# Gresham College Main logo

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**Success of the Smelliest?**

**The Search for Human Pheromones**

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Whether or not humans have pheromones is a hotly debated topic. If you search the web for ‘pheromones’, you will find many sites which claim human pheromones are real and will try to sell you some to make you irresistible. My aim this evening is to explore the science behind the claims: sadly, they are completely unfounded. However, I think that humans might well have pheromones. To find them we should restart our search on a sound footing. The techniques are ready even though the task will be a challenging one.

A fully referenced paper on human pheromones, covering the ideas in this talk, can be found in a freely available, open access article: Wyatt, TD (2015) The search for human pheromones: the lost decades and the necessity of returning to first principles. *Proc Roy Soc B:* 282: 2014.2994. (<http://bit.ly/1eIGZxN> )

## What pheromones are

Pheromones are chemical signals between members of the same species. This is true for animals across the animal kingdom. They are species-wide signals. Some males for example might have more than others, and females might find these more attractive, but the signal is the same molecule or molecule in all males. Pheromones work just as well underwater as in air. Although the first pheromone to be chemically identified was a single molecule, pheromones are usually particular combinations of molecules. Pheromones can be short range as well as long range. If we were to find them in humans they might be involved in the final stages of foreplay (if at all). Across the animal kingdom, pheromones are generally detected by the sense of smell (the sense of olfaction). There are good evolutionary reasons for this because of the way that smells are processed in the brain.

The first animal pheromone to be identified, in 1959, was the result of decades of work by a team of chemists led by Adolf Butenandt, who had already received a Nobel Prize for his work on the chemistry of human hormones. He established the ‘Gold Standard’ for how you identify a pheromone. The bioassay (a repeatable and measurable response) he used to track down the molecule was the male silkmoth wing fluttering in response to the female pheromone (Photo credit, female and male silkmoth Sam Woo, UC Davis). He made a very good choice in the silkmoth because he needed a lot of them – half a million female moths, using the chemistry of the time. This was before gas chromatography and mass spectroscopy revolutionized the way we can separate and identify the molecules in biological samples. He went through the steps of extracting the molecules, using the wing fluttering bioassay to keep track of the activity, identified the molecule, synthesized it, and showed that the synthetic molecule elicited the same response as the original extract. In this way he ‘closed the circle’ to show that the synthetic molecule was the female signal.

## How pheromones evolve

Pheromones evolve from cues. What’s the difference between a cue and a signal? Mosquitoes use the smells we give off as cues to find us but our smells are not an evolved signal to attract mosquitoes (we would rather they left us alone). Within a species, molecules that start off as cues can evolve into signals if responding to such cues gives a selective advantage. For example, goldfish sex pheromones (chemical signals) are related to female goldfish hormones. So how did hormones become adopted, co-opted as pheromones? What we think happened is that hormones were leaking out from the females, through their gills and in their urine, as they developed their eggs ready to release them at spawning. Any mutant males that could smell these molecules (smell works just as well under water as in air) would have a selective advantage because they would get to the female first when she released her eggs. Males were selected for greater and greater smell sensitivity for these molecules and also greater receptor specificity so they did not get stimulated by similar molecules. Then gradually, over evolutionary time, females would be selected to produce more of the molecules, which would now be a signal. There seems to have been later selection to produce slightly different molecules to avoid confusion with the signals from other species.

To recap, what we are looking for in a pheromone is a molecule or molecules that elicit the same response as the natural stimulus, at the concentrations used by the species.

