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ABSTRACT: Chemical ecology has grown as a scientific discipline from its earliest days of tracking the exquisitely potent chemistry of insect pheromones to a deep understanding of the molecular, physiological, and behavioral interactions governed by naturally occurring small molecules. The current practice of the field relies on knowledge of genomes and gene expression patterns, protein biology, and small-molecule chemistry, providing illustrations of ecological and evolutionary patterns in natural communities.

Some 65 years ago, one of us elucidated the structure of nepetalactone,[11] a novel monoterpene in the essential oil of the mint species, *Nepeta cataria*, commonly called "catnip" (Figure 1). This is a compound that intrigues not only domestic cats but also leopards, cougars, lynxes, lions, and tigers, inducing a variety of playful behaviors. The significance of these behaviors is unknown, and the plant/animal chemical interaction it represents remains a minor mystery. Nevertheless, the realization initially by biologists and much later by chemists that organisms can respond behaviorally as well as developmentally to small molecules emitted by other organisms has given rise to a highly diversified and fundamentally important body of science at the interface between chemistry and biology. We would like this perspective to encourage present and future colleagues to explore this subject of biotic chemical communication in new and exciting directions.

To introduce the subject about how organisms gain information about their environment, we might ask how we humans know what is going on around us? Most obviously, we look and we listen. We sense the properties of our environment through a small number of distinct channels. The information we gather plays a crucial role in enabling us to react adaptively to changing conditions. Which information-gathering channels are particularly influential in the lives of each species depends on their natural history. Although we depend heavily on sight and sound to learn about their environment, we are also sensitive to changes in ambient temperature and to information gathered by our sense of touch.

In addition to these, there are the chemical senses, taste and smell, which are unique because they depend on the generation, transmission, detection, and characterization of molecular entities. They are important to just about all species, from microbes to mammals. The study of how organisms interact chemically in nature has come to be called chemical ecology. This discipline is of importance to areas of human endeavor as diverse as medicine, agriculture, forestry, and environmental sustenance, but it is still in its infancy. Although few researchers consider themselves chemical ecologists, anyone working on bacterial quorum sensing (microbial chemical ecology[2]), the human microbiome, plant-fungal symbiosis, vector-borne diseases of plants or animals, insect defense mechanisms and pheromonal communication, the control of agricultural pests, bark beetle/forest relationships, red tides, and countless other chemical interactions in nature is contributing to this field.

