CHAPTER 2

CHEMICAL COMMUNICATION

Five major challenges in the post-genomics age

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Abstract. Chemical signals play an important role in the behaviour of most, if not all, organisms, but we still have much to learn about this mode of communication. Here I examine some of the major challenges to understanding chemical communication, especially for vertebrates, and consider how genomics, proteomics, metabolomics, and other '-omics' sciences and technologies provide new opportunities to address many of these challenges. First, one of the major challenges of this field is to better understand the kinds of information chemical signals provide. A second challenge is to unravel the proximate mechanisms that control chemical communication (i.e., the production and composition of chemosignals and olfactory recognition). Progress has been advancing rapidly in these areas, especially since the genes that encode odorant receptors were discovered, but there is still much to learn. Third, most research is focused on mechanisms, but there are major unsolved questions regarding the evolution of chemical communication. In particular, we still do not know how signals can evolve to become honest and reliable. A fourth major challenge is to better understand the role of chemical communication in the behaviour of our own species, and integrate this work into the social sciences. The final major challenge is to develop a field of applied chemical signalling that addresses problems in agriculture, medicine and the environment. In particular, we need to determine how chemical pollutants in our environment disrupt biological chemical signalling systems and potentially affect the health of humans and wildlife (ethotoxicology and ecotoxicology).
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INTRODUCTION

Chemical communication is a universal feature of life that occurs at all levels of biological organization, including regulation of cells and organs within the body, as well as social behaviour and ecological interactions among individuals (Agosta 1992). The terminology used for communication is constantly evolving, and so for clarification, I will use the term semiochemicals for chemicals used for information conveyance, and the term pheromones for those semiochemicals used for intraspecific communication. Pheromones play an important role in the behaviour of a wide variety of organisms, from moths to elephants (Wyatt 2003). Chemical cues provide several possible advantages compared to other sensory modalities (Doty 1986). They can be used in situations in which visual cues are unavailable, for

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example, and they provide spatial information, such as space occupancy. A problem with chemical signals is that they are more difficult to observe or measure than visual or acoustic ones, and therefore they remain less understood. There are many unsolved mysteries about chemical communication. My aim here is to review some of the main challenges for chemical-communication research, with an emphasis on mammals and other vertebrates, and consider how genomics and other '-omics' technologies offer opportunities to solve some of these problems.

DETERMINING THE KINDS OF INFORMATION ENCODED IN CHEMICAL SIGNALS

Odour can reveal much information about an individual, including sex, diet, social status, individual and group identity, reproductive condition, age, health, fear and other emotional states (Wyatt 2003). Scent marks and many other semiochemicals can be thought of as *extended phenotypes* (Dawkins 1983), though we know little about the genetics of semiochemical production. It has been suggested that pheromones and other chemical cues provide indicators that advertise a male's health and resistance to disease to potential mates, functionally analogous to the colourful secondary sexual traits of birds (Penn and Potts 1998). An individual's scent not only provides an indicator of infection**,** it also appears to indicate the activation of immunity (Zala et al. 2004). It is unclear how this occurs, though odour has long been used to diagnose a variety of diseases (Penn and Potts 1998). Odour also provides an indicator for assessing genetic relatedness and genetic compatibility of potential mates (Penn 2002), though it is also unclear how this occurs. Wilson (1970) suggested that in vertebrates individual identification is the most important message used in chemical communication, and there has been an increasing interest in determining whether individuals have unique chemical fingerprints or odourtypes (Beauchamp and Yamazaki 2003).

UNRAVELLING THE MECHANISMS CONTROLLING CHEMICAL COMMUNICATION

Chemical signals convey an amazing amount of information, and so one of the major challenges is to determine how this occurs. In particular, we need to know more about the compounds that are involved, how they are produced, and how olfactory organs are able to 'decode' information from chemical signals.

