

What Will It Take To Find a Human Pheromone?

Alla Katsnelson

Despite decades of research into chemical communication, researchers are no closer to determining whether a human pheromone exists.

In 1991, at a conference sponsored by a fragrance company called the Erox Corporation, two University of Utah scientists presented research on a tantalizing pair of chemical compounds provided by the company. They reported that in a few dozen human volunteers, the molecules androstadienone and estratetraenol activated the vomeronasal organ (VNO)—an olfactory organ that senses pheromones in many animals—in a sex-specific manner. The company patented these molecules as putative human pheromones. About ten years later, University of Chicago biopsychologist Martha K. McClintock and a colleague tested the molecules' ability to affect the emotional states of men and women. The results, when published in 2000, did not support Erox's claim: "It is premature to call these steroids human pheromones", the authors concluded. But the paper brought the compounds to the attention of the scientific community.

The human body naturally produces androstadienone and estratetraenol, but the compounds' activity as pheromones—substances produced and emitted by one individual of a species as signals affecting the behavior or physiology of another individual of the same species—has never been rigorously demonstrated. Also, most researchers today agree that the VNO in humans, located just behind the nasal septum, is vestigial, an organ that's no longer of use to today's *Homo sapiens*. Even so, these molecules pop up in studies to this day. Tristram Wyatt, a zoologist at the University of Oxford, has tracked every mention of them in the scientific literature, following their trail either to McClintock's 2000 paper or the 1991 symposium paper in which they were first presented. "It's as if the company just plucked these molecules from the air", Wyatt says. "And people simply took them on trust, so the idea of doing proper chemistry and experiments was short circuited."

Wyatt and other researchers have long tried to address a thorny question: Do human pheromones actually exist?



Credit: Can Stock Photo Inc./Rustenchik

Despite more than half a century of vigorous debate on the topic, there's still no answer in sight. Indirect evidence suggests they might. For Wyatt, one strong indicator is that humans develop a permanently strong body odor during puberty; it's at least possible that such odors act as chemical signals, as they do in some other sexually mature mammals at breeding times. The fact that other mammals have pheromones suggests that we may, too. Knowing either way would shed light on this primal and poorly understood communication system.

To date, though, no actual molecules that serve as human pheromones have ever been identified—in part because molecules falsely called pheromones diverted the search, but also because identifying the molecular basis of human behaviors is exceedingly complicated. A close look at the history of research on pheromones in other mammals reveals some of the challenges in the search and why settling the question will be no easy matter.

■ ON THE PHEROMONE TRAIL

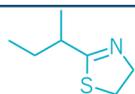
By definition, pheromones are detected by the olfactory system and affect behavior either directly or by influencing the hormone system of a member of the same species. (Molecules that serve as signals to members of a different species are called kairomones.) In order for a molecule to count as a pheromone, the behavior it elicits must be innate, not learned, and the chemicals must be able to signal anonymously to any member of the species, rather than providing an olfactory fingerprint of any one individual. The classic example is bombykol, which is released by female silkworm moths, *Bombyx mori*, to attract mates. Researchers first speculated that animals use pheromone-based

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communication in the late 19th century, but it took until 1959 to identify bombykol and to coin the term “pheromone” itself. Since then, pheromones have been identified across the evolutionary tree—including in spiders, lobsters, fish, frogs, snakes, sheep, deer, dogs, rabbits, elephants, and of course, mice, in which much of the research on mammalian pheromones has been conducted.

It wasn't long after pheromones' initial discovery that researchers began to look for them in humans. In a 1971 *Nature* commentary, Oxford University physician Alex Comfort exhorted researchers to apply analytical chemistry techniques such as gas chromatography to search for human pheromones in order to “open a new chapter in reproductive pharmacology at a time when it is badly needed.” Comfort's piece ran in response to a *Nature* study published just a few months earlier by McClintock, then an undergraduate at Wellesley College, which found that the menstrual cycles of women who lived together tended to synchronize. Since then, multiple publications have cast doubt on this so-called “dormitory effect”, attributing the observation to errors in research design and data collection. At the time, however, the study was heralded as the first demonstration of the existence of human pheromones.