Chemical ecology is a six-decade-old discipline[3] with roots two centuries old. On 6 May 1870, French naturalist Jean-Henri Fabre incarcerated a newly emerged great peacock female moth, *Saturnia pyri*, to discover, after a few experiments, that she produced a scent undetected by the human nose, which was a powerful attractant for males of the same species.[4] Soon after that, New York entomologist Joseph A. Lintner reached a similar conclusion while observing the spicebush silk moth, *Callosamia promethea*. He then envisioned a practical application for these scents and reached out to chemists for help: "cannot chemistry come to the aid of the economic entomologists in furnishing at moderate cost the odorant substances needed?"[5] It is highly unlikely that he was aware that bola spiders were already producing moth sex pheromones to attract male moths, the first pheromone-based attract-and-kill system.[6] Evidence also suggests that Sir John Ray noted that the female peppered moth, *Biston betularia*, attracted males with
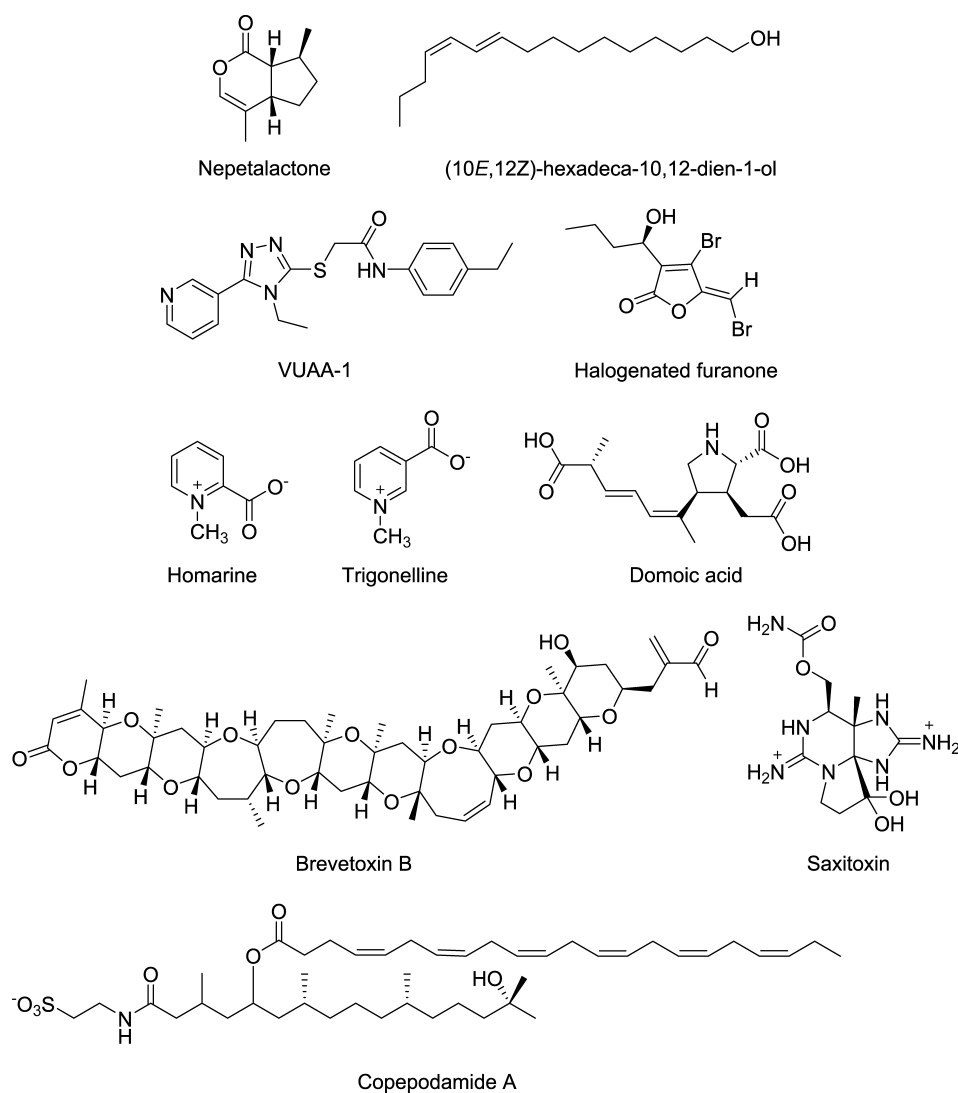
Figure 1. Sampling of molecules that function in ecological interactions among living organisms.

her scent.[77] Prior to Fabre's experiments, other pioneers in chemical ecology, including Ernst Stahl, had already suggested the importance of plant secondary metabolites in plants' interactions with the environment, including herbivorous insects. Their ideas were rejected by contemporary physiologists who dominated biological research at the time.[8] Science is neither a democratic process in which the majority wins, nor a popularity contest. It may take time to convert skeptics and for the minority to prevail.

Chemical ecology sensu stricto started when, after three decades of research, Adolf Butenandt unraveled the chemistry of the female scent from the silkworm moth that attracts males: (10E,12Z)-hexadeca-10,12-dien-1-ol (common name, bombykol; Figure 1).[9] After all, chemical ecology is the study of the chemical languages, cues, and mechanisms controlling interactions among living beings, including communication among individuals of the same species and between organisms and their environment.[3] Butenandt envisioned that unraveling the chemistry behind moth attraction would open an entirely new field of research. Because they have now embarked on "language studies", they first needed a dictionary to catalogue technical terms. Karlson and Butenandt[10] coined the term pheromone to describe a chemical signal like the silkworm

moth's scent that Butenandt had discovered. Then, they and others started adding more entries like semiochemicals, literally meaning chemicals that carry signal/excitement. The term pheromone refers only to semiochemicals involved in communication between individuals of the same species, but could be separated into alarm, aggregation, and sex pheromone. The cousin to the term pheromone, allelochemicals, refers to communication between different species. For example, a plant-derived compound may fend off herbivorous insects and, therefore, is named allomone because it benefits the producer (emitter). Flowers' semiochemicals that attract pollinators are called synomones because they benefit both emitter and receiver. A parasitoid may use a pheromone signal to find a host; thus, in this context, the semiochemical is called kairomone because it benefits the receiver and is disadvantageous to the emitter. The chemical vocabulary in chemical ecology built up rapidly. As with any other languages, the definitions have been refined over time. The accepted term for aggregation pheromone for sex procurement is now aggregation-sex pheromone.[1111]