Determining the chemical composition of semiochemicals

The vast majority of semiochemicals of interest remain to be chemically identified. A variety of techniques are used for chemical analyses, especially *combined gas chromatography and mass spectroscopy* (GC-MS). Finding biologically active compounds, however, is like finding needles in haystacks, and one way to narrow down the possibilities is to use the olfactory organs of animals as sensors for determining the bioactivity of compounds. In arthropods the bulk of olfactory

neurons are contained in filamentous antennae from which so-called 'electroantennograms' (EAGs) can conveniently be recorded. This method has successfully been used for the identification of pheromones in numerous insects. More recently electrophysiological activity recorded from the olfactory bulb has been used as a biosensor signal in mammals (Lin et al. 2005). Chemical identification of active components among the many peaks in a complex chromatogram is still not an easy task, however, and conducting library searches to get clues about the identity of a compound from its mass spectrum (and retention time) is just one step in this process. After chemical identification, pheromones can then be synthesized using techniques in organic chemistry, and then confirmed using behavioural or neurological bioassays. Another problem is that chemical analyses are often just qualitative, identifying the presence or absence of compounds, even though there is potentially a great deal of information contained in quantitative levels of odorants, the ratios of multiple components (multicomponent pheromones and multivariate fingerprints), and the dynamic expression of these compounds. Fortunately, though, new developments in analytical chemistry are making it possible to obtain quantitative chromatographic data. This is largely due to improvements in solventless sampling techniques, such as open-tubular trapping (OTT), solid-phase microextraction (SPME) and particularly stir-bar-sorptive extraction (SBSE) (Baltussen et al. 2002; 1999; Soini et al. 2005). Moreover, recent advances in *chemometrics* offer powerful statistical analyses, such as pattern recognition, that are used to discover and quantify compounds of interest in complex chromatographic profiles (Brereton 2003). Chemical identification of the compounds is necessary; but it is not sufficient as we also need to understand how these compounds are produced.

Determining how semiochemicals are produced

This problem is becoming easier to solve due to the increasing availability of high-throughput tools from genomics, proteomics, metabolomics and other -omics technologies (Box 1). These have proved useful for determining the structure of carrier molecules (lipocalins) that bind and transport volatile compounds to urine and saliva (Timm et al. 2001; Spinelli et al. 2002). Determining the metabolic origins of individual odours is likely to be complicated because complex communities of commensal microflora probably play an important role (Albone et al. 1977). Commensal microflora is still not well described for any species, largely because the majority cannot be cultured in the laboratory. However, recently developed molecular genetic tools are successfully being applied to solve this problem (PCR-DGGE profiling) (Tannock 2002). Identification of compounds and determining the origin of their production will help to understand the underlying mechanisms; however, we also need to better understand the receiver side of communication.

Determining the molecular basis for olfaction

Determining how chemical signals are detected, processed, and how they trigger the perception of smell has been an extremely difficult problem. Olfaction has long been the least understood of all the senses, but progress has been advancing rapidly, especially since Buck and Axel discovered the olfactory receptor (OR) genes (a discovery for which they recently received a Nobel Prize) (Buck and Axel 1991). Tools from genomics and other -omics sciences have subsequently been helping to improve our understanding of olfaction (Young and Trask 2002). OR proteins bind odorant molecules and then initiate neural responses that trigger the perception of smell (De Bruyne in press). OR genes comprise one of the largest known gene families, with 900 (humans) to 1500 (mice) loci, scattered throughout the genome. The current paradigm is that each olfactory neuron expresses a single allele of a single OR gene through some sort of allelic selection process during development, but an exception has recently been reported for *Drosophila* (Goldman et al. 2005). It is unclear how the nervous system turns signals from olfactory neurons into the perception of smell – and how it integrates input from multiple sensory modalities, though these problems are gradually being solved. Unravelling the molecular basis for olfaction will be a major advancement (De Bruyne in press), but even this is not sufficient to understand chemical communication fully because we ultimately need to explain how such complex mechanisms evolved. For example, OR genes are highly polymorphic in sequences and copy numbers, and yet it is completely unclear how natural selection maintains this enormous diversity.

DETERMINING HOW CHEMICAL SIGNALS EVOLVE AND CONVEY RELIABLE INFORMATION

One of the central problems for the study of animal communication is explaining why signals can evolve to become honest and reliable (Maynard-Smith and Harper 2003). Not all signals are honest, of course, as there are many examples of deceit and manipulation. For example, male moths are attracted to the pheromones of conspecific females, and bolas spiders in two independent lineages have evolved the ability to synthesize moth pheromones, which they use to lure male moths (Stowe et al. 1995). However, signals are usually reliable because otherwise receivers would ignore them, and the signalling system would cease to exist. Signalling should lead to a dynamic co-evolutionary ‘arms race’ between signallers and receivers, with signallers evolving ways to cheat and manipulate others, and receivers evolving mechanisms to resist manipulation and ‘mind-read’ signallers (Krebs and Dawkins 1984). This arms race model is surely correct, at least when signallers and receivers do not have mutual interests, though it has not been tested to my knowledge.

