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How Sea Slugs Fall in Love

BY SCOTT CUMMINS

A sex attraction pheromone is like a mix between Chanel No. 5 and Viagra. The only catch is you have to be a sea slug.

The mating ritual of the sea slug is a peculiar affair. Occurring at the ocean depths, these soft-bodied sea creatures gather in their hundreds for what might be the finale to their short lives. But in doing so, they ultimately guarantee the continuation of their species.

In many ways life is about finding a mate, and humans aren't the only species in the animal kingdom to send love messages in an attempt to attract that perfect partner. My research has revealed that to get the party started, sea slugs have developed an ingenious and potent solution by releasing a powerful cocktail of attraction molecules.

It's a big world out there, and many lonely near-sighted creatures might never find each other were it not for powerful love messages in the form of sex attraction pheromones – chemicals released by one animal that prompt sexual behaviour in another. Pheromones play an important role in virtually all animals' sex lives, but normally we are completely unaware of their presence. However, thanks to scientific ingenuity and advances in analytical equipment, researchers have been able to eavesdrop and decode thousands of these once-secret attraction

We know most about pheromone communication in land-dwelling animals such as insects, rodents, swine, sheep, goats and cattle. How potent are they? A single female Bombay moth carries enough pheromone to lure 1 billion billion males at close range. Meanwhile, the silkworm moth can detect pheromones from other silkworms up to 11 km away.

Pheromones are also essential in the marine world. However, although the world's oceans are home to more than 80% of all living organisms, our knowledge of how marine animals use pheromones in this vast environment is relatively limited. While we recognise numerous pheromone-induced animal attraction behaviours, the precise chemical composition of these messages is often largely unknown. This is particularly concerning given that over 90% of all marine species dwell on the ocean bottom, where vision is at best limited and a single rock can be home to several major groups such as corals, crustaceans, molluscs and sponges. For many marine animals here, love is blind and pheromones must be essential.

The sea slug *Aplysia* (Fig. 1) is a typical

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example of how the survival of near-blind sea creatures relies almost exclusively on their sense of smell. This sleek, snail-like animal is a little denser than the ocean, about 30 cm long, and emits a purplish ink cloud if disturbed. Although they lack shells, *Aplysia* are in fact molluscs – related to scallops, oysters and snails. Of the 38 species of *Aplysia* found throughout the world, Australia is the home to several, where they can often be observed swimming around in seaside rock pools.

The decision to study marine attraction pheromones in the love-life of a sea slug may seem unusual at first, but several factors implicated them as the ideal animal for this type of study. Most important was that they are no stranger to researchers. Due to their simple nervous system and abnormally large brain cells, *Aplysia* have long been a favourite to brain scientists. Just ask Eric Kandel, who shared the 2000 Nobel Prize in Physiology or Medicine for his pioneering work using the sea slug species *Aplysia californica* as a model for how memories are formed in the human brain.

Like many animals that perceive the world through smell, sea slugs have a remarkably high percentage of brain cells devoted to its sniffing organs, which are located on the animal's antennae-like olfactory stalks. This accumulated knowledge made them a prime candidate for pheromone research, and the discovery of sea slug love.

The Sea Slug "Brothel"

One of the most bizarre sights in animal behaviour is the breeding aggregations formed by certain sea slugs. While they spend most of their year-long lives cruising the ocean alone, every summer a strange and mysterious assembly takes place – a ritual that has been going on for millions of years. This gathering normally leads to breeding aggregations called "brothels", in which dozens of individuals lay eggs, mate, and often trade sexual roles.

Sea slugs are hermaphrodites, and therefore it is physically possible for them to act as a male and female at the same time, both donating and receiving sperm. Typically this takes the form of mating chains (Fig. 2) that can last for days.

Sea slug sperm is released in an immature state, so the recipient animal must store transferred sperm until it is capable of fertilisation. Then, it mixes stored sperm with its own eggs and lays the eggs.

But exactly how these sea slugs signal each to other that it is time to gather remained a mystery for many years. One observation may have been the key to understanding this.

Prior to the gatherings, a single animal initially lays a spiralled egg mass of long, yellow strings, or cordons, containing millions of tiny (but sometimes unfertilised) eggs. It was thought that a potent pheromone was released precisely when they lay eggs, and this provokes these normally solitary and sexually self-sufficient creatures to aggregate and mate. Absolute proof of this could not be produced for many years.

Eventually, marine researchers Sherry Painter and Gregg Nagle of the University of Texas Medical Branch provided the proof by employing a T-maze behaviour test. The T-maze is an experimental maze shaped like the letter "T". The animal is placed in the base of the "T" and is allowed to explore the maze and choose to enter either the right or left arms.

This is often a slow and labour-intensive process but in time it was concluded that the sea slug egg mass had irresistible properties. Placed alone in a tank of seawater, it can draw a crowd of several dozen animals. Tests showed that a non-laying animal was not attractive while an egg-layer was insatiably attractive.

Using that clue to refine our search, the next several years of research focused on finding the substance that elicited this dramatic attraction response.

Decoding the Love Message

Attraction pheromones in terrestrial environments are typically composed of small, volatile fatty acids so that they can float in the air. In the marine environment, however, larger water-soluble molecules can be employed.

The difficulties associated with finding and analysing air-borne pheromones are also encountered in the marine environment. The most significant relates to low concentrations, contamination, or breakdown of the molecules prior to analysis. Fortunately, we knew exactly when the pheromones were being released – during egg laying – so these problems could be minimised.

First we collected the seawater surrounding freshly laid egg masses and then separated the molecules out of the mixture by liquid chromatography – a technique for separating components in a liquid mixture based on their size and shape. Going back to the T-maze, purified sample fractions had to be painstakingly tested individually to determine which fraction contained the attractive pheromone component.

Finally, after hundreds of animals were tested, one small protein revealed itself from the others and was subsequently named "attractin". While non-laying animals were normally not attractive, when attractin was given to them, as we

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may apply cologne, they became immediately attractive to other sea slugs.

Attractin marked the first discovery of a water-borne, protein-based pheromone in invertebrates. Tests revealed that attractin acts like a mix between Chanel No. 5 and Viagra. Not only did it induce attraction, but it was a powerful aphrodisiac to stimulate mating as a male.

The power behind attractin's potency became clearer following the revelation of its chemical composition and three-dimensional shape by nuclear magnetic resonance. Most proteins fold into a unique three-dimensional shape that determines their function, and attractin seemed perfectly shaped as a marine signalling molecule. Small molecules are often very susceptible to being broken down in the harsh conditions of the ocean, but attractin formed a highly resilient, soluble and compact structure that could efficiently move throughout this environment.

Our understanding of other pheromone systems, particularly those used by insects, told us that most attraction pheromones consist of a cocktail of molecules. Often, the addition of one or two components to single compounds vastly improve the species specificity of a chemical signal. A similar situation is found in mice, where two compounds combined in urine are essential for a functional male signal.

Other Pheromones

We quickly turned our attention from attractin to the task of finding whether other pheromones are important to sea slug attraction. It was not surprising for us to discover that the sea slug attraction message actually consisted of a cocktail of proteins.

To discover the other pheromones in the message, a slightly different approach was taken that initially involved genetic analysis followed by the T-maze test to again provide definitive proof of pheromone attraction activity. The final attraction message was subsequently decoded and found to consist of an additional three small proteins that we suitably named "enticin", "temptin" and "seductin".

In a surprising development, the individual proteins failed to attract. However, when combined with attractin, they form a powerful message that helps slugs get lucky (Fig. 3).

Attractin, enticin, temptin and seductin are each likely to play a slightly different role. One might be saying, "I'm an attractive sea slug looking for company," while another could be setting up a rendezvous.

Regardless, what's almost certain is that temptin binds to the other components to ensure that the message is protected and is efficiently and discreetly sent to the correct address – another sea slug.

Strangely, this message is rather promiscuous in that it can attract multiple species of *Aplysia*. However, upon meeting they realise their love can never be consummated and they avoid any cross-species mating.

Like other known attraction pheromones, these are effective at low doses and over large distances. For example, the sea slug pheromone attractin evokes robust mating behaviour at concentrations equivalent to one teaspoonful dissolved in an Olympic-sized swimming pool. That makes it more than 1000 times stronger than typical sex hormones coursing through the human bloodstream.

The Future

Sea slugs have been and will continue to be a rich source of information to enlighten us on the secrets of marine pheromone communication. But protein pheromones are probably used by many marine and freshwater animals for communication, and we have only just begun to identify them to exp ore their activities.

Now that we understand how attraction pheromones are used in sea slugs – what they are, how they detect them and how they influence their behaviours – we have begun to use this wisdom to find others that may enhance the management of similar marine animals. It may even resolve some puzzles with potentially lucrative implications, including the question of how scientists might persuade commercially important molluscs, such as cultured abalone, to spawn when their keepers want them to. A recent finding by our international research group has discovered that squid, although highly visual, can also communicate via protein pheromones.

Pheromones may also form the basis for powerful new tools to eliminate pest species. Already insect researchers use pheromones to eliminate insect pest species. In a similar context, coercing sexually active marine species away from potential partners could be an important application.

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Figure 3. The sea slug language of love – a rather promiscuous attraction message

Figure 2. Sea slug mating chains. The sea slug in front acts as the female to the one directly behind it. Photos: Anne DuPont (left), and Leanne and David Atkinson (right)

Figure 1. The sea slug *Aplysia* secretes a potent pheromone that initiates mating
Photo: Eva McClure