

18. Exploitation of olfactory-mediated behaviour

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Abstract

Intra- and interspecific communication between arthropods and their blood hosts is to a large extent mediated by chemical cues. The information provided in this book shows the many and significant advancements in our knowledge of these interactions, from DNA regulation to natural interactions of wild arthropod populations and their hosts. Recent information from molecular genetics, neurobiology, behavioural ecology and chemistry have resulted in understanding how these organisms are affected by small molecules, mostly produced by the vertebrate host, and that the insects and ticks respond to blends of odorants rather than single cues. Whereas CO₂ is a universal kairomone for these blood-feeding arthropods, other host-derived odorants are often required to attain behavioural responses similar to those observed with natural hosts. The collective data described in this book provide opportunities and challenges for integration of olfactory tools as novel interventions for the control of these arthropods, many of which are vectors of infectious diseases or nuisance insects. Examples of such a strategy are provided, notably with mosquitoes, as these are among the group of arthropods for which alternative control strategies are most urgently required. Finally, the gaps in research that need to be addressed are discussed, focusing on identification and formulation of semiochemicals, sampling technologies and the integration of this in existing control programmes.

Keywords: semiochemicals, blood-feeding arthropods, chemical communication, challenge, vector control, attractant, repellent

Introduction

Chemical communication is an important mechanism of intra- and interspecific communication of arthropods. This book, which reviews the role of olfaction in vector-host interactions, shows progress made in our understanding of this process in blood feeding insects and ticks, from cellular level to population. Whereas the role of olfactory cues in host seeking of disease vectors was known for many decades, the regulatory mechanisms of this behaviour remained largely hidden until recent advancements in the fields of molecular genetics, analytical chemistry and visual technology. These technologies allowed for the discovery of olfactory genes and examination of the genetic regulation of gene expression in several disease vectors. In sensory physiology advances in technology and chemistry have made detailed studies possible on the activation of olfactory processing from the periphery to the insect brain. Advanced knowledge on insect behaviour led to the development of tools with which flight behaviour and responses to olfactory cues can be accurately assessed in laboratory and field. This book provides numerous examples of these advances over a wide range of insects and ticks. The collected information should be used for the development of tools that can be exploited for the control of vector-borne disease transmission and reduction of biting nuisance by blood-feeding arthropods, and several examples of these will be discussed in this concluding chapter.

Molecular regulation of olfactory behaviour

The recent publication of the genomes of several disease vectors (2002: *Anopheles gambiae* Giles *sensu stricto*; 2006: *Culex pipiens quinquefasciatus* Say; 2007: *Aedes aegypti* (L.); 2007: *Pedicularis*

humanus Linnaeus; 2008: *Ixodes scapularis* Say (see www.vectorbase.org for all genomes) creates unprecedented opportunities for studying the genetic and cellular regulation of insect behaviour. For example, mechanisms that affect the expression of olfactory genes in mosquitoes can now be studied in detail, allowing for a comprehensive understanding of the insect response to selected odorants (Touhara and Vosshall 2009, Chapter 2 in this volume). In addition, recent data on odorant binding proteins (OBPs) demonstrate the significance of these molecules in transporting volatile chemicals through the sensillum fluid to the receptor membrane, but also how gene silencing, using RNAi technologies, can fundamentally alter these processes, suggesting behavioural changes that went hitherto unexplained (Pelletier and Leal 2009, Sengul and Tu 2008, Zhou *et al.* 2008). The challenge is how this information can be maximally exploited for vector-borne disease control. For example, it has been suggested that silencing of selected olfactory genes might lead to mosquitoes that no longer recognise human odours, or not any vertebrate indeed. Exploitation of this knowledge would require the stable transformation of genetic traits to produce lines of mosquitoes with altered host-seeking behaviour and/or host-seeking strategies, so that fewer bites would occur on humans. We can then envisage the release of such mosquitoes in natural ecosystems to replace wild sibling populations as a contribution to disease control. In recent years, the use of such genetically modified vectors has been debated intensively as the socio-ethical and legal aspects of this technology remain unresolved (Benedict *et al.* 2008, Knols and Louis 2004, Scott *et al.* 2002, 2008). The World Health Organization, through its Tropical Disease Research (TDR) programme, discussed this topic at length during a meeting in May 2009, and it is expected that the organisation will provide guidance on the use of this technology in disease-endemic countries (Mumford *et al.* 2009). As the focus of genetic modification for vector-borne disease control appears to be more on the alteration of vector competence than on other biological traits (Catteruccia *et al.* 2009), the development of mosquitoes with altered biting behaviour is at present remote.