On the advice of one of us and about a century after Lintner's appeal, the New York Agricultural Experiment Station (Cornell College of Agriculture) recruited a charismatic chemist,

Wendell Roelofs, and the State University of New York at Syracuse hired Robert (Milt) Silverstein to launch a chemical ecology program. Roelofs, Silverstein, and many other chemists throughout the world started deciphering at a large pace chemical communication among various agricultural pests, particularly moths, and helping economic entomologists develop new trapping, attract-and-kill, and mating disruption strategies. As chemical ecology gained momentum, evolutionary entomologists started accepting the work of Stahl and others and recognized the essential role of plant secondary metabolites in plant−insect interactions, thus broadening the field.[8]

As many sex pheromones were characterized and synthesized, physiologists, ethologists, and neurobiologists were excited to join and broaden the field of chemical ecology to study multiple facets of insect chemical communication. Neurophysiologists, first within the group of Dietrich Schneider, identified single cells in insect antennae that detect pheromone constituents and demonstrated how multiple chemical signals are integrated in the brain. Schneider developed the electroantennogram and single sensillum recording (SSR) techniques, which help unravel the remarkable sensitivity and selectivity of the insect's olfactory system.[12,13][12,13] When combined with gas chromatography (e.g., gas chromatography-electroantennographic detection (GC-EAD), GC-SSR), these techniques also facilitated the identification of new pheromone systems. In the 1970s, Schneider joined one of us (JM), Carl Djerassi, Thomas Odhiambo, and Koji Nakanishi to launch the International Centre of Insect Physiology and Ecology and to bring chemical ecology to the African continent. Subsequently, many chemical ecology centers of excellence were formed throughout the world, which are epitomized by the Max Planck Institute for Chemical Ecology founded in 1996. Thus, chemical ecology research has been performed on all continents, including Antarctica, wherever scientists can observe and access organisms for experimentation.

Using wind tunnels and synthetic pheromones, ethologists were able to dissect the intricacies of odorant-mediated navigation in insects. The uniqueness of the insect's olfactory system triggered the interest of biochemists and molecular biologists. Chemical ecology is now addressing the molecular basis of insect olfaction, although the pioneer of the "molecular basis" was indeed Butenandt. In the last three decades, we learned that the reception of pheromones and other semiochemicals in insects is mediated by olfactory proteins, particularly odorant-binding proteins (OBPs), which are also named pheromone-binding proteins (PBPs) when the odorant is a pheromone: odorant receptors (ORs), odorant-degrading enzymes (ODEs), ionotropic receptors (IRs), and sensory neuron membrane proteins (SNMPs).[14][14] The roles of IRs and SNMPS are still to be fully elucidated. OBPs and PBPs bind hydrophobic odorants and form binding protein−semiochemical complexes that help solubilize the odorants for proper transportation through the aqueous environment in sensilla in peripheral organs like antennae, maxillary palpi, and others. OBPs are essential for the sensitivity of the insect's olfactory system and may play a part in selectivity, although ORs are the ultimate gatekeepers of selectivity.[a] Once a signal is conveyed, it must be terminated rapidly, with ODEs assisting at least in part with inactivation via enzymatic degradation.

To identify semiochemicals for possible applications in agriculture and medical entomology, chemical ecologists apply bioassay-guided approaches for the isolation of active ingredients, which may be augmented with electrophysiological approaches, such as gas chromatography-electroantennographic detection (GC-EAD). Although GC-EAD may provide a "shortcut" to the active ingredients, a solid and consistent bioassay is still needed to avoid false-positives. A compound may generate an electrical signal and be behaviorally inactive. The explosive advancement in our understanding of the molecular basis of insect olfaction in the last two decades opened the door for reverse chemical ecology, a term coined for the screening of olfactory proteins as another shortcut toward active semiochemicals.[15] In short, olfactory proteins are identified from the genome of a receiver and utilized as molecular targets to fish out potential attractants rather than isolating these attractants from the sender (as in conventional chemical ecology approaches). This noninvasive process is particularly suitable for studying endangered species as well as those species whose behavior leads to cumbersome bioassays for isolation and identification of active ingredients. Recently, OBPs identified in the genome of the giant panda (Figure 2)

Figure 2. In a reverse chemical ecology approach, odorant-binding proteins (OBPs) have been recently used to study chemical communication in the giant panda. Putative sex pheromones were identified on the basis of their affinities for OBPs as well as structural features of OBP−ligand interactions. Illustration created by Steve Oerding (UC Davis, Information and Educational Technology, Academic Technology Services).

have been used in binding assays to identify putative sex pheromones and to gain a better understanding of how this vulnerable species interacts with the environment.[16] Similarly, ORs have been used to screen a library of compounds to select, on the basis of OR activation, mosquito oviposition attractants.[17−19] Like the use of GC-EAD, a bioassay to confirm function is still needed, but is now performed with a reduced number of samples. Additionally, this reverse chemical ecology approach may lead to discovery of physiologically relevant compounds, which would never be identified using a conventional chemical ecology approach. One such example is the discovery of Orco agonist, *N*-(4-ethylphenyl)-2-[[4-ethyl-5-

pyridin-3-yl-1,2,4-triazol-3-yl)sulfanyl]acetamide, VUAA-1 (Figure 1).[20] It is highly unlikely that this compound from a library of non-natural products would ever lead to measurable behavior because VUAA-1 cannot activate the intact olfactory system due to its low volatility. Yet, its discovery opened new avenues for the study of OR interactions and functions.

Although our understanding of the molecular basis of terrestrial insect communication has grown dramatically, chemical communication in aquatic insects lags behind. For those insect species, like mosquitoes, with immature larvae living in an aquatic environment and adults being terrestrial, we have learned a great deal about how adults communicate with the environment and find oviposition sites, but little progress has been made toward elucidating chemical communication in the aquatic environment. This differs remarkably from our current knowledge of the chemical communication in the sea.

Although the creatures living in the world's oceans look very different from those on land and are surrounded by water rather than air, chemistry is just as important a tool for communication under water as above it. Molecules produced by one organism travel through water to be sensed by others or act in surface-mediated interactions enabling a wide range of organisms to interpret the world around them. For example, marine bacteria seeking to form biofilms on algal surfaces are thwarted by halogenated furanones secreted from algal pores that confuse bacteria and interrupt their production of biofilm matrix (Figure 1).[21] Tiny mud crabs hiding in the interstitial spaces between oyster shells read the risk of predation by larger blue crabs via the chemical signature of their predator's urine, judging a predator most dangerous if its urine is rich in trigonelline and homarine, associated with consumption of mud crabs (Figure 3).[22] In other words, tiny animals can chemically sense what their predators have recently eaten and behave most cautiously around predators whose last meal was one of their own kinds.

