvae of *P. lewisii* is comparable to the larval velar retractor that exists at the time of ontogenetic torsion in the vetigastropod, *Haliotis kamtschatkana*. However, the post-metamorphic columellar muscle of *P. lewisii* is derived exclusively from part of the second-formed muscle, which is comparable to the second-formed pedal muscle system in the vetigastropod. I conclude that the post-metamorphic columellar muscle of *P. lewisii*, is not homologous to the larval velar retractor of the vetigastropod, *H. kamtschatkana*.

Keywords: Gastropoda; larva; programmed cell death; muscle; heterochrony; 3D reconstruction

1. INTRODUCTION

The relationship between development and evolution has been a subject of speculation since the last century and is currently an area of much renewed interest (Atkinson 1992; Gilbert *et al.* 1996; Hall 1992; Raff 1996). Altered development generates structural novelties that are the raw material for evolution, but the construction of novelties must be successfully integrated into conserved aspects of the developing body plan (Müller & Werner 1991). In order to understand how this occurs at the cellular, molecular, and genetic level, investigations of the developmental basis of evolutionary change must be built on accurate, comparative information about the dynamic form of developing structures.

Gastropod molluscs were the inspiration for one of the earliest proposals for evolution through developmental modification. Garstang (1929) suggested that a mutation affecting a pair of shell-attached retractor muscles in the larva of a pre-gastropod might have generated a twist in the larval body that established torsion, the defining characteristic of gastropods. Garstang's proposal later received empirical support from studies by Crofts (1937, 1955), although aspects of Garstang's theory and Crofts' data have been contested (Thompson 1967; Salvini-Plawen 1980; Bandel 1982; Pennington & Chia 1985; Page 1997*a,b*). Nevertheless, there is a widespread assumption

that torsion in early gastropods reversed the left–right positions of a bilateral pair of larval retractor muscles, which were developmental precursors of a pair of adult shell muscles. This premise underlies speculation about the evolutionary derivation of retractor muscles in extant gastropod groups (Fretter 1969; Haszprunar 1985, 1988), and of interpretations of muscles that left scars on fossils of early gastropods or gastropod relatives (Harper & Rollins 1982; Runnegar 1981). The premise also figures in the hypothesis of a paedogenic origin of caenogastropods from a vetigastropod-like ancestor (Fretter 1969).

Most members of the Caenogastropoda have a single retractor muscle, called the columellar muscle, that is attached to the central coiling axis (columella) of the shell (Fretter & Graham (1994), who also cite exceptions). According to current opinion, the caenogastropod columellar muscle is derived from a similar muscle in the larval stage, which in turn is a homologue of the post-torsional left retractor muscle in larvae of vetigastropods and patellogastropods (Fretter 1969; Haszprunar 1985, 1988). This interpretation sees correspondence between the caenogastropod columellar muscle and the muscle that Crofts (1937, 1955) implicated in the generation of ontogenetic torsion in vetigastropods and patellogastropods. Recently, however, ultrastructural studies have shown an ontogenetic sequence of two shell-attached retractor muscle systems in a vetigastropod (*Haliotis*

kamtschatkana) and a number of nudibranch gastropods (Page 1995, 1997b). A first-formed system of one or two retractor muscles is later supplemented by a second muscle system that projects mainly into the foot.

The present study asks: do caenogastropods also show evidence of two, sequentially initiated developmental programmes for shell-attached retractor muscles? Observations on feeding larvae of *Polinices lewisii* (Gould, 1847) show that this is the case. Furthermore, the adult columellar muscle of *P. lewisii* is derived from only a portion of the second-formed larval muscle. The first-formed muscle, which is morphologically similar to the muscle implicated in ontogenetic torsion in vetigastropods (the post-torsional left retractor muscle), is not retained into the post-metamorphic stage of *P. lewisii*.

2. MATERIALS AND METHODS

Egg masses of *Polinices lewisii* were collected from the south-east end of Vancouver Island on the Pacific coast of Canada. The planktotrophic larvae were cultured at 20–22 °C according to the method of Pedersen & Page (1998). Development stages were anaesthetized, fixed, and embedded in Epon resin as described by Page & Pedersen (1998).

Larvae were fixed at hatching and at 6, 9, 14, 19, 25 and 35 d post-hatching. A metamorphic stage and juveniles at 8 d after metamorphic loss of the velum were also fixed. Sections of either 0.75 or 1 µm thickness were stained with Richardson's stain (Richardson *et al.* 1960), and examined by light microscopy. Thin sections were stained with uranyl acetate and lead citrate and examined with a Hitachi 7000 transmission electron microscope (TEM).

