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Potential Multiple Functions of a Male Sea Lamprey Pheromone

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Introduction

The sea lamprey is a useful animal model for understanding the role of olfactory communication in reproduction (Teeter, 1980; Li *et al.*, 2003a). Our research has focused on characterizing the structure and function of pheromones that signal mating readiness in adults. Sexually mature sea lampreys are virtually blind (Manion and Hanson, 1980), cease feeding (Larson, 1980) and likely coordinate mating and spawning activities through chemical cues (Li *et al.*, 2003a).

Previous studies have indicated that both male and female sea lampreys release pheromones to attract individuals of the opposite sex (Teeter, 1980). We reasoned that the male pheromone would be technically advantageous to isolate and identify. This pheromone has to be released in a large quantity to be effective because males move into the spawning grounds first and then release a pheromone to guide the females to their nests (Teeter, 1980), which are often built in rapids where flow rates range between 0.5 and 1.5 m/s (Manion and Hanson, 1980). Further, identification of a male sea lamprey pheromone could provide a model system for studies of male pheromone signaling in fish. Several hormonal pheromones have been identified in female teleosts but not in males (for a review, see Stacey and Sorensen, 2002).

Structure and function of male pheromone compounds

Two novel bile acids, 7α,12α,24-trihydroxy-3-one-5α-cholan-24-sulfate (3kPZS; Li *et al.*, 2002; Yun *et al.*, 2002) and 7α,12α-dihydroxy-5α-cholan-3-one-24-oic acid (3kACA; Yun *et al.*, 2003), have been postulated to be components of the male pheromone based on several lines of evidence (Li *et al.*, 2003a). First, these two compounds are released only by male lampreys after the onset of spermiation (Yun *et al.*, 2002, 2003; Siefkes *et al.*, 2003b) and it is only spermiating males that releases the male pheromone (Li *et al.*, 2002; Siefkes *et al.*, 2003a,b). These results corroborated previous findings that sexually mature males release a pheromone that attracts sexually mature females (Teeter, 1980) and that water washings from spermiating males are more stimulatory to adult olfactory organs than those from pre-spermiating males (Bjerselius *et al.*, 1996).

Secondly, when measured with electro-olfactogram (EOG) recording, mature female sea lampreys detect 3kPZS and 3kACA at concentrations as low as 10^{-12} and 10^{-10} molar, respectively (Siefkes and Li, 2004). Thirdly, at $\sim 1.7 \times 10^{-10}$ molar, 3kPZS induces robust preference and search behaviors in ovulated females placed in a two choice maze (Li *et al.*, 2002). These behaviors are the same as those induced by washing of spermiating males (Li *et al.*, 2002; Siefkes *et al.*, 2003a,b). In a natural spawning stream, it appears that 3kPZS induces upstream movement at an even lower concentration (Siefkes and Li, unpublished). Evidently, 3kPZS is the main component of the pheromone that is released by spermiating sea lamprey males to attract ovulated females to their nests (Li *et al.*, 2002).

Another male bile acid, 3kACA, may also be a component of the male pheromone (Li *et al.*, 2003a; Yun *et al.*, 2003). The timing of 3kACA release is synchronized with 3kPZS (Yun *et al.*, 2002; Siefkes *et al.*, 2003b). The exact role of this compound has yet to be determined. Our current hypothesis is that 3kACA may regulate the reproductive endocrine system to promote sexual maturation in conspecific individuals and thus augment the behavioral responsiveness of adult females to 3kPZS. There is also evidence indicating that 3kCAC functions as a minor component to induce search behaviors typically induced by 3kPZS in ovulatory females (unpublished results).

Production and release of 3kPZS and 3kACA

It seems that 3kPZS and 3kACA may have evolved as specialized pheromone signals (Li *et al.*, 2002, 2003a). They are not needed for lipid digestion because adult lampreys do not feed (Larson, 1980). Yet, spermiating male hepatocytes contain higher levels of immunoreactive 3kPZS and 3kACA than pre-spermiating male hepatocytes (Siefkes *et al.*, 2003b). Since adult lampreys do not have bile ducts or gall bladders (they are lost during transformation; Youson, 1985), bile acids are not likely released directly into intestine; rather, they are probably transported via the bloodstream to kidney and gills where they are released into the water. Our experiments indicate that only washings from the anterior portion of the male lamprey are immunoreactive for male bile acids, whereas urine contained only a trace amount of 3kPZS (Yun *et al.*, 2002). In addition, washing collected directly off the gills contained male bile acids and pheromone potency (Siefkes *et al.*, 2003b).

The selection pressure to produce a signal that can be detected from a great distance in rapidly flowing water may have favored the evolution of a bile acid derivative as a pheromone—as opposed to the gonadal hormones that commonly act as pheromones in teleost fish (Stacey and Sorensen, 2002)—since bile acids can be produced in relatively large quantities due to the synthetic efficiency of the liver (Li *et al.*, 2002). This gill-based release route is potentially very efficient and direct because the hepatic veins carry blood directly to the heart and because all the blood from the heart goes through the gills. However, sulfated steroidal compounds typically are excreted through the urinary tract and only unconjugated compounds are shown to diffuse through gill epithelium (Vermeirssen and Scott, 1996). Clearly, a mechanism other than passive diffusion must have had to evolve in lampreys to excrete a sulfated bile acid, 3kPZS, into water via their gills. Male sea lamprey gills develop profuse glandular cells with secretory papillae during spermiation (Siefkes *et al.*, 2003b). These cells were first found in European river lampreys (*Lampetra fluviatis*) and speculated to excrete 'sex substances' (Pickering, 1977). We have shown that in the sea lamprey, the 'sex substances' include 3kPZS and 3kACA (Siefkes *et al.*, 2003b). The molecular mechanism for active transportation of male bile acids through the gills has not been elucidated. Further, synthetic pathways for 3kPZS and 3kACA have not been studied.

Potential application of the male lamprey pheromone

A major impetus that drives the quest for identifying sea lamprey pheromones has centered on their potential applications in lamprey management (Teeter, 1980; Li *et al.*, 2003b). The sea lamprey is an invasive species in the Laurentian Great Lakes of North America where it has caused dramatic and undesirable changes throughout the fish community (Smith and Tibbles, 1980). Our results to date indicate that interference with the male bile acid pheromone system offers an attractive target for selective and environmentally benign control of the sea lamprey. A recent study indicates that traps baited with spermiating males capture up to 87% of the ovulating females, whereas empty traps and traps baited with non-spermiating males capture no females (Johnson *et al.*, 2004). This study confirms a previous report of the 'infallible' practice of French fishermen who use male lampreys to bait traps to capture females (Fontaine, 1938). Whether synthetic 3kPZS and 3kACA can replicate the male washings in trapping ovulatory females has not yet been determined. None the less, 3kPZS has been found to induce female behaviors similar to those induced by spermiating male washings (Li *et al.*, 2002).

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