Even single-celled algae called phytoplankton appear to have a "nose" for sensing the chemistry of their enemies, without having a true nose (or any organs at all). These plantlike cells receive and transmit chemical information, which shapes the continent-sized marine ecosystems they inhabit. Phytoplankton are responsible for approximately half of Earth's photosynthesis and represent a major food source for animals living in the open ocean. Some phytoplankton congregate to form massive blooms, visible from space, whose individual cells manufacture toxins that harm marine animals. These toxins are diverse and complex in their molecular structures, consisting of products of polyketide (e.g., brevetoxin B), isoprenoid, and amino acid biosynthesis (e.g., domoic acid and saxitoxin), with most such molecules produced by only one or a few algal species and found only in certain aquatic habitats (Figure 1). The chemistry of toxic algae has a rich history of discovery coupled with detective work to uncover biological function: the impacts of exposure to algal toxins have been explored mostly in the context of neurological risk to human health and marine wildlife. It is not clear to biologists why phytoplankton evolved to produce such toxic chemicals, although one hypothesis is that these toxins function as chemical defenses against specific predators such as microscopic animals (zooplankton). Zooplankton including copepods feast on a wide variety of phytoplankton, but appear to avoid certain toxic species; in some habitats, copepods have evolved partial tolerance of toxins in their diets after many generations of exposure to toxic algae.[23,24]
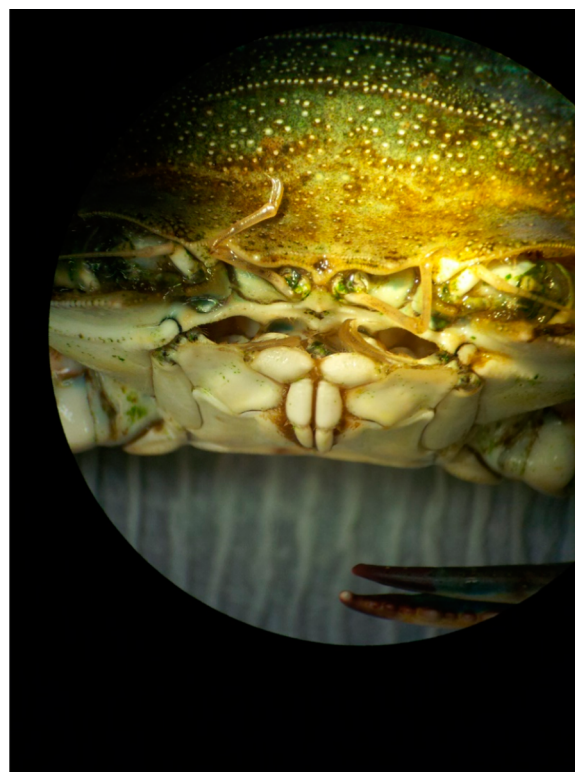

Figure 3. Blue crab *Callinectes sapidus* whose urine induces fear in prey organisms, with prey behavioral response proportional to the concentration of particular urinary metabolites (photo courtesy of Remington Poulin, Georgia Institute of Technology).

How do zooplankton sense algal toxicity, to avoid consuming these otherwise nutritious cells? It is possible that predatory copepods directly sense algal toxins via chemoreceptors on their antennules, but those toxins typically remain inside algal cells, whereas copepods reject certain kinds of toxic cells without swallowing them. Perhaps zooplankton sense other features of toxic algae, such as surface glycoproteins or diffusible odors associated with toxic cells. Chemosensory processes of marine organisms are poorly understood compared to their terrestrial counterparts, but play a substantial role in marine ecosystem function.

Like copepods, phytoplankton also possess chemosensory capabilities, responding to their copepod predators much the way mud crabs do to metabolites in their blue crab predators' urine. When phytoplankton sense enhanced concentrations of copepod-specific taurolipids (e.g., copepodamide A; Figure 1), which are probably unavoidable products of copepod digestion, this predator-specific information triggers a response that varies for each kind of phytoplankton.[25,26] Some phytoplankton respond to copepod cues by severing connections among chains of cells, releasing individual cells, which are less obvious to copepods than chains of multiple cells.[27] Others respond to small, ciliated predators by growing large colonies that cannot be engulfed by predatory ciliates.[27,28] A few phytoplankton species upregulate their own toxicity upon sensing copepod cues, further discouraging predators from consuming them.[29] As with the fear induced in mud crabs when exposed to predator urine, the most powerful response in phytoplankton comes from exposure to copepods that have recently eaten phytoplankton.

In fact, it has long been known that the toxicity of algal blooms is highly variable worldwide, and only in the last decade has it become apparent that a substantial portion of that variation is due to chemical sensing of predator risk. It appears that phytoplankton have evolved to turn up their toxicity when it is most needed, an example of induced defense, which has been frequently observed among trees, shrubs, and herbs in response to insect attack. Although planktonic creatures have been studied by scientists since the first microscopes revealed their intricate and diverse forms, the taurolipids excreted by copepods that phytoplankton use to measure risk were unknown until 2015. Since their discovery, these compounds have been detected at picomolar concentrations, potent enough to induce phytoplankton toxicity and break up chains, in ocean water around the world. It turns out that microscopic marine organisms are more sensitive chemists than those of us with mass spectrometers and high-performance liquid chromatographs in our labs. To discover a new class of compounds with planetwide environmental impacts, we had to follow the noses of the phytoplankton.