Neurophysiology

Recent advances in the neurophysiology of olfaction of disease vectors focused mostly on mosquitoes (Chapters 3 and 4) although studies on sandflies (Chapter 9) and kissing bugs (Chapter 14) also provided new insights in chemoreception of these insects. *Drosophila melanogaster* Meigen serves as a universal model insect for our understanding of processing of olfactory information in insects. Studies on *Ae. aegypti*, *An. gambiae sensu stricto* and *Cx. quinquefasciatus* demonstrate many similarities with *D. melanogaster* in olfactory processing, confirming the validity of using this species as a model for our understanding of insect olfaction. Whereas olfactory receptor cells can be broadly tuned to a wide range of chemical cues (Chapter 3, this volume and Carey *et al.* 2010), internal processing of these signals, perhaps governed by olfactory binding proteins (OBPs) and interaction at the neural membrane cause a first selective barrier for the action of semiochemicals, which is further regulated at the primary olfactory centre in the brain (Chapter 4). Functional dimorphism has been described for insects from different orders, but for mosquitoes this still has to be described. If so, this might explain the observed differences in olfactory responses of mosquitoes with respect to mating, host seeking and oviposition. It is interesting that odours, and specifically odour quality, ultimately are encoded by spatial patterns of activation in defined sets of glomeruli (Chapter 4). Such information needs to be linked to behavioural studies in which the behavioural response of insects to qualitatively different blends of odorants are being examined (Bernier *et al.* 2007, Jawara *et al.* 2009, Okumu *et al.* 2010a, Smallegange *et al.* 2009, Syed and Leal 2009, Williams *et al.* 2006). Whereas in recent years significant advances have been made in our understanding of the olfactory processing of odour cues of vectors, there is a lack of congruence between these results and those of the behavioural essays reported elsewhere in this volume

(Chapters 7 and 8). For rapid assessment of the quality of candidate odorant cues as behaviourally relevant, we propose the development of a model that would predict the behavioural outcome of the signal-transduction cascade of semiochemicals.

Flight behaviour

It is now generally accepted that blood-feeding insects respond to host odours, which as kairomones serve to guide the insects to the host. In this volume mosquitoes were selected as an example for reviewing upwind flight behaviour (Chapter 6). During the flight, mosquitoes use both chemical and visual cues, while temperature is detected only within a short range from the host. The effect of carbon dioxide during upwind flight has become better understood in recent years but the overall effect of blends of kairomones on in-flight behaviour, however, remains to be clarified. For example, CO₂ is sensed from a long distance, but we do not know over which distance other chemical cues are being detected and cause a behavioural effect. This is puzzling, as the principles of odour dispersal predict that packages of odour move downwind in a concentration similar to that at the source (Murlis *et al.* 2000). In a wind tunnel, Dekker *et al.* (2005) demonstrated that the yellow fever mosquito *Ae. aegypti* responded to homogeneous plumes of host odour and to turbulent plumes of CO₂. A similar phenomenon was shown to occur in the malaria mosquito *An. gambiae s.s.* (Spitzen *et al.* 2008). Recent semi-field and field studies showed that host odours alone attracted few mosquitoes to odour-baited traps, and that addition of CO₂ increased the catch significantly (Qiu *et al.* 2007, Schmied *et al.* 2008), providing support for these laboratory findings. These results suggest that mosquitoes sense a host from a distance with CO₂ as a principle component and that close-range host orientation is guided by other host odours, principally derived from the skin. It remains a challenge to elucidate whether such host odours induce upwind flight behaviour from a distance (Chapter 6). Tools such as windtunnels provided with imaging recorders, capable to record flights at 6,000 images per second (Anonymous, 2010) and equipped with on-line analysis software, as well as the use of electric nets for recording host-seeking flights in a natural environment (Chapters 6 and 12) suggest that details of odour-modulated flight behaviour of insects will rapidly advance. This, in turn, provides opportunities to better understand the role of specific host cues in the host-seeking strategy of disease vectors. Indoor host-seeking behaviour has remained largely unexplored, yet given the tendency of many vectors to bite indoors (endophagy) this deserves more focus. Exactly how host-seeking insects behave in a 'no-wind' environment could serve as a means to interfere with odour-modulated behaviour indoors and optimise methods to divert, trap, or repel insects close to hosts.