## What are not pheromones?

Smells are certainly very important to us. But not every smell is a pheromone even though the word is used for ‘Pheromone parties’ (you may have read about these in the press, around Valentine’s Day of course). Basically the idea is that you wear a T-shirt for 24 hours. You go to the party and put the T-shirt into a numbered plastic bag. And then you go around the tables, opening bags and sniffing the T-shirt inside. It’s all a bit of an ice-breaker. If you find a T-shirt whose smell you like (the bags are also colour-coded for gender of the T-shirt donor), you photograph yourself with the bag, number showing and put it up on the private party website. And if the wearer of the T-shirt likes you, he or she can approach you or get in contact – or vice versa. It’s based on some wonderful work done originally on inbred mice which showed that mice found potential mates that were different from themselves more attractive as mates. The choice could be made by smells somehow related to the immune system. We don’t know why the immune system makes us give off different smells, but it does. Claus Wedekind in Switzerland tried out the idea on humans. If you tissue-type lots of students, as if they were going to be organ donors, the female students seemed to prefer the smell of T-shirts worn by male students that were immunologically different from the choosing women students. These men would be poor organ donors but they would have made wonderful children together. However, these individual smells are not pheromones. The reason they are not pheromones is that these are not the same molecules in each person. It’s why we can tell people apart by smell, because these are individual difference in the smells they give off.

## Why would we expect humans to have pheromones?

Well the main reason for me is that we are mammals. And if there is one thing you can say about mammals is that they are smelly. In Darwin’s 1871 book *Sexual Selection and the Descent of Man*, he writes about the wonderful smells that are given off in the breeding season by male elephants and goats. And he would have said that our changes in smell at puberty indicate that probably there's something to do with sexual signalling going on and of course it's also that age when we get all the hair in the groin (the pubic hair) and the armpits. Those are fabulous places for the bacteria to develop to produce the smells that we know and love.

So with any other mammal, we'd be looking for pheromones. And mammals do have pheromones. There's the one in pigs probably, and lots in mice which seem to match the classic definition of pheromones. Some are large protein molecules like darcin and ESP1 in mice.

## How good is the human sense of smell?

It has been proposed that perhaps our sense of smell is too weak for us to have pheromones. For many years it was argued that we were microosmic and that this meant a poor sense of smell. (Microosmic simply means that our olfactory bulb is relatively small as a percentage of brain volume). But our olfactory bulb, although it might be tiny compared with the rest of our brain, is larger than the entire brain of a mouse.  That's something that Gordon Shepherd at Yale has commented on in his wonderful book called *Neurogastronomy*. He argues that actually we're very good smellers. It's simply that we haven't asked the right questions. We might not be as sensitive as a dog but we're very good at distinguishing smells. A paper last year suggests we may be able to distinguish more a trillion different odour molecule combinations (though remembering them is another matter).

## What's wrong with these putative human pheromones?

There's simply no evidence for these molecules being pheromones.  The story of these molecules is quite remarkable. There were two waves of these putative human pheromones.  The first of these was in the 1970s, with the two molecules androstenone and androstenol. The second wave takes us from 1991 to the present with two molecules androstadienone (AND) and estratetraenol (EST).  This was based on a conference paper with no evidence for the molecules whatsoever.

What about the first wave? Androstenone and androstenol are found in pigs, produced in the male's saliva. It seems to prompt the female to dip her back and make it easier for the boar to mount. A small amount *was* found in human armpits so some scientists jumped to the conclusion that it must be a pheromone in humans. But there was no good bioassay evidence that the molecules did anything to humans. However, what made it popular was probably that you could buy the molecules in a spray can (farmers needed it for pig husbandry). So there were some experiments with spraying it under chairs in a dentist's waiting room and seeing if men and women were more likely to sit on the chairs that had been sprayed.

The second wave came not from a real scientific discovery but instead from the US EROX Corporation which had a patent on the use of molecules they claimed were human pheromones. They launched the molecules androstadienone (AND) and estratetraenol (EST) at a conference they sponsored in Paris in 1991 to which leading olfactory scientists were invited. At the conference EROX Corporation-sponsored scientists claimed that the molecules were pheromones but no details at all were given of how these molecules were extracted, identified or bioassayed and shown to be pheromones (Monti-Bloch & Grosser 1991). The only details were the words 'These putative pheromones were supplied by EROX Corporation'.