How do phytoplankton sense copepod odors, without a true nose? They must have receptors that have yet to be discovered, which allow them to interpret the information from zooplankton exudates, turning on the phytoplankton's biosynthetic apparatus for toxin production, chain disruption, colony growth, and other behaviors or physiological changes that have yet to be discovered. At the foundation of this complex biology is simple communication of small molecules diffusing through the water column. Answering these questions will allow scientists to better predict the toxicity of algal blooms, how much of the Earth's fixed carbon will be consumed in the oceans, in what circumstances to expect the evolution of algal toxicity and the evolution of resistance in predators. Research in chemical ecology will lead us to greater insights into the molecular mechanisms of cellular biology and animal behavior.

We are just beginning to pay close attention to the fact that small molecules are constantly carrying valuable bits of information by land, sea, and air. Chemists working in collaboration with organismal biologists are continually discovering completely new interactive roles played by small molecules. Right now, in part as a consequence of limited funding opportunities, the level of research effort devoted to chemical ecology is shockingly small. The International Society of Chemical Ecology has fewer than a thousand members, whereas the American Chemical Society boasts more than 150 000. Yet numerous critical scientific questions remain, including how the earliest occupants of our planet interacted via chemical signals and cues, how pheromones have contributed to human social organization, and how chemical communication among microbial constituents of our own guts regulates human health and behavior. We know that knowledge of chemical ecological interactions is essential to our understanding of the biotic world. We also know that the application of chemical ecological ideas to current problems in medicine, agriculture, forestry, and many other human endeavors can have enormous applied consequences. Perhaps we will even one day figure out what those cats are doing!

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Notes

The authors declare no competing financial interest.

■ ADDITIONAL NOTE

[a]ORs form ion channels with obligatory odorant receptor co-receptors, Orco, which are embedded in the dendritic membrane of olfactory receptor neurons (ORNs, also referred to as olfactory sensory neurons, OSN). Signal transduction starts once semiochemicals interact with OR units forming these ion channels.

■ REFERENCES

(1) Meinwald, J. The Degradation of Nepetalactone. *J. Am. Chem. Soc.* **1954**, *76*, 4571−4573.

(2) Kolter, R. Reflections on the history of microbial chemical ecology. *Microbe Mag.* **2010**, *5*, 201−205.

(3) Leal, W. S. Reverse chemical ecology at the service of conservation biology. *Proc. Natl. Acad. Sci. U.S.A.* **2017**, *114*, 12094−12096.

(4) Fabre, J.-H. *The Life of the Caterpillar*; Dodd, Mead, and company: New York, 1913; p 382.

(5) Lintner, J. A. In *A New Principle in Protection from Insect Attack*, Western New York Horticultural Society Proceedings, 1882; Vol. 27, pp 52−66.

(6) Stowe, M. K.; Tumlinson, J. H.; Heath, R. R. Chemical mimicry: bolas spiders emit components of moth prey species sex pheromones. *Science* **1987**, *236*, 964−967.

(7) NCBS Chemical ecology: a brief history. https://www.ncbs.res.in/ChemicalEcology/BriefHistory (accessed Dec 24, 2017).

(8) Hartmann, T. The lost origin of chemical ecology in the late 19th century. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 4541−4546.

(9) Butenandt, A.; Beckmann, R.; Stamm, D.; Hecker, E. Uber Den Sexual-Lockstoff Des Seidenspinners Bombyx Mori - Reindarstellung Und Konstitution. *Z. Naturforsch. B* **1959**, *14*, 283−284.

(10) Karlson, P.; Butenandt, A. Pheromones (Ectohormones) in Insects. *Annu. Rev. Entomol.* **1959**, *4*, 39−58.

(11) Cardé, R. T. Defining attraction and aggregation pheromones: teleological versus functional perspectives. *J. Chem. Ecol.* **2014**, *40*, 519−520.

(12) Kaissling, K. E. Pheromone Reception in Insects: The Example of Silk Moths. In *Neurobiology of Chemical Communication*; Mucignat-Caretta, C., Ed.; CRC Press: Boca Raton, FL, 2014.

(13) Schneider, D. 100 years of pheromone research, an essay on Lepidoptera. *Naturwissenschaften* **1992**, *79*, 241−250.

(14) Leal, W. S. Odorant reception in insects: roles of receptors, binding proteins, and degrading enzymes. *Annu. Rev. Entomol.* **2013**, *58*, 373−391.

(15) Leal, W. S. Pheromone reception. *Top. Curr. Chem.* **2005**, *240*, 1−36.

(16) Zhu, J.; Arena, S.; Spinelli, S.; Liu, D.; Zhang, G.; Wei, R.; Cambillau, C.; Scaloni, A.; Wang, G.; Pelosi, P. Reverse chemical ecology: Olfactory proteins from the giant panda and their interactions with putative pheromones and bamboo volatiles. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114*, E9802−E9810.

(17) Zhu, F.; Xu, P.; Barbosa, R. M.; Choo, Y. M.; Leal, W. S. RNAi-based demonstration of direct link between specific odorant receptors and mosquito oviposition behavior. *Insect Biochem. Mol. Biol.* **2013**, *43*, 916−923.

(18) Leal, W. S.; Barbosa, R. M.; Xu, W.; Ishida, Y.; Syed, Z.; Latte, N.; Chen, A. M.; Morgan, T. I.; Cornel, A. J.; Furtado, A. Reverse and conventional chemical ecology approaches for the development of oviposition attractants for Culex mosquitoes. *PLoS One* **2008**, *3*, No. e3045.

(19) Choo, Y. M.; Xu, P.; Hwang, J. K.; Zeng, F.; Tan, K.; Bhagavathy, G.; Chauhan, K.; Leal, W. S. Reverse chemical ecology

approach for the identification of an oviposition attractant for Culex quinquefasciatus. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115*, 714−719.

(20) Jones, P. L.; Pask, G. M.; Rinker, D. C.; Zwiebel, L. J. Functional agonism of insect odorant receptor ion channels. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 8821−8825.

(21) Manefield, M.; de Nys, R.; Kumar, N.; Read, R.; Givskov, M.; Steinberg, P.; Kjelleberg, S. A. Evidence that halogenated furanones from Delisea pulchra inhibit acylated homoserine lactone (AHL)-mediated gene expression by displacing the AHL signal from its receptor protein. *Microbiology* **1999**, *145*, 283−291.

(22) Poulin, R. X.; Lavoie, S.; Siegel, K.; Gaul, D. A.; Weissburg, M. J.; Kubanek, J. Chemical encoding of risk perception and predator detection among estuarine invertebrates. *Proc. Natl. Acad. Sci. U.S.A.* **2018**, *115*, 662−667.

(23) Teegarden, G. J. Copepod grazing selection and particle discrimination on the basis of PSP toxin content. *Mar. Ecol.: Prog. Ser.* **1999**, *181*, 163−176.

(24) Avery, D. E.; Dam, H. G. Newly discovered reproductive phenotypes of a marine copepod reveal the costs and advantages of resistance to a toxic dinoflagellate. *Limnol. Oceanogr.* **2007**, *52*, 2099−2108.

(25) Selander, E.; Kubanek, J.; Hamberg, M.; Andersson, M. X.; Cervin, G.; Pavia, H. Predator lipids induce paralytic shellfish toxins in bloom-forming algae. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112*, 6395−6400.

(26) Lindström, J.; Grebner, W.; Rigby, K.; Selander, E. Effects of predator lipids on dinoflagellate defence mechanisms - increased bioluminescence capacity. *Sci. Rep.* **2017**, *7*, No. 13104.

(27) Selander, E.; Jakobsen, H. H.; Lombard, F.; Kiorboe, T. Grazer cues induce stealth behavior in marine dinoflagellates. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 4030−4034.

(28) Long, J. D.; Smalley, G. W.; Barsby, T.; Anderson, J. T.; Hay, M. E. Chemical cues induce consumer-specific defenses in a bloom-forming marine phytoplankton. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 10512−10517.

(29) Selander, E.; Thor, P.; Toth, G.; Pavia, H. Copepods induce paralytic shellfish toxin production in marine dinoflagellates. *Proc. R. Soc. B* **2006**, *273*, 1673−1680.