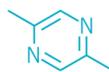
That same year, a thousand miles away, a young Czech-born analytical chemist named Milos Novotny joined the faculty at Indiana University. Novotny's main gig was developing analytical separation methods such as gas chromatography–mass spectrometry, but a senior colleague, Marvin Carmack, soon introduced him to a consuming side interest: chemical communication among mammals. Novotny reached out to a leading biologist in this area, Wesley Whitten at the Jackson Laboratory, to propose a collaboration. Whitten was an outdoorsman; when Novotny and Carmack came to visit, he took them on walks along snowy trails, pointing out markings of foxes. The hikers bagged some yellow snow, shipped the samples to Novotny's lab for analysis, and found that they could differentiate males from females by volatile compounds in their urine.



2-sec-Butyl-4,5-dihydrothiazole



Dehydro-exo-brevicomin



2,5-Dimethylpyrazine

These molecules, found in mouse urine, were credited as the first mammalian pheromones, but questions remain about whether they truly are pheromones.

They then began to study mice, developing techniques to profile the molecular constituents of mouse urine and

various gland secretions. Ultimately, they hit on a pair of molecules—a heterocyclic compound, 2-sec-butyl-4,5-dihydrothiazole, and a terpene derivative, dehydro-exo-brevicomin—that provoked aggression in male mice and were attractive to females. In adult female mouse urine, they also identified 2,5-dimethylpyrazine, a molecule produced by the adrenal gland that delayed puberty in juvenile females, perhaps acting as a signal to individuals that the population was too large to favor reproduction. “It's an embarrassingly simple compound—something a chemist would yawn over”, Novotny notes, “but it had a very distinctive odor, as many pyrazine derivatives do.”

■ A DIFFICULT ROAD

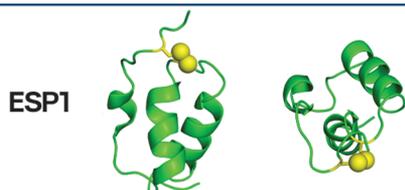
These and a handful of other volatile molecules identified during the 1980s are credited as the first mammalian pheromones. Today, their status is less clear. Some later work failed to find them in different laboratory mouse strains and couldn't replicate their behavioral and reproductive effects. Several other proposed pheromone candidates have spurred similar debates, reflecting how slippery research on mammalian pheromones can be.

While insect pheromones are generally single molecules that promote well-defined behaviors—attracting mates, swarming, or egg-laying, for example—mammalian pheromones often work in combinations, says C. Ron Yu, a neuroscientist at the Stowers Institute for Medical Research, and their effects can change with context. Also, pheromones change behaviors, and mammalian behaviors are complicated. Tests can probe things like mating and aggression in mice, Yu explains, but since these behaviors are always performed in the presence of other animals, in most cases you can't just wave a molecule in front of a mouse's nose and track how it responds. “If we don't know which behavior to screen, we don't know which compound is responsible for it, and without knowing the compound it's tough to identify the function of receptors”, he says.

And if screening for these behaviors is difficult in a lab mouse, it's exponentially more difficult in humans and other primates. In the early 1970s, researchers claimed to have identified sex pheromones in Old World primates that directly affected mating behavior; aliphatic acids in the vaginal secretions of rhesus monkeys primed with estrogen were reported to trigger male attraction, but these so-called copulins were later discredited because the behavioral responses could not be reproduced. “I just had a graduate student write to me asking about human pheromones, saying she was interested in pursuing copulins”, says George Preti, an analytical organic chemist at Monell Chemical Senses Center. “I told her not to waste her time.”

The irresistible draw of pheromone research is the prospect of tracing the whole chain of action, from pheromone and behavior to receptor and brain circuitry, Wyatt says. Yet very few mammalian pheromones have been so definitively nailed. One problem is that most researchers study pheromones from the perspective of behavior, neuroscience, or molecular biology, says Kazushige Touhara at the University of Tokyo. But there are few chemists on the job to isolate and study the molecules themselves. “If more people were working on the chemistry, the entire field would speed up”, he says.