I think nothing would have happened subsequently had not a renowned biologist, Martha McClintock at the University of Chicago, published a paper on the apparent effects of AND and EST on mood in women and men. The reason for using the molecules was simply on the basis of the 1991 EROX-sponsored paper. Her paper (Jacob and McClintock 2000) gave instant credibility to AND and EST.

Since the year 2000 there have been more than 40 experimental papers on the effects of AND and/or EST, about 3-4 papers each year and there are hundreds of citations to these.  The papers are from good scientists and in reputable journals and they usually cite the Jacob and McClintock (2000) paper and use the same concentrations. But there's no more evidence now than in 2000 that AND and EST are human pheromones: zero evidence.

How did this happen? I think the reason probably comes back to a few factors. First, AND and EST are easily purchased. These molecules quickly had a market with scientists. Second, it removed the need for labs and chemistry. So if you were in a psychology department you could buy the molecules and start doing serious experiments. You didn't need a spare chemist working with you, a real chemist. And then you had a growing scientific literature, almost an echo-chamber that assumed that these molecules were putative human pheromones. It was a self-referential literature. And of course when papers were sent out to peer review, the reviewers were other scientists who were using the molecules and making the same assumptions. It looks as though no one was going back to that 1991 paper as I have done and looked for the methods - where they would have found only that single line. They might have wondered why they were devoting so much time and grant money to studying something that was basically a will­‑‘o‑the‑wisp.

I think another problem was positive publication bias, a bit of a tongue twister. It is a phenomenon that has been noted in psychology but really applies across the sciences (and social sciences too): only new and exciting results get published in the journals. And we're all guilty of this, me too.

Only positive results are likely to get published in psychology. We are rewarded for exciting results, not for getting it right.  As a result we probably rush to publication, given the pressures for careers and grants. Caution is not rewarded - and the most prestigious journals demand the most clear cut and exciting results to even consider a paper for publication (it may be no surprise that more papers in these journals seem to be later retracted too). Another problem affecting biology in general, is the lack of replication; experiments don't get repeated by other labs.

## How should we research pheromones in the future?

I think we need a new start and we need to think of ourselves as if we were a newly discovered mammal.

Armpits are a favourite place to look for possible pheromone molecules. I've confessed in a TED talk that mine are wonderful but I'm not going to share them today. A good reason to look in armpits is that they are characteristic of the great apes, a point made by David Stoddart in his book *The Scented Ape* and by Alan Dixson in a more recent excellent book on primate sexuality, including humans.

Where do the smells come from? They are produced from odourless precursors secreted into the armpit by our apocrine glands then particular bacteria break them down specifically into the smells we know and love. There's some lovely work done by some Swiss scientists to try to create molecules to give to the bacteria so that when they break these new molecules down, we are left with some nice smells instead.

But more than 20% of the world's population produces very little of these molecules. And that's because they have white dry earwax, because they are homozygous AA for a gene *ABCC11*. The A gene variant has another effect: it is also characterized by armpits that don't smell. The mutation occurred about 15,000 years ago in NE Asia. More than 97% of people in China, Korea, and Japan there have the non-smelly version of the gene and do not produce the molecules that are characteristic of the armpits of people like me. It doesn't seem to stop them finding partners, so the armpit smells don't seem to be essential. There's nothing wrong with being less smelly.

So I think we ought to look all over the human body. It may turn out that rather than the apocrine glands in the armpit and elsewhere, the sebaceous glands may be important. These glands are important sources of odours in other mammals. In humans, the ear canals, the nostrils, the lips, the buccal mucosae, the breasts, the prepuce and the genital region all contain specialized sebaceous glands.

Having taken a sample, you need to separate the molecules by gas chromatography (GC) so you can see what's there. However, the GC reveals that there are great numbers of molecules in mammal secretions. This is quite a haystack if you trying to find some molecules which might be pheromones. And you may be looking for not just one molecule but probably a couple of molecules which work together.