Behavioural assays

The study of odour-modulated behaviour of flying insects is, by nature, complex as the target organism moves fast through the natural environment such that direct observations are highly unreliable. This complexity is even enhanced as we consider that odour plumes are highly motile and affected by wind (Chapter 6, this volume). A consensus seems to have been reached among scientists that this behaviour should be studied at different levels: (a) under controlled conditions in the laboratory, (b) in semi-field enclosures, where the insects can be observed in a simulated near-natural environment and (c) in the field. Examples of these studies are reported in this volume, with mosquitoes, sandflies, stable flies, blackflies, tsetse flies, kissing bugs and ticks as target arthropods. The latter two groups require different observation tools as they move by walking and, hence, are more easily observable by the researcher. In the cascade of behavioural observations, the order of laboratory – semi field – field is desirable, but as the use of semi field systems is of recent origin (Ferguson *et al.* 2008, Knols *et al.* 2002), many studies move directly from

the laboratory to the field. Video recording can be used for detailed studies of in-flight behaviour (Beeuwkes *et al.* 2008, Cooperband and Cardé 2006, Dekker *et al.* 2005, Gibson and Brady 1985, 1988), and may reveal behavioural manoeuvres associated with particular olfactory cues (Dekker *et al.* 2005, Spitzen *et al.* 2008) and advanced software is available that enables detailed analysis of flight paths according to essential behavioural parameters (e.g. see www.noldus.com).

Several standard assays for laboratory studies have emerged, which provide robust tools for examining the behavioural response of blood-feeding insects and ticks to sensory cues such as odour, heat, humidity and visual objects. For the study of mosquito behaviour, a wind tunnel or olfactometer is widely used, and using these devices, semiochemicals that affect the behaviour of mosquitoes have been identified. The most recent studies report odour blends of chemicals identified from the natural volatiles of blood hosts (Bernier *et al.* 2007, Okumu *et al.* 2010a, Chapter 7 in this volume). Although this work initially focused on tsetse flies, it is encouraging to note that these studies are rapidly expanding to other blood-feeding species, leading to several promising odour blends with which these insects can be manipulated. Laboratory studies now produce blends of odorants to which the insects are attracted or by which they are repelled, depending on the odour composition. The challenge now is to provide proof that these blends are also effective in the field, as was done for tsetse flies. Only then can these studies be justified and perhaps improved to provide a large range of odorant cues with which nuisance insects and disease vectors can be effectively controlled. The recent study by Okumu *et al.* (2010a) in Tanzania and field studies in Brazil (Chapter 17, this volume) as well as a field study in Scotland (Logan *et al.* 2009) demonstrate that such cues are effective against different mosquito and biting midge species on several continents. Ongoing studies are expected to lead to a rapid expansion of the available odour cues affecting these insects.