Computer-aided three-dimensional (3D) reconstructions of nerve, muscle, and gut components in transversely sectioned larvae fixed at hatching and at 25 d post-hatching were generated using Jandel's PC-3D software. The reconstructions of a hatching larvae shown in figure 1c–e were prepared from sequential sets of both thick and thin sections. Each set consisted of two thick sections of 0.75 µm each, which were cut with a diamond knife and stained for light microscopy, followed immediately by six thin sections of 80 nm each. The thin sections were examined with a TEM to provide unambiguous identification of nerve and muscle components in the previous pair of thick sections. The second thick section of each set was photographed, nerve and muscle components were identified by consulting electron micrographs of the thin sections, and the photographs were aligned by reference to two parallel nicks in one side of the Epon resin block; this side was trimmed so that it was parallel to the long axis of the transversely sectioned larva (a modification of the technique of Page (1997a)). Profiles of selected anatomical components within each photograph were traced with a digitizing tablet. The interval between the stacked section profiles of a hatching larva shown in figure 1c–e, is 2 µm and the reconstructions are derived from a total of 65 traced sections. It was not necessary to use the TEM to identify nerve and muscle components in histological sections of 25-day-old larvae. Reconstructions of the 25-day-old larva shown in figure 1f–h were prepared from tracings of anatomical profiles in every tenth section cut at 1 µm thickness; the interval between adjacent structural profiles is therefore 10 µm and data from a total of 43 sections are shown.

3. RESULTS

(a) Overview of larval anatomy and development

Larvae of *Polinices lewisii* consist of a cephalopodium and visceropallium. The cephalopodium of hatching larvae includes a small foot and head, the latter bearing the mouth and two-lobed velum (figure 1a). The ciliated velum is a larval structure that, in *P. lewisii*, enables both swimming and capture of microalgae. The visceropallium includes most of the digestive tract encased within a sac of mantle epidermis that secretes a calcareous, external shell. An in-pocketing of mantle epithelium forms the mantle cavity, which contains the anus and osphradium.

During the larval stage, the foot and mantle cavity enlarge greatly and each velar lobe subdivides to produce a velum with four long arms (figure 1b). The growing shell coils asymmetrically such that the first-formed shell whorls protrude as a low spire from the right side of the body (orthostrophic coiling). Metamorphosis can be induced at 35 days post-hatching by sand collected from the adult habitat (Pedersen & Page 1998).

All larval and post-metamorphic stages of *P. lewisii* are able to retract the cephalopodium into the shell, and an operculum on the back of the foot closes the shell aperture. The withdrawal behaviour is effected by two shell-attached muscles, one developing before the other. I will use the terms 'larval retractor muscle' (LRM) for the first-developed muscle and 'pedal muscle' for the later-developing muscle. Reconstructions of the LRM in a hatching larva are shown in figures 1c–e. Reconstructions of the retractor muscle system in a 25-day-old larva are shown in figures 1f–h.

(b) Larval retractor muscle (LRM) in hatching larvae

The 15 muscle cells of the LRM in hatching larvae are anchored on the shell via specialized mantle epithelial cells. The muscle trunk is attached on the left side of the bottom of the shell, from where it extends anteriorly along the left side of the stomach and lower oesophagus. Individual muscle cells then spread out around the mid-oesophagus (figure 2a) and insert onto the head and mantle. Insertions are onto epithelial basal laminae or on connective tissue investing other muscle cells.

Six muscle cells of the LRM reach to the dorsal part of the head (figure 1c). Cells 1 and 2 insert onto the dorsomedial edges of the right and left velar lobes. Cells 3 and 4 insert onto intrinsic muscles extending into the left velar lobe, whereas cells 5 and 6 reach beneath the oesophagus to insert primarily onto intrinsic fibres extending into the right velar lobe. A branch of cell 5 also inserts onto the ventrolateral mantle epithelium. As cells 5 and 6 reach towards the right side, they pass through the contorted loop of the visceral connective (figure 1c), a nerve tract that links some of the major ganglia of the nervous system.

Muscle cells 7 and 8 travel together over the oesophagus to insert onto the duct cell of the right protonephridium and on the sensory osphradium (figures 1d, 2a), which are both associated with the epithelium lining the mantle cavity. The duct cell of the left protonephridium is the main insertion target of muscle cell 9 (figures 1d, 2a).