So how can you reduce the number of molecules that you need to test in the bioassay? One way is to compare chemical profiles of, for example, males and females.

Using this comparative technique, a team in Japan discovered the male pheromone molecules in goats. The male pheromone molecules, produced by skin on the head of the male, are responsible for switching on oestrus in females after the long summer break. The researchers put a shower cap on the head of a male goat and used a ‘molecular sponge’ under the cap to collect the molecules given off.

As the production of many male pheromones in mammals is dependent on testosterone, the researchers compared the GC profiles of samples collected from intact males and castrated male goats - and looked for molecules that were missing in the castrated males. The comparison allowed the researchers to put together a cocktail of 18 molecules as a candidate pheromone. One of the molecules, 4-ethyloctanal, reproduced most of the natural activity in the bioassay of females but not completely so there may be other molecules needed for the complete pheromone.

In studying humans, I think the obvious thing is to compare adult males and adult females. We could also compare the molecules given off by adults and pre-pubertal individuals. This would limit the range of molecules that we would need to study and identify. It would still be a huge task but still easier than trying to identify and test every molecule, which is the alternative. Nonetheless we will to need to go through the whole systematic process for identifying a pheromone as outlined by Butenandt for the silkmoth pheromone.

I think one of the big problems is going to be bioassays, the simple repeatable experiments to measure responses. In the case of the goat pheromone, female hormonal responses were used. In the silk moth, wing fluttering by the male gave the bioassay. If we were going to look at sex pheromones in humans, we have a problem as we still don't know that much about human sexual behaviour. And this is before we consider cultural differences.

The other problem is that not only that studies of human sexual behaviour are hard to get funding for, so is the study of smell (olfaction). There are some indications as to the low importance society gives smell research.  For example, the founding date of United Kingdom's organization for blind people was 1868, for deaf people 1911, but not until 2012 for people affected by smell and taste disorders (Fifth Sense). We don't treat the loss of the sense of smell as important as a loss as the loss of our other senses. In terms of being able to work that may be true but in terms of quality of life, our sense of smell is incredibly important. Sadly, this low value given to the sense of smell seems to be reflected in levels of funding for researching it.

## A possible human mammary pheromone

I want to end this talk with what I think may be the most promising example for a human pheromone. Babies learn their mother's individual smell and every mother is different. Babies also respond to something that is the same in each mother, not different, and this may be a mammary pheromone. It has not been identified yet although a French team led by Benoist Schaal in Dijon are working on it now. This secretion is from the areola glands around the nipple. We all have them.  In a lactating mother, these glands secrete mixture of milk and sebaceous gland secretions.

If you put the secretion, taken from any mother, on a glass rod and put it under the nose of any sleeping baby, it will start ‘rooting’ to find the nipple, and either purse its lips or even stick out its tongue and start to suck. There's a correlation between the number of areola glands around the nipple and the speed of starting suckling by the baby: the more glands the mother has, the quicker the baby starts to suckle.  Poor suckling in the first 24 hours of life has a lasting effect on survival. That's because those first milk meals contain the colostrum with antibodies and hormones that the baby needs. If we could find and then synthesise the molecules involved then it would be really useful in cases such as encouraging premature babies to suck on teats or other babies if they find it difficult to start suckling.

There are number of reasons to think that the mammary pheromone might be the first to be found. First is that we have a good bioassay - a sleeping baby, secretion on a glass rod and a behaviour to measure. It's also that of all the life-stages of humans, it is the one that is least affected by culture and learning.  We know it is not culture-free as babies in the womb also learn what is good to eat from the molecules such as those from garlic that make their way into the amniotic fluid.  So the baby is primed to like the foods that will flavour their mothers’ milk. Nonetheless, in terms of all the ways culture differs between human societies, babies probably have the closest we get to natural behaviour.

The real story of human pheromones is just beginning. Thank you.

## Further reading

(full references can be found at <http://bit.ly/1eIGZxN> )

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