There are at least three explanations for the evolution of stable and reliable signalling. First, *the handicap principle* suggests the counter-intuitive notion that honest signals can evolve precisely *because* they are costly to produce and cannot be faked (Zahavi and Zahavi 1997). It has been suggested that chemical signals are strategic handicaps that provide honest indicators of a male’s quality to rivals and potential mates (Penn and Potts 1998). Contrary to what has become widely

assumed, however, the handicap principle is not the only explanation for reliability. A second explanation for how reliable signals can evolve, is when the signal and receiver have *common interests* in the outcome of their interaction. For example, species recognition signals used in mate choice to avoid hybridization can be honest and cheap because there is no benefit to cheating. Similarly, signals among cells within the body need not be costly to be honest as they generally have shared interests. Third, signals can be honest when they provide an *index* of some aspect of the organism, such as size, that is unmodifiable and therefore the signaller simply cannot lie. For example, it has been suggested that odour cues provide an honest indicator of health and disease because the volatile metabolic by-products of an immune response and disease are impossible to disguise (Penn and Potts 1998). The task of determining the reliability and costs of chemical signalling has only just begun, which includes measuring the energetic costs, and ecological costs, such as exposing the owner to greater risks of predation or parasitism.

DETERMINING THE ROLE OF CHEMICAL COMMUNICATION IN HUMAN BEHAVIOUR

Although the existence of human pheromones remains controversial, there is increasing evidence that volatile chemical signals influence human behaviour (Hays 2003; Stoddart 1990; Wysocki and Preti 2004). We need to know more about the types of information that humans convey by scent, and especially how other individuals respond to chemical signals. For example, a woman's scent indicates whether she is ovulating or not (Singh and Bronstad 2001), though we do not know how this affects the behaviour of other female of male individuals. An individual's odour changes when a fearful situation is perceived (Ackerl et al. 2002), but it is not known whether this triggers fear or anxiety in other individuals (such as 'fear pheromones'). There are very few examples of chemical signals affecting another individual's physiology or behaviour. The best examples are some unknown pheromones that somehow synchronize women's menstrual cycles (McClintock 1971), and yet it is unclear whether menstrual synchrony is functional or even occurs under natural situations. There appear to be pheromones that induce hormonal changes and trigger changes in emotions and moods (Jacob and McClintock 2000). There is evidence that odour plays a role in kin recognition, including a study that found that infants move towards scent from their mother's breast (Porter 1998), individual recognition, and mate choice (Penn 2002).

Although they appear to exist, no human pheromones have been chemically identified to date, and so this presents an important challenge. This challenge is similar to identifying the active ingredients in useful herbal medicines, such as isolating digitoxin in foxglove (Wysocki and Preti 2004). The human axillae are probably functional analogues to scent glands of other mammals (Stoddart 1990). For example, in humans protein (lipocalin) molecules carry odorants to the axillae, where they are metabolized and made volatile by commensal microflora (Spielman et al. 1995), which seems to be analogous to major urinary proteins (MUPs) and other carrier proteins used by other mammals. The greatest progress has been

unravelling the mechanisms controlling olfaction, and there is now overwhelming evidence that the vomeronasal organ (VNO) is not functional in human adults (Wysocki and Preti 2004). This means that human pheromones must be detected by the main olfactory bulb, despite popular misconceptions that pheromones are only detected by the VNO.

Chemical communication in humans has largely been ignored, though this situation is changing and human pheromones are attracting increasing attention. Integrating chemical-communication research into the social sciences will be easier as the artificial barriers between the human and natural sciences are breaking down. There will likely be more interest in chemical communication as researchers find more applications for our own species, such as in medicine.

DETERMINING HOW POLLUTANTS DISRUPT CHEMICAL SIGNALS

One of the most difficult challenges is to use our understanding of chemical communication to address applied problems, such as in medicine, agriculture and the environment. For example, in medicine, artificial chemical sensors or e-noses are currently being developed to diagnose diseases, such as cancer, via a patient's breath or urinary odour (Turner and Magan 2004).