Semiochemicals

For many years, odorant baits for mosquitoes consisted of carbon dioxide (CO₂) or live hosts (Takken and Knols 1999). Detailed research on tsetse flies and screwworm flies, however, resulted in the identification of chemical cues to which these insects respond by attraction or aversion (Cork and Hall 2007, Vale 1993, Chapter 12 in this volume). Inspired by these developments, studies on odour baits for other vectors were actively initiated, and in the preceding chapters of this volume the state-of-the-art of these studies are reviewed. Carbon dioxide acts as a universal kairomone (activator and attractant) for blood-feeding arthropods (Chapter 5, this volume), which suggests an evolutionary-safe adaptation to vector-host interactions, as all vertebrates exhale this compound. Any response to CO₂ is expected to lead the arthropod to a live host. However, CO₂ is not a kairomone that arthropods can exploit to satisfy their intrinsic preference for biting specific host species. For several mosquito species, which express a strong anthropophilic behaviour, studies focused on the chemical identification of human odorants and on selection of those compounds that cause a behavioural response in the mosquito (Bernier *et al.* 2000, 2002, Logan *et al.* 2009). This has led to the publication of blends of odorant chemicals that cause attraction of *Ae. aegypti* (Bernier *et al.* 2003) and *An. gambiae* (Okumu *et al.* 2010a, Smallegange *et al.* 2009). Both species are important vectors of arboviruses such as dengue, yellow fever, and Chikungunya (*Ae. aegypti*) and malaria (*An. gambiae*), and the availability of attractive blends opens the way for improved surveillance of these vectors, as well as for the development of removal trapping systems. In Chapter 17 (this volume) a practical application for the surveillance of *Ae. aegypti* in Brazil is described, which is the immediate result of the detailed chemical, physiological and behavioural studies reviewed in other chapters of this book. Compared to mosquitoes, the identification of kairomones for other vectors has been less intense, but recent data on biting

midges (Chapter 10, this volume), kissing bugs (Chapter 14, this volume) and sandflies (Chapter 9, this volume) suggest that these insects can soon also be sampled using attractive odour baits. It has been suggested that only baits that are as attractive as natural host odours can be applied for the successful manipulation of vectors, such as for example the control of tsetse populations in southern Africa (Hargrove 2005), and that baits that are less attractive than natural hosts are not relevant for vector control. We argue that a constant removal of fractions of vector populations over time will cause a significant reduction in density irrespective of the degree of attractiveness, although the speed of population reduction will be much higher when the baits are powerful. This of course on condition that the target vector population does not develop a change in behaviour by selection. We are not aware of publications of behavioural resistance as a result of olfactory manipulation, and suggest that the probability of this to occur is small as the insects can usually feed on alternative hosts (Killeen *et al.* 2001), which express a different odour profile.

Pathogen-induced effects on olfactory behaviour

The few reports on manipulation of vector behaviour by pathogens remains intriguing, as it is evolutionary advantageous for these microorganisms to control their transmission from host to vector and *vice versa*. Although there is much evidence for the manipulation of feeding behaviour by pathogens, the olfactory evidence of these interactions remains limited to studies on *Plasmodium* and *Leishmania* (Chapter 16, this volume). Recent work on vector manipulation by viruses in plants demonstrated convincingly that the pathogens affect the composition of semiochemicals that guide their insect vectors to the plant (Mauck *et al.* 2010) and match earlier work on malaria vectors (Lacroix *et al.* 2005). It is, however, not known whether these effects apply to a broad range of vector-borne microorganisms and of those where such behavioural manipulation was found, the nature of the semiochemicals involved is unknown to date. Yet, detailed knowledge of these interactions would provide insight in specific cues that attract vectors to their hosts, and can be used for vector manipulation.

Future challenges and prospects

Our knowledge on the role of olfactory cues in host-seeking behaviour of blood-feeding arthropods has made significant advances in the last decade (this volume). Tools for the detailed observation and analysis of this behaviour are now being used by a large group of scientists, which is expected to answer questions that have eluded us this far. The field of research has broadened, to include molecular genetics, cellular regulation of signal transduction, analytical chemistry, behavioural ecology and parasite-vector interactions. While these studies answer fundamental questions about the role of odorants in arthropod behaviour, several challenges need to be resolved. For example, the sampling of blood-feeding insects relies currently on crude tools such as traps, knock-down sprays, resting collections or the human biting catch (Chapters 8, 11 and 12, this volume). The rationale for trap design is often unknown, and studies as described by Cooperband *et al.* (2006) show the importance of understanding the way insects approach a trap while responding to host volatiles. All mosquito traps currently marketed operate a strong suction airflow to draw insects into a collection bag. The disadvantage of such traps is that they require a power source to operate the fan, which is often lacking in rural areas where such traps need to be operated. Research into passive trapping systems or even targets should receive more attention. Recently, for the first time, odour-baited stations for mosquitoes have been field tested (Okumu *et al.* 2010b). Although still not practical and in need of a CO₂ source provided from a pressurised gas cylinder, this study marks the first step towards effective use of attractive kairomone blends for operational use in developing countries. Such systems can be used as a 'lure and kill' tactic,

