HYDROPHOBIC LARVAL SHELLS: ANOTHER CHARACTER FOR HIGHER LEVEL SYSTEMATICS OF GASTROPODS

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ABSTRACT

Higher level relationships within the Gastropoda are difficult to determine, in part due to the paucity of identified synapomorphic characters. Larval shell hydrophobicity may be a useful additional character for gastropod family systematics. A survey of 57 species indicates that larval shell hydrophobicity is common or ubiquitous in pyramidellids, opisthobranchs, and marine pulmonates but is unknown in patellogastropods, vetigastropods, and caenogastropods. The taxonomic distribution of hydrophobic larval shells is consistent with the hypothesis that it is a heterobranch synapomorphy. Unfortunately the condition in key 'lower' heterobranchs such as architectonicids and valvatoids is unknown.

INTRODUCTION

Malacologists have yet to come to a consensus on higher level relationships within the class Gastropoda (Bieler, 1992). Discerning relationships is difficult because derived characters shared among these many disparate groups are scarce. Considerable attention has recently been focused on the relationships among groups within the 'lower' heterobranchs: the architectonicids, pyramidellids, mathildids and others (Haszprunar, 1985, 1988; Ponder, 1991; Warén, 1993). These groups have traditionally been viewed as morphological mosaics of prosobranch and opisthobranch characters, but they also share several derived characters. The presence of a pigmented mantle organ (PMO), ciliated strips in the mantle cavity, chalazae in the egg masses, heterostrophic shells, and a unique osphradial type unite several families within this group and suggest relationships with opisthobranchs and pulmonates (Haszprunar, 1985, 1988; Ponder, 1991; Robertson, 1985; Warén, 1993).

Despite the clear monophyly of the heterobranchs, the relationships among 'lower' hetero-branchs are still not well resolved (Bieler, 1992; Mikkelsen, 1996; Wise, 1996). This low resolution is due to both the small number of putative homologies that are phylogenetically informative at this level, and inadequate available character information for many groups. Because anatomical characters traditionally used for gastropod systematics are often absent or not informative for lower heterobranchs (Mikkelsen, 1996; Wise 1996), it is important to investigate unconventional characters. Descriptions of gastropod embryology and larvae are beginning to yield such potentially useful characters for systematics (van den Biggelaar & Haszprunar, 1996; Robertson, 1985). Useful characters should be consistent within taxa, vary among taxa and should not be obviously functionally dependent on other characters used at the same level of analysis. Larval shell hydrophobicity may be one such unconventional systematically useful character.

Larval shells of many heterograstropods and opisthobranchs are hydrophobic (an unfortunate but standard term); in other words they are physically characterized by a surface that is water repellent. Because calcium carbonate is hydrophilic, hydrophobic shells are probably due to lipids and non-polar proteins in either the shell's organic matrix or the periostracum. As a consequence of this, larvae with hydrophobic shells frequently become trapped at the air-water interface in standing cultures and survival is significantly reduced unless measures are taken to break or reduce the surface tension. In order to explore the systematic usefulness of hydrophobic larval shells, I investigated its distribution among marine gastropods using a combination of my observations and published reports of larval hydrophobicity.

MATERIALS AND METHODS

I observed development type and larval shell hydrophobicity. Adult *Crepidula fornicata* (Linnaeus,

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1758) (Caenogastropoda) and *Boonea bisuturalis* (Say, 1822) (Pyramidellidae) were collected in the summer of 1993 at Woods Hole, Massachusetts, U.S.A. All other specimens were collected at San Juan Island, Washington, U.S.A. between 1994 and 1996. Eggs and egg masses or capsules were kept in small glass bowls at ambient sea temperature (8–12°C) until hatching. Larvae were reared in unstirred 0.45 μm-filtered sea water and fed a mixture of *Isochrysis galbana* and *Rhodomonas* sp. All observations were of live larvae of known parents. Larvae that became trapped at the air-water interface were counted as hydrophobic while those that were never stuck at the surface were not considered to be hydrophobic. When placed on a slide with a cover slip, larvae with hydrophobic shells also became trapped at the air-water interface along the edge of the water drop.

I also surveyed published accounts of larval shell hydrophobicity. Species were scored as hydrophobic if the author stated that veligers became trapped in the surface tension or if special methods of reducing the surface tension were necessary to successfully rear larvae. Unfortunately, many studies of larval development do not mention hydrophobicity or the specific conditions of larval culture and could not be used for this survey. Because hydrophobic shells cause higher mortality in cultures of long-lived feeding larvae than in ephemeral non-feeding larvae, studies of feeding larvae were more likely to mention hydrophobicity.

RESULTS

Larval shell hydrophobicity is clearly associated with taxonomic category (Tables 1 and 2; three-way G-test, $53.1 < G < 64.1$, df = 2, $p << 0.005$). None of the patellogastropod, veti-gastropod, or caenogastropod larvae had hydrophobic shells; neither those that I reared nor those described in published records. In contrast, it is clear from both my observations and published accounts of larvae (Table 1) that larval shell hydrophobicity is widespread among pyramidellids, opisthobranchs, and marine pulmonates. Although few non-feeding (4) or direct developing (2) opisthobranch larvae were included in the survey, hydro-phobic larval shells were not convincingly associated with larval type, independent of taxonomy (Table 2; three-way G-test; $0 < 0 < 11.7$, df = 1).

Microscopic observations of larvae in culture dishes and on microscope slides with cover slips showed that when hydrophobic larvae swim near the surface they become trapped with the shell extending above the water surface. Larvae of other species often swim at the surface but never become trapped. Additionally, dead larvae and empty larval shells of hydrophobic species were also hydrophobic, while shells of other larvae were not. Larvae that are not normally hydrophobic do, however, become trapped in the surface tension after being fixed in 10% formalin. These observations provide further evidence that larvae become trapped at the surface as a result of shell surface properties and not larval behaviour.

DISCUSSION

The distribution of hydrophobic larval shells among gastropods suggests that it is a derived character shared by pyramidellids, opistho-branchs and marine pulmonates. Its distribution is congruent with, but not identical to the distribution of pigmented mantle organs (PMOs), heterostrophic larval shells, ciliated strips in the mantle cavity, chalazae in the egg masses, and unique osphradial and sperm morphology (Haszprunar, 1985, 1988; Ponder, 1991; Robertson, 1985; Warén, 1993). Because a phylogenetic hypothesis that resolves the relationships among the 'lower' heterobranchs is not currently available, none of these putative homologies can be tested for congruence on a cladogram (Patterson, 1982). However our inability to demonstrate homology *a priori* does not preclude a character's use in phylogenetic analyses (Hennig, 1966).

Before larval shell hydrophobicity can be used as a character in a thorough phylogenetic analysis additional taxonomic sampling is necessary. The state of this character needs to be determined for other lower heterobranchs such as architectonicids and mathildids, and the representation of tropical species needs to be increased. Shell hydrophobicity and other such larval characters might also be useful in determining the problematic relationships of groups such as pteropods for which adult characters have not been particularly informative.

One of the consequences of hydrophobic larval shells is that larvae in laboratory cultures tend to clump together and become trapped in the surface film. Trapped larvae die unless they are quickly submerged. Amio (1955) thought that tiny air bubbles inside pulmonate and opisthobranch larval shells cause them to aggregate at the surface. However microscopic observations show that this is not true (pers. obs. and R. Robertson, pers. com.). Additionally, agents like cetyl alcohol that chemically reduce surface tension can be used to prevent larvae from becoming trapped.

Table 1. Systematic list of gastropod larval type and hydrophobicity.

Species	Larval type*	Hydrophobic	Reference†
Patellogastropoda			
Lottia digitalis (Rathke)	nf, p	n	
Lottia pelta (Rathke)	nf, p	n	
Tectura scutum (Rathke)	nf, p	n	
Vetigastropoda			
Calliostoma annulatum (Lightfoot)	nf, p	n	
Calliostoma ligatum (Gould)	nf, p	n	
Diodora aspera (Rathke)	nf, p	n	
Haliotis kamschatkana Jonas	nf, p	n	
Margarites pupillus (Gould)	nf, p	n	
Tegula funebralis (Adams)	nf, p	n	A. Moran, pers. com.
Caenogastropoda			
Crepidula dorsata Broderip	f, p	n	
Crepidula fornicata (Linnaeus)	f, p	n	
Fusitriton oregonensis (Redfield)	f, p	n	
Lacuna variegata Carpenter	f, p	n	D. Padilla, pers. com.
Lacuna vincta (Montagu)	f, p	n	
Littorina scutulata Gould	f, p	n	D. Padilla, pers. com.
Trichotropis cancellata Hinds	f, p	n	per. obs. and B. Perner, pers. com.
Pyramidelliae			
Boonez impressa (Say)	nf, p	y	White Kitting & Powell, 1985
Odostomia columbiana (Dall & Bartsch)	f, p	y	
Cephalaspida			
Acteocina canaliculata (Say)	f, p	y	Mikkelsen & Mikkelsen, 1984
Gastropteron pacificum Bergh	f, p	y	Hurst, 1967
Haloa rotundata (A. Adams)	f, p	y	Amio, 1963
Haminaea callidigenita Gibson	nf, p, b	y	
Haminaea solitaria (Say)	f, p	y	Harrigan & Alkon, 1978
Haminaea vesicula Gould	f, p	y	
Melanochlamys diomedea (Bergh)	f, p	y	
Philine aperta (Linnaeus)	f, p	y	Hansen & Ockelmann, 1991
Anaspida			
Aplysia brasiliana Rang	f, p	y	Strenth & Blankenship, 1978
Aplysia californica Cooper	f, p	y	Kriegstein, Castellucci & Kandel, 1974
Aplysia dactylomela Rang	f, p	y	Switzer-Dunlap & Hadfield, 1977
Aplysia juliana Quoy & Gaimard	f, p	y	Switzer-Dunlap & Hadfield, 1977
Aplysia parvula Guilding	f, p	y	Amio, 1963
Dolabella auricularia (Lightfoot)	f, p	y	Switzer-Dunlap & Hadfield, 1977
Stylocheilus longicauda (Quoy & Gaimard)	f, p	y	Switzer-Dunlap & Hadfield, 1977
Saccoglossa			
Elysis chlorotica (Gould)	f, p	y	Harrigan & Alkon, 1978
Elysia hedgpethi Marcus	f, p	y	
Nudibranchia			
Aeolidacea			
Berghia verrucicornis (Casta)	f, p	y	Carroll & Kempf, 1990
Hermissenda crassicornis (Eschscholtz)	f, p	y	
Tenellia pallida (Nordmann)	f(?), p	y	Eyster, 1979
Arminacea			
Janalus barbarensis (Cooper)	f, p	y	
Armina californica (Cooper)	f, p	y	Hurst, 1967

Table 1. (*Cont.*)

Species	Larval type*	Hydrophobic	Reference†
Dendronotacea			
Dendronotus sp.	f, p	y	
Doto amyra Marcus	nf, p, b	y	Goddard, 1996
Doto coronata (Gmelin)	f, p	y	Kress, 1975
Doto fragilis (Forbes)	f, p	y	Kress, 1975
Doto pinnatifida (Montagu)	f, p	y	Kress, 1975
Melibe leonina (Gould)	f, p	y	
Tethys fimbria Bohadsch	f, p	y	C. Mills, pers. com.
Tritonia diomedea Bergh	f, p	y	
Doridacea			
Adalaria proxima (Alder & Hancock)	nf, p	y	Todd, Bentley & Havenhand, 1991
Archidoris montereyensis (Cooper)	f, p	y	
Doridella obscura Verrill	f, p	y	Perron & Turner, 1977
Rostanga pulchra MacFarland	f, p	y	
Triopha catalinae (Cooper)	f, p	y	
Pulmonata			
Siphonaria gigas Sowerby	p	y	R. Emlet, pers. com.
Siphonaria japonica (Donovan)	p	y	Amio, 1963
Siphonaria palmata Carpenter	p	y	R. Emlet, pers. com.

* nf = non-feeding, f = feeding, p = planktonic, b = benthic.
†Personal observations unless otherwise noted.

Table 2. Summary of relationships between larval type and hydrophobicity.

	Hydrophobic		Not hydrophobic	
Taxon	Feeding	Non-feeding	Feeding	Non-feeding
Patello- and Veti-gastropod*	0	0	0	9
Caenogastropod	0	0	7	0
Opisthobranch and Pyramidellid	33	4	0	0

* Patellogastropoda and Vetigastropoda are combined here for statistical power and because their larval development shares some peculiar features not present in other gastropod groups.

The functional consequences of hydrophobic larval shells in nature are unclear. Cloney & Hansson (1996) suggest that hydrophobicity is actually disadvantageous to planktonic larvae because it causes them to become trapped by the surface tension and to stick to debris. They interpret the surface coverings of ascidian, polychaete and sipunculan larvae as hydrophilic adaptations to reduce the risk of becoming trapped by the surface tension. However, the presence of hydrophobic exoskeletons on other small marine invertebrates such as amphipods, barnacle cyprids, some infaunal bivalves and adult eulimids suggests that hydrophobicity is not universally detrimental. In fact Highsmith (1985) suggested that rafting in the surface film may be an effective dispersal mechanism.

The distribution of larval shell hydrophobicity among gastropods sheds little light on either of these ideas. If hydrophobicity is indeed detrimental to small planktonic animals, long-lived larvae like those of *Odostomia columbiana* Dall and Bartsch, 1907 with a planktonic period of over two months (Collin & Wise, in press.) might be expected to have reduced hydrophobicity. However I found no obvious relationship between planktonic period or larval type and hydrophobicity. Pyramidellids and opisthobranchs with long- or short-lived, feeding or non-feeding larval phases were all hydrophobic, while non-hetrobranch gastropods which encompass similar variation in larval types were never hydrophobic. It is also unlikely that becoming caught

in the surface film would greatly alter the dispersal potential of planktonic larvae, as it would benthic juveniles and adults. Because many opisthobranchs have internal adult shells and nudibranchs lose their shells entirely at metamorphosis, the effects of the larval shell on these life stages may be negligible. Surface hydrophobicity may also effect fouling of the shell since it has been shown to alter settlement preference of fouling organisms (Mihm & Banta, 1981). Before the ecological and evolutionary consequences of shell hydrophobicity can be evaluated further it will be necessary to determine if hydrophobic larvae get caught at the surface of the ocean or if natural turbulence or surfactants prevent this from happening.

A more refined measurement of hydrophobicity beyond simple presence or absence could increase the usefulness of this character. Hurst (1967) found qualitative variation in hydrophobicity among species and changes in the strength of hydrophobicity during develop-ment. These conclusions were based on her impressions of the propensity of the larvae to get trapped at the surface and the difficulty with which they were dislodged. Such changes may reflect differences in larval attributes other than hydrophobicity. For example, larval behavior may influence the frequency with which larvae contact the surface. Additionally, the force required to dislodge an object from the surface tension depends on its size and shape, both of which vary among species and change during development. Quantitative comparisons of hydrophobicities among species would be useful but hydrophobicity is extremely difficult to measure for surfaces the size of larval shells (Mittal, 1993; pers. obs.). An alternate approach to assay variation among taxa would be to determine the biochemical composition of larval shell matrix or periostracum for representatives of different clades (see Ghiselin et al., 1976 for an analysis of adult shell matrix).

Note: It has come to my attention that larvae of Epitonium albidum (Orbigny, 1842) have hydrophobic shells (Robertson, 1983). This is not the only developmental character that suggests heterobranch affinities for Epitonium: the egg capsules are connected by chalazae and the larvae have a pigmented mantle organ (Robertson, 1983). Perhaps a reevaluation of the systematic relationships and development of the Epitoniidae would be a fruitful area of research.

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