Chemical-communication research has surprising implications for toxicology. There are an increasing number of chemical pollutants in our environment and in our bodies, and many of these are not toxic or carcinogenic, and yet they cause numerous other problems, such as altering sexual development. The problem is that they are chemically similar to the body's own hormones (estrogen mimics) or they otherwise disrupt the body's own internal chemical signals (Colborn et al. 1996b). These so-called endocrine-disrupting chemicals (or EDCs) impact endocrine, neural, immune and behavioural responses. In an outstanding book on the topic, called *Our Stolen Future,* Colborn et al. (1996b) point out that "The key concept in thinking about this kind of toxic assault is *chemical messages.* Not poisons, not carcinogens, but *chemical messages.*" (p. 204; italics added). Since then, several studies have found that pheromones and other semiochemicals are negatively affected by EDCs (Zala and Penn 2004; Fox 2004). The impact of these endocrine-disrupting chemicals for humans and wildlife is still controversial, though this has become the focus of the new interdisciplinary fields of ecotoxicology and ethotoxicology. Recently, researchers have increasingly been applying tools from -omics technologies to address problems in ecotoxicology (Robertson 2005).

CONCLUSIONS

Many vertebrate species, including our own, use chemistry to communicate, though exactly how is still rather mysterious. The increasing number of new tools available in analytical chemistry, chemometrics, molecular biology, and genetics, are leading to exciting new discoveries. These new technologies provide unprecedented opportunities, but they also create a new set of problems. For instance, we need to find ways to analyse statistically the enormous amount of complex data generated

from chromatographic profiles and DNA microarrays. Also, they will not replace the crucial role of theory: as one researcher, Christer Löfstedt, points out, "to obtain an interesting answer from your research, it helps to ask an interesting question!". There are numerous other important problems in chemical communication that I did not address here. Perhaps, the most important problem is clarifying all of the links that make up chemical communication, from pheromone production by the emitter on one end, to olfactory reception by receivers on the other, in a single model organism, such as house mice (Emes et al. 2004). A more integrated understanding of chemical communication will require insights into ecology and evolution. The problem is that we still know little about the ecology and evolution of house mice and other model organisms, as the importance of ecology and evolution for understanding the 'design' of these organisms and their genomes is not generally appreciated. Therefore, organisms whose ecology and evolution are well-studied would make excellent subjects for a genome project, and could become models for studying chemical communication.

Box 1. *Chemical communication in the post-genomic era*

The increasing availability of high-throughput tools from genomics and other -omics sciences and technologies allows researchers to measure gene expression (transcriptomics) and to determine protein structure (proteomics) and metabolic profiles (metabolomics). These tools help to identify gene products (transcripts, proteins, metabolites) in a sample, and examine quantitative dynamics in biological systems (Kell 2004).

Genomics is already being applied to address ecological questions about chemical communication (ecogenomics) (Berenbaum and Robinson 2003; Dicke et al. 2004). These -omics technologies are just beginning to be applied to address animal behaviour (Pennisi 2005), the evolution of behaviour (behavioural ecology) (Feder and Mitchell-Olds 2003; Fitzpatrick et al. 2005), and the evolution of social behaviour (sociogenomics) (Robertson 2005). Sociogenomics is a sub-discipline of behavioural genomics, or what could be called 'ethological genomics' or 'ethogenomics'. Combined with improved phenotyping tools, ethogenomics and sociogenomics have the potential to become core disciplines for chemical-communication research, linking chemistry and physiology on one end with ecology and evolution on the other.

The various -omics sciences and technologies offer new opportunities to investigate chemical communication; however, they also generate such massive datasets that new methods for managing, processing and analysing data are required (bioinformatics). Sir Peter Medawar (1982) argued that "…there is an epoch in the growth of a science during which facts accumulate faster than theories can accommodate them…" (p. 29). The post-genomics age appears to be just such an epoch, as it is becoming increasingly difficult to keep up with the explosion of data and facts! Still, to better understand highly complex systems, such as the genome and metabolism, proper data handling and analysis are crucial, and there is increasing interest in applying modelling techniques from *systems biology* (Kell 2004; Provart and McCourt 2004). Perhaps theoretical approaches from systems biology could also help to understand more complex problems in chemical communication.

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