Touhara’s chemosensory signaling lab managed to lay out the full picture for one mouse pheromone—exocrine gland-secreting peptide 1 (ESP1). They found that secretions from male mice from a minor tear gland called the extraorbital lacrimal gland, located under the ear, triggered VNO activity in females. “It was really an accident”, Touhara says. The gland was so understudied that they could barely find mention of it in the literature. And yet, it makes sense as a source of pheromones, he explains. Watch a mouse or a cat grooming and you’ll see it rubbing around its ears with its paw; that motion could facilitate the spread of secretions onto the animal’s fur. The researchers isolated the molecule in 2005, but it took five more years to determine its role in enhancing females’ sexual receptivity and to identify the receptor.



The protein ESP1 is produced in a tear duct in mice. It is one of the only molecules whose complete mechanism as a mammalian pheromone has been traced. This research was originally published in *The Journal of Biological Chemistry*: Yoshinaga, S.; et al. Structure of the mouse sex peptide pheromone ESP1 reveals a molecular basis for specific binding to the class C G-protein-coupled vomeronasal receptor. *J. Biol. Chem.* **2013**, *288*, 16064–72. Copyright 2013 American Society for Biochemistry and Molecular Biology.

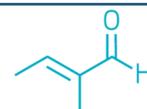
Unlike Novotny’s and some other proposed pheromones, ESP1 is a peptide, not a volatile small molecule. Touhara believes that, originally, all land-dwelling animals’ pheromones were volatiles, but that mice and certain other terrestrial animals evolved a more durable, protein-based marking system that signals through an animal’s touching its nose to the marked area. These can act alone or with volatile pheromones. A handful of other peptides identified since then are widely considered to be pheromones in mice. A male pheromone, *darcin*, named after Mr. Darcy in Jane

Austen’s *Pride and Prejudice*, belongs to a protein family called mouse urinary proteins. Steroid derivatives—sulfated steroids and, more recently, carboxylic acid steroids, both in females—have also been proposed, as have bile acids in feces of both sexes. Touhara’s team also proposed another ESP-family peptide, ESP22, as a pheromone in juvenile males. Advances in chromatographic techniques for purifying such peptides as well as in methods for identifying receptors have spurred recent progress, but researchers say they’ve barely scratched the surface in understanding this type of chemosensory communication, even in the laboratory mouse.

■ HUMAN HORIZONS

Despite the challenges the work will bring, Wyatt and Preti both argue that it’s time to turn back to humans. After decades wasted on molecules such as androstadienone and estratetraenol, researchers should launch a concerted effort to analyze human secretions to try to finally settle the question of whether they contain pheromones, they say. Some studies have provided hints that pheromone communication exists. Preti and his colleagues, for example, showed that extracts of sweat from men’s underarms stimulated in women a rush of luteinizing hormone, the molecule that triggers ovulation. And Israeli researchers reported that smelling women’s tears reduced both testosterone levels and feelings of arousal in men. But a serious search for the specific chemicals modulating these and other effects has never been done.

Preti and his long-term collaborator Charles Wysocki, also at Monell, envision a wide-ranging study in which compounds, once isolated, would be tested in bioassays that looked for mood alteration or changes in luteinizing hormone. Preti says the place to start looking is the armpit. “The mechanism of odor production there is similar to other mammalian communication systems, where volatile small molecules are carried to the surface by a protein”, he says. So far, the duo has not managed to get such a study funded, but Preti is convinced that analytical chemistry is advanced enough to yield valuable results.



2-Methyl-2-butenal

This rabbit pheromone guides newborn rabbits to their mother’s milk.

Wyatt, however, proposes a different starting point. If human pheromones do exist, the interaction between mothers and babies would be a prime place to look for them,

he says: The elicited behavior would be relatively easy to observe, and since the subjects are infants, it would definitely be innate. Indeed, a French team that identified a rabbit mammary pheromone, 2-methyl-2-butenal, has more recently observed that [human newborns](#) inhale more and move their mouths seeking food in response to breast secretions from lactating women. “If those molecules could be found”, Wyatt says, “it would be really exciting, because it would give you the confidence to start looking for much more difficult things in humans.”

Alla Katsnelson is a freelance contributor to [Chemical & Engineering News](#), the weekly newsmagazine of the American Chemical Society.