whereby insects are attracted to an insecticide-treated resting site. Alternatively, insects can be contaminated with biological control agents such as entomopathogenic fungi, through a 'lure and contaminate' strategy (Lwetoijera *et al.*, in press).

An important goal of research on olfactory behaviour of arthropods is to understand how odour cues can be used operationally. Many blood-feeding arthropods are disease vectors, and hence are targeted for control. The work on tsetse flies has convincingly shown that odour cues can be used to control disease vectors (Torr *et al.* 2005), but currently there are no examples of other disease vectors that can be controlled in this way.

Much work reviewed in this book concerns the role of kairomones in vector-host interactions, and for some vector species effective strategies are available that are used for their control (Chapter 12, this volume). An older, and more widely applied strategy of vector manipulation is the use of repellents such as *N,N*-diethyl-3-methylbenzamide (DEET) (Katz *et al.* 2008). DEET has been in use for more than 50 years, and is effective against mosquitoes, sand flies and ticks. Because of its relatively long persistence and high efficacy, it is not only used as protection against nuisance mosquitoes but also as personal protection against malaria and dengue vectors. Other compounds like IR3535((R)) and Picaridin have been shown to provide a similar degree of protection as DEET. Recently, olfactory receptors for DEET were reported, providing exciting prospects for behavioural manipulation for other candidate chemicals with repellent action (Ditzen *et al.* 2008, Syed and Leal 2008). For instance, it is known that at low concentrations 1-octen-3-ol is attractive to some mosquito species and tsetse flies, but that higher concentrations cause a repellent effect. A different, but repellent effect was observed with carboxylic acids in anopheline mosquitoes, which was overcome by the addition of ammonia + lactic acid to the acid mixture (Smallegange *et al.* 2005). From these studies it is suggested that semiochemicals can be strategically applied for behavioural manipulation of disease vectors, opening the way for push-pull strategies as have been developed for herbivorous insects (Agelopoulos *et al.* 1999, Khan *et al.* 2008).

The emergence and spread of infectious diseases is studied using models and for vector-borne diseases these include the interaction between vectors, parasites and hosts (Anderson and May 1992). The vector-component in these models is, however, surprisingly inaccurate as vector behaviour is often not taken into account. For example, vectors exhibit different biting behaviour depending on physiological stage, age and infectious stage. The preceding chapters provide evidence that behavioural responses to blood hosts can be much affected by these factors, and therefore should be included in these models. Models can also be used to assess the impact of behavioural manipulation, for instance by removal trapping or the impact of a push-pull system. Agent-based models have recently been developed to assess the efficacy of bed net interventions on a micro scale, and such models can similarly be developed for removal trapping (Gu and Novak 2009). We suggest that field studies on the risk of vector-borne disease incorporate a behavioural component, so that better use can be made of behavioural manipulation through vector control or through personal protection.

This book focuses on advances in our knowledge on olfactory mediation of arthropod-host interactions. To study these interactions in natural situations in the field, sampling systems are needed to assess the behavioural response to semiochemicals. Although the efficacy of the many tools used for the collection of arthropods is not discussed here, it is obvious that such tools are essential in behavioural studies. A growing number of semiochemicals is becoming available for behavioural manipulation of nuisance insects and disease vectors and proof of principle of their operational use is becoming available (Okumu *et al.* 2010b). We suggest that the private pest

control sector becomes more closely involved in this work, so that the concept of arthropod manipulation with semiochemicals can be further developed. One successful example of such a development is that of the insecticide-treated bed nets, where the private sector became closely involved in disease control (Lengeler *et al.* