4.05 Pheromones in Vertebrates

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4.05.1 Introduction

4.05.1.1 Definition of a Pheromone

About 50 years ago, Karlson and Lüscher coined the term 'pheromone' to describe chemicals that are 'excreted to the outside by an individual and received by a second individual of the same species in which they release a specific reaction.'[1] They created this term in conjunction with the then recent structure elucidation of 10*E*, 12*Z*-hexadecadien-1-ol (bombykol), a long-chain 16-carbon alcohol that female silkmoths (*Bombyx mori*) produce to attract mates. Notably, the concept is a biological one. Since that discovery many hundreds of structures with similar types of activity on conspecifics (members of the same species) have been identified, and it is now commonly accepted that most animals use chemicals to mediate a wide variety of intraspecific social interactions. Many hundreds of invertebrate pheromones have been identified. However, in spite of compelling evidence that with the possible exception of birds, vertebrates commonly use chemical cues to communicate (exchange information) with conspecifics, only a handful of vertebrate pheromones have been identified. The complication seems to be related to the biological complexity of vertebrates whose large brains and complex sensory systems allow them to process more complex cues and use them in complex and subtle manners that are difficult for biologists to measure. Indeed, many mammalian pheromones might best be considered as subtle modulators, rather than drivers, of behaviors. Thus, the original definition of a pheromone has been frequently revisited and many revisions suggested;[2–4] however, no new definition has gained acceptance and the term, pheromone, is now commonly used to describe almost any chemical cue that mediates information transfer between members of the same species and to which organisms are in some manner predisposed to respond. We use this broad definition here.

4.05.1.2 Biological Activity of Pheromones: Defining Principles

Pheromones are typically defined by the biological actions they induce, which may be either behavioral and/or physiological and need not always be immediate. To understand pheromones, one must understand their biological function. These functions are as diverse as the life histories of the species that use them. Generally, the actions of pheromones are species specific, although there are exceptions because the ecology of animals well-developed olfactory organ. The identity of the putative cues used by hagfish remains totally unknown. The first 'true' vertebrates were the Ostracoderms, a group of jawless cartilaginous fishes from which modern jawed vertebrates evolved. Lampreys are the only surviving members of this group and approximately 40 species exist today. One of these, the sea lamprey, has been studied for pheromonal function. This species relies heavily upon unique, sulfated steroids, as reviewed next.

4.05.2.1 Sea Lamprey

The sea lamprey (*Petromyzon marinus*) has a fascinating migratory life history.[14] It breeds in coastal freshwater streams where its males build simple nests, which females locate using pheromones. Both die a few days after mating. Surviving eggs hatch into filter-feeding, blind larvae that burrow in stream bottoms and grow at varying rates for 3–20 years before eventually metamorphosing into a parasitic form having eyes, a very developed nose, and a sucker-like rasping mouth. Parasitic phase lampreys leave their streams and enter the ocean (or large lakes in the case of landlocked populations) where they locate and prey on other fishes. Sea lampreys grow rapidly and mature within 1–2 years, before returning in the spring to streams to spawn (and die). The tasks of finding suitable spawning streams and then mates are essential and demanding processes. Both of these activities are mediated by potent, now well-understood, pheromones. Both the gender-specific sex pheromone and gender-neutral migratory pheromone systems have been the subject of considerable study because the sea lamprey is an invasive species in the North American Great Lakes. The sea lamprey invaded the Great Lakes about a century ago and the fisheries there have become severely threatened by this species.[15]

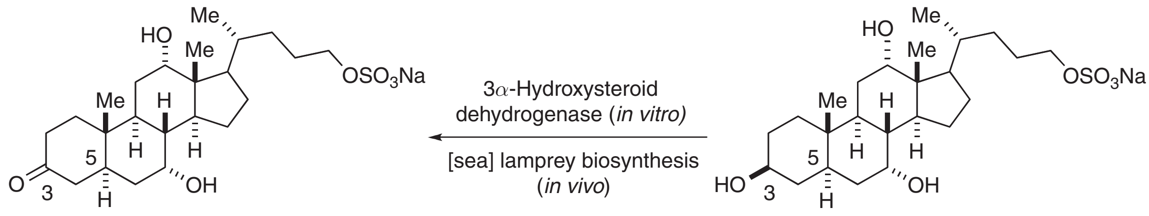
4.05.2.1.1 Male sex pheromone: Biology

During upstream migration, male and female sea lamprey undergo final maturation and develop behavioral responsiveness to the odor of mature conspecifics of the opposite sex during which time these cease responding to larvae and their odor.[16–19] Males build simple nests and females follow with spawning lasting only a few days. French lamprey fishermen, originally caught females by placing males into traps, a response that has recently been duplicated and attributed to pheromonal odors.[18] While male sea lamprey release attractants in their urine,[18] more recent work has focused on components released through the gill.[16] Using a fractionation scheme based upon EOG recording and Y-mazes, Li and coworkers discovered that male sea lamprey release a substance that is attractive to females. That new compound was shown to be a ketone and was named 3-ketopetromyzonol sulfate (3K-PS, **1**). EOG recording has shown that this steroid is detected by adult lamprey at approximately $10^{-12}\,\mathrm{mol\,l^{-1}}$ and that it is attractive to ovulated females in laboratory mazes and in streams.[20–22]

Behavioral studies suggest that 3K-PS is synergized by the presence of other steroidal odorants, perhaps including another ketone, 3-keto-allocholic acid. Immunoassay shows that nonspermiated males (whose odor does not attract ovulated females in the maze) do not release appreciable quantities of 3K-PS, whereas spermiated males release large quantities of immunoreactive 3K-PS. It is estimated that the quantity produced is sufficient to create a large active space (the volume of water that contains detectable concentrations of pheromone) of greater than $10^6\,\mathrm{l\,h^{-1}}$.[16,23] Not surprisingly, this cue is being explored for use in lamprey control in the Great Lakes.[24] Presently, the lamprey is the only vertebrate for which identified pheromones are being actively considered for managing wild animals.

4.05.2.1.2 Male sex pheromone: Chemistry

To isolate the putative male sex pheromone, the organic content of water conditioned by spermiating male sea lampreys was concentrated by passage through a C18 resin. Chromatographic purification, guided by EOG recording and mass spectrometry, provided a sufficient quantity of material for NMR analysis. Thereby the structure of 3K-PS, **1** was deduced. This was confirmed by preparation of an authentic sample of **1** through the action of 3α-hydroxysteroid dehydrogenase on petromyzonol sulfate (PS, **2**) (Equation 1). The latter was identified over 40 years ago as a major constituent of the larval sea lamprey bile.[25] It is interesting that, like many higher organisms, the sea lamprey uses a bile acid family consisting of allocholic rather than cholic acid (i.e., having a 5α- rather than 5β-hydrogen substituent) derivatives. PS (**2**) is the probable biosynthetic precursor to the male sex pheromone component 3K-PS (**1**), and it is also noteworthy that PS has been found nowhere else in nature other than in this group of ancient vertebrates.



1 3-Ketopetromyzonol sulfate (3K-PS) **2** Petromyzonol sulfate (PS)

(1)

4.05.2.1.3 Migratory pheromone: Biology

Migratory adult lampreys are now known to locate suitable riverine spawning habitat using a pheromone released by stream-dwelling larval lampreys. So important is this cue that lampreys cannot find suitable streams if their olfactory systems are ablated (blocked). Indeed, the vast majority of adult lacustrine lampreys select and then spawn in only a few streams, all of which have very high densities of larvae.[26] This ecological strategy makes good evolutionary sense for a species whose juveniles may become widely distributed and whose adults need to quickly locate suitable spawning/nursery habitat because the presence of such habitat correlates strongly with the presence (odor) of larval conspecifics living in it.[15] Although the presence of a migratory pheromone system in sea lamprey was first suggested by historical fisheries records in the Great Lakes, which showed that eradication of larvae from streams using poisons resulted in reduced adult migration, direct evidence has come from behavioral laboratory studies. These key studies have used mazes and natural waters into which larval odors have been added (**Figure 1**).[19] First, low but realistic concentrations of larval lamprey

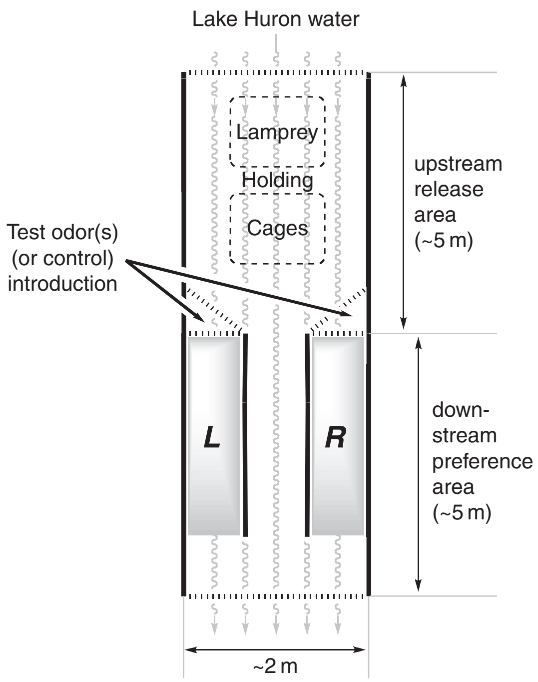


Figure 1 Schematic of the behavior maze used to guide isolation and identification of the sea lamprey pheromone.[19,27,28] Lake water mixed with a small amount of nonlamprey river water flows slowly from top to bottom at a depth of *c*.10 cm in this two-choice maze, where preference of adult lamprey is assessed by measuring the ratio of time in the right channel to time in the left (t_R/t_L), while odor is into one side or the other.