Figure 1. (a) Sketch of a hatching larva of *Polinices lewisii*, left ventrolateral aspect. (b) Sketch of a larva competent to metamorphose, right lateral aspect. Sketches are not drawn to same scale. (c)–(e) Reconstructions of LRM tracts (blue; numbers identify muscle cells as described in the text), nervous system (red; pedal and osphradial ganglia omitted), and oesophagus (green) in hatching larva of *P. lewisii*; one profile of the velum is shown in brown. (c) Dorsal view showing dorsal head tracts. (d) Dorsal view showing mantle tracts; duct cells of protonephridia in yellow. (e) Ventral view showing left lateral oesophageal tract (cells 10 and 11) and ventral tracts (cells 12–15). (f)–(h) Reconstructions of retractor muscle in a 25-day-old larva; colour coding as in the hatching larva. (f) Dorsal view showing dorsolateral velar tracts. (g) Ventral view showing ventromedial and ventrolateral tracts of the pedal muscle. (h) Ventral view showing pedal tract of pedal muscle. Abbreviations: col, columellar attachment site of pedal muscle; dl, dorsolateral velar tracts; es, oesophagus; f, foot; lcg, left cerebral ganglion; pe, pedal tract; pr, protonephridium; rcg, right cerebral ganglion; sbg, sub-oesophageal ganglion; sh, shell; spg, supra-oesophageal ganglion; vc, visceral connective; ve, velum; vl, ventrolateral velar tract; vm, ventromedial velar tract.

The remaining muscle cells of the LRM, like cells 5 and 6, pass dorsal to the sub-oesophageal limb of the visceral connective (figures 1e, 2b). Cells 10 and 11 insert onto the left side of the distal oesophagus, medial to the left cerebral ganglion, but there are no counterparts on

the right side of the oesophagus (figure 1e). Muscle cells 12 and 13 run into the protruding ventral lip of the mouth (figures 2e,g), where they insert onto connective tissue investing a concentration of intrinsic muscle fibres in this area (figure 2h). As noted previously by Fretter

Figure 2. (a) Transverse section through a hatching larva of *P. lewisii* showing muscle cells (not all cells are numbered) of LRM distributed around the mid-oesophagus; boxed area is shown in figure 1b; orientation arrows indicate dorsal (D) and right (R) sides. Scale bar, 20 μ m. (b) TEM of boxed area in figure 1a, but photographed from a neighbouring thin section; note muscle cells 11–14 of the LRM passing dorsally to sub-oesophageal portion of the visceral connective (arrows). Scale bar, 5 μ m. (c) TEM of degenerating muscle cell from a nine-day-old larva. Scale bar, 2 μ m. (d) Longitudinal section through far left side of a six-day-old larva showing attachment plaque (arrow) of the LRM on a non-columellar area of the shell. Scale bar, 40 μ m. (e) Longitudinal mid-sagittal section through a six-day-old larva; large arrow indicates the site shown in figure 1f; boxed area contains a portion of both the LRM and the forming pedal muscle, which are shown at a higher magnification in figure 1g. Scale bar, 40 μ m. (f) TEM of attachment junctions (arrows) between a pedal muscle cell and mantle epithelium underlying the columella. Scale bar, 0.5 μ m. (g) TEM of boxed area in figure 1e; but photographed from a different specimen. Cells of developing pedal muscle (large arrows) pass outside the sub-oesophageal portion of the visceral connective; cells of the LRM (arrowheads) pass inside this connective and extend to intrinsic muscles within the ventral lip of the mouth; asterisk indicates a dying muscle cell. Scale bar, 10 μ m. (h) Enlargement of the distal end of the LRM fibre (asterisk) from figure 1g showing its connection with intrinsic muscles within the ventral lip. Scale bar, 2 μ m. (i) TEM of visceral connective passing between cells of the pedal muscle and LRM. Scale bar, 2 μ m. Abbreviations: e, mantle epithelium; es, oesophagus; f, foot; i, intestine; im, intrinsic muscles; li, ventral lip of mouth; lrm, LRM; mc, mantle cavity; mo, mouth; os, osphradium; pm, pedal muscle; pp, propodium of foot; pr, duct cell of protonephridium; sc, secretory cells; st, stomach.

(1972) in a number of other caenogastropod larvae, many intrinsic muscle fibres radiate from the ventral lip ('mentum') into the foot and ventral margin of the velar lobes. Muscle cells 14 and 15 extend to the left and right sides, respectively, of the junction between the foot and head, where they connect with a bilateral pair of cephalopedal muscles that extend from the apex of the head to the epithelium underlying the operculum. A similar set of cephalopedal muscles has been illustrated by Fretter (1972) for larvae of the caenogastropod, *Lacuna vincta*.

(c) *Remodelling of the LRM and differentiation of the pedal muscle*

Programmed cell death begins to modify the LRM soon after hatching. Destruction begins with cells 14 and 15, which are absent or degenerating in nine-day-old larvae (figure 2c). In addition, the trunk of the LRM migrates to the forming columella of the growing shell. Although the muscle trunk is still attached to a non-columellar region of the shell in six-day-old larvae (figure 2d), by nine days all surviving LRM cells are anchored on the presumptive columella. The distance travelled by the attachment plaque of the LRM is not great because the original attachment is on the left side of the hatching shell, close to where the initial body whorl overlaps the shell umbo. The inner surface of this overlap on the left side of the hatching shell is the apex of the future columella.

In hatching larvae, a few cells of the future pedal muscle have acquired clusters of myofilaments that are visible in electron micrographs. By six days, the pedal muscle is obvious in sections prepared for either light or electron microscopy (figures 2e–g,i), and the cells are attached to mantle epithelium underlying the forming columella (figure 2f). Therefore, for a short period before the LRM has transferred to the columella, the attachment plaques of the LRM and pedal muscle are clearly separate.

Myosin filaments within LRM cells are shorter than those within the cells of the pedal muscle, although accurate measurements from thin sections are difficult when filaments are long. A mean value of 1.3 µm (coefficient of variation 11.4%) was obtained for ten, apparently intact, myosin filaments photographed from random locations within the LRM of 9- and 19-day-old larvae. By contrast, myosin filaments of 4.5 µm were routinely observed within cells of the pedal muscle. Furthermore, alignment of Z-bodies within LRM cells produces a striation pattern, whereas striations are not obvious in pedal muscle cells (compare figures 3a and 3b). A possible exception to these generalizations about the fine structure of LRM and pedal muscle cells is described below.

The pedal muscle consists of three unambiguous tracts: a pedal tract and two ventral velar tracts. The large pedal tract (figure 1h) inserts on epithelium underlying the operculum and on intrinsic muscle fibres within the foot (figure 3c). The ventromedial velar tract (figures 1g, 3d,e) extends to the mid-ventral lip of the mouth, where it connects with intrinsic velar fibres running into the two ventral arms of the velum. The ventrolateral velar tract (figures 1g, 3d,e) subdivides distally and the branches extend processes to the developing, post-metamorphic buccal mass and to the left and right side of the ventral

lip, where they connect with intrinsic fibres extending into the ventral velar arms. All three of the pedal muscle tracts travel ventral to the sub-oesophageal portion of the visceral connective (figures 1g,h, 2i), which distinguishes them from all LRM cells in this region (figure 1e, 2g,i).

In 19-day-old larvae, the ventral velar tracts of the pedal muscle coexist with cells 2, 10, 11, and 13 of the LRM, as determined by muscle cell insertion sites and trajectories relative to the sub-oesophageal portion of the visceral connective (figure 3d). However, by 25 days post-hatching, when the larval phase is two-thirds completed, these LRM cells are absent (compare figure 3d and 3e).

A pair of dorsolateral velar tracts, each consisting of many muscle cells, is present in older larvae (figures 1f, 3c). However, it is unclear if these are elaborations of the LRM muscle or if they are components of the pedal muscle developmental programme. Cells 3 and 4, and cells 5 and 6, of the LRM in hatching larvae have trajectories that are identical to the many-fibred, dorsolateral velar tracts on the left and right sides, respectively, of 25- and 35-day-old larvae (compare figure 1c and 1f). If the dorsolateral tracts are indeed elaborations of the two LRM tracts, then these are the only LRM tracts that acquire additional muscle cells during development. Myosin filament lengths and arrangement of Z-bodies within muscle cells of the dorsolateral tracts are similar to those within LRM cells.

Finally, as the shell grows by orthostrophic coiling, the columella, with its attached pedal muscle, is rotated out of the right side of the body whorl. Concurrently, the shell attachment of the enlarging pedal muscle spreads along the ventral lip of the shell from the right-sided columella to the far left side of the shell aperture (figures 1f and 1g). The anatomy of the retractor muscle in 25-day-old larvae, as shown in the reconstructions in figure 1f–h, remains the same until metamorphosis, although additional fibres are added. This muscle does not have well-defined tracts into the mantle, but its fibres connect with an extensive subepidermal plexus of muscle fibres within both the mantle and the cephalopodium.

(d) *Metamorphic remodelling*

In conjunction with metamorphic loss of the velum, the four muscle tracts extending to the velar lobes in late-stage larvae are almost entirely destroyed. In sections through metamorphosing larvae, the four velar tracts are replaced by four masses of degenerating tissue (figure 3f). Disorganized muscle fibres are distinguishable within these masses, which may also include degenerating epithelium and intrinsic muscles of the velum. A small group of fibres that extends anteriorly in conjunction with the ventrolateral velar retractor, and which inserts onto the differentiating buccal mass of the post-metamorphic stage (figure 3f), is retained through metamorphosis together with the pedal tract.

4. DISCUSSION

Two retractor muscles are constructed during the development of *Polinices lewisii*: the LRM and the pedal muscle. The post-metamorphic columellar muscle is derived from a portion of the larval pedal muscle only. This portion consists of the pedal tract and a small

Figure 3. (a) TEM showing myofilament arrangement in LRM cells. Scale bar, 0.5 μm . (b) TEM showing myofilament arrangement in pedal muscle cells. Scale bar, 0.5 μm . (c) Histological longitudinal section through a 19-day-old larva of *P. lewisii*; note dorsolateral velar tract and the large pedal tract extending into the foot and inserting onto the operculum. Scale bar, 50 μm . (d) Transverse section through the sub-oesophageal portion of the visceral connective in a 19-day-old larva showing dorsolateral velar tracts, ventromedial velar tract, ventrolateral velar tracts, and the pedal tract; muscle cells 2, 10, 11, and 13 of the LRM are still present. Scale bar, 30 μm . (e) Transverse section through sub-oesophageal area of the visceral connective in a 25-day-old larva showing the same muscle tracts as labelled in figure 1d, but the LRM cells are absent; arrowheads indicate haemocytes. Scale bar, 30 μm . (f) Transverse section through a metamorphic stage showing four degenerating tissue masses (asterisks). Arrowhead indicates a small bundle of muscle fibres that is retained through metamorphosis. Scale bar, 75 μm . Abbreviations: cg, cerebral ganglion; dl, dorsolateral velar tracts; eg, oesophageal gland (rudiment); es, oesophagus; mc, mantle cavity; op, operculum; pe, pedal muscle tract; plg, pleural ganglion; pg, pedal ganglion; r, radular sac; sbg, subintestinal ganglion; vc, visceral connective; vl, ventrolateral muscle tract; vm, ventromedial muscle tract.

bundle of fibres that inserts on the post-metamorphic buccal mass. The first-formed LRM makes no contribution to the post-metamorphic columellar muscle.

The developmental pattern of two sequential retractor muscle systems in *P. lewisii* is very similar to the pattern described in ultrastructural studies on a vetigastropod, *Haliotis kamtschatkana*, and on a number of nudibranch

gastropods (Page 1995, 1997b). The pattern also exists in the feeding larvae of two other caenogastropods, *Lacuna vincta* and *Nassarius mendicus* (L. R. Page, unpublished data). Furthermore, anatomical characteristics suggest that the LRM systems in these species are homologous, whereas the pedal muscle systems form a separate homologous group. In each case, the LRM develops first, has

very short myosin filaments, includes some fibres that pass through the visceral connective, has insertion sites within the head and mantle, and does not insert on epithelium underlying the operculum. The pedal muscle develops after the LRM, has longer myosin filaments, projects mainly into the foot where some fibres insert directly on sub-opercular epithelium, and does not penetrate the visceral connective (with the possible exception of the right dorsolateral velar tract in *P. lewisii*).

Despite the similarities between the LRM and pedal muscles of these gastropods, there are also some notable differences. For example, the LRM of *H. kamtschatkana* lacks a tract to the mid-ventral oral lip, and the LRM is divided into two trunks having different attachment sites on the shell. In addition, there are differences between *P. lewisii* and nudibranch gastropods regarding the timing of LRM destruction relative to metamorphosis. In *P. lewisii*, the LRM is destroyed gradually and this begins soon after hatching. In the nudibranch larvae that have been studied ultrastructurally, the LRM tracts survive throughout the larval phase until they are destroyed at metamorphosis (Bonar & Hadfield 1974; Page 1995).

The Vetigastropoda, Caenogastropoda, and Heterobranchia (which includes nudibranchs) are major gastropod groups that have distinct fossil records dating from the Palaeozoic (Peel 1987; Tracey *et al.* 1993). The existence of two sequential programmes for retractor muscle morphogenesis in gastropods belonging to each of these groups suggests that the pattern is a basal and conserved characteristic of the gastropod *Bauplan*. It will be important to test this hypothesis by extending the ultrastructural analysis of muscle morphogenesis to other members of this large molluscan group. Furthermore, it might be possible to differentially characterize the LRM and pedal muscle systems using molecular markers or patterns of gene expression. Degnan *et al.* (1997) have recently constructed a probe for a muscle tropomyosin gene from *Haliotis rufescens*, and have characterized patterns of tropomyosin gene expression during the development of this species. However, expression was not studied prior to metamorphic competence, and possible expression in non-retractor muscle systems (e.g. within muscles of the developing buccal mass) requires clarification.

The retractor muscle system in late-stage larvae of *P. lewisii* has a similar anatomy to retractor muscles in late-stage larvae of several other caenogastropod species described by Fretter (1972). Fretter (1972) noted that the ventral velar tracts are destroyed during the metamorphosis of *Lacuna vincta* and *Crucibulum spinosum*, but some fibres extending to the subradular membrane of the buccal mass are retained. I observed similar metamorphic events for *P. lewisii*, but, in addition, the dorsal velar tracts in *P. lewisii* are also destroyed. However, the observation that the retractor muscle in late-stage larvae of at least one caenogastropod is mostly a different muscle than the retractor of hatching larvae has no precedent. Depending on the interpretation of the dorsolateral velar tracts in late-stage larvae of *P. lewisii*, the retractor muscle at this stage is either exclusively pedal muscle or a hybrid of the pedal muscle and part of the LRM. Regardless, the LRM and pedal muscle have temporally distinct

developmental programmes, they both undergo remodelling after their first appearance, and the LRM makes no contribution to the post-metamorphic columellar muscle.

Results of this study, if found to be widespread among the Caenogastropoda, have several important implications with respect to gastropod evolutionary theory. First, these data indicate that the columellar muscle of adult *P. lewisii* is not homologous to the larval muscle in *Haliotis kamtschatkana* (a vetigastropod) that has been implicated in generating ontogenetic torsion in other vetigastropods (see Page 1997b). The LRM system is absent in the post-metamorphic stage of *P. lewisii* and its homologue in *H. kamtschatkana* also undergoes extensive destruction during larval and metamorphic development.

Second, whereas ontogenetic torsion in *H. kamtschatkana* reverses the left–right positions of two LRM trunks, the pedal muscles in *H. kamtschatkana*, develop after ontogenetic torsion. I suggest that only the latter muscle system is homologous to the single pedal muscle in larvae of *P. lewisii*, which subsequently gives rise to the post-metamorphic columellar muscle. According to this interpretation, it is inappropriate to refer to the columellar muscle of *P. lewisii* as a post-torsional muscle, implying that it derives from a pre-gastropod muscle that resided on the opposite side of the body (see Haszprunar 1985).

Finally, results presented here and in ultrastructural studies on other gastropods erode the validity of a theory that invokes paedogenesis to explain the evolutionary emergence of caenogastropods from a vetigastropod-like ancestor (Fretter 1969). This theory is based on Crofts' (1937, 1955) suggestion that a post-torsional left larval retractor muscle in *Haliotis tuberculata* (a vetigastropod) develops before a post-torsional right muscle. Fretter (1969) interpreted the single larval and adult retractor muscle of caenogastropods as a homologue of the post-torsional left retractor in larval vetigastropods. She proposed that caenogastropods may have emerged as a persistent vetigastropod-like developmental stage, in which the right post-torsional shell muscle failed to form. However, the adult columellar muscle of *P. lewisii* is not homologous to the LRM (post-torsional left shell muscle) of *H. kamtschatkana*. In both these gastropods, the one or two adult shell muscles are formed from a muscle system that begins to acquire myofilaments only after the LRM system is fully differentiated. Unlike *P. lewisii* and most other caenogastropods, *H. kamtschatkana* has two adult shell muscles, but these muscles acquire myofilaments simultaneously during development. These data weaken the justification for the paedogenesis hypothesis, as formulated by Fretter (1969).

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