EVIDENCE THAT PETROMYZONTID LAMPREYS EMPLOY A COMMON MIGRATORY PHEROMONE THAT IS PARTIALLY COMPRISED OF BILE ACIDS

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Abstract—This study examined whether the larval pheromone employed by adult sea lamprey (Petromyzon marinus) to locate spawning streams and known to be at least partially comprised of bile acids is also employed by other lamprey species. Both production and release of lamprey-specific bile acids, and sensitivity to them were examined in a wide variety of species. High pressure liquid chromatography and electrospray ionization/mass spectrometry (ESI-MS) found gallbladders from 10 species of European and North American lamprey to contain large quantities of petromyzonol sulfate (PS) together with much smaller quantities of allocholic acid (ACA) and petromyzonol (P). Evaluation of holding waters from three of these species using ESI-MS found all to contain large quantities of PS and lesser quantities of ACA in similar ratios. Electro-olfactogram recording from the olfactory systems of three parasitic lamprey species found all to detect PS and ACA with high sensitivity. Behavioral studies using migratory adult sea lamprey found them to be attracted to the odors of heterospecific larvae as well as conspecific larvae, both of which contained similar amounts of PS and ACA. Finally, adult silver lampreys (Ichthyomyzon unicuspis) were also found to be attracted to the odor of larval sea lamprey. Together, these results demonstrate that PS and ACA are commonly produced and released by larval petromyzontid lampreys and likely used as part of a common evolutionarily conserved pheromone. This scenario is reasonable because lampreys share similar larval and spawning habitat requirements, and their larvae derive no apparent benefit from producing compounds that serve as an attractant for adults.

Key Words—Pheromone, migration, lamprey, Petromyzontidae, petromyzonol sulfate, allocholic acid, petromyzonol, evolution, species-specificity.

1For ease of discussion and to be consistent with existing literature on biliary compounds, we use the term “bile acid” to describe all biliary steroids even though some such as petromyzonol are alcohols.

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The sea lamprey, *Petromyzon marinus*, is an ancient cartilaginous fish that spends its larval life in freshwater streams, metamorphoses and emigrates into oceans or large lakes where it parasitizes other fish, grows and matures, and then migrates back into streams to spawn (Applegate, 1950; Purvis, 1980). Sea lampreys are dispersed great distances as parasites and make no attempt to return to their natal streams (Bergstedt and Seelye, 1995). Instead, a variety of evidence has shown that adult sea lamprey locate streams using innately recognized odorous cues, a critical component of which is a pheromone released by stream-resident larval lampreys (Sorensen and Vrieze, 2003). Not only are adults unable to locate streams efficiently if their olfactory sense is impaired (Sorensen and Vrieze, 2003), but the capture rate of adults entering streams correlates with the presence of larvae living in them (Moore and Schleen, 1980). Further, larval sea lamprey odor is attractive to conspecific adults at low, biologically relevant concentrations (Vrieze and Sorensen, 2001). It seems reasonable that adult sea lampreys have evolved to employ this strategy because the presence of larvae correlates with the presence of nursery habitat and by default, spawning habitat as well.

The sea lamprey migratory pheromone is known to be at least partially comprised of bile acids produced by larval conspecifics. Several studies have shown that larval sea lamprey produce and release significant quantities of the sulfated 24-carbon bile acid, petromyzonol sulfate (PS: 3α, 7α, 12α, 24-tetrahydroxy-5α-cholan-24-sulfate), and its probable precursors, allocholic acid (ACA: 3α, 7α, 12α-trihydroxy-5α-cholan-24-oic acid) and petromyzonol (P: 3α, 7α, 12α, 24-tetrahydroxy-5α-cholan) (Li et al., 1995; Polkinghorne et al., 2001). Enzyme-based high performance liquid chromatography (HPLC) suggests that larvae release PS at about three times the rate of ACA, which is released in greater amounts than P (Polkinghorne et al., 2001). Further, electrophysiological recording has shown that the adult olfactory system detects both PS and ACA with extreme specificity and sensitivity (picomolar thresholds; Li et al., 1995; Li and Sorensen, 1997). Petromyzonol does not appear important to the pheromone because its detection threshold is relatively high (about $10^{-8}$ Molar (M); Li and Sorensen, 1997). Lastly, and most importantly, migratory adult sea lampreys are attracted to low ($10^{-10}$ M) concentrations of mixtures of PS and ACA in both laboratory and raceway mazes although not as strongly as to larval holding water (Bjerselius et al., 2000; Vrieze and Sorensen, 2001). Recent HPLC fractionation studies of extracts from larval holding water suggest that there is at least one unknown component in the pheromone that is bile acid-like (Sorensen et al., 2003).

Lamprey pheromone systems are of interest for several reasons. First, the sea lamprey is a significant pest; it invaded the Great Lakes and is now the subject of a substantial control program that is exploring pheromones for use in control (Li et al., 2003; Sorensen and Vrieze, 2003; Twohey et al., 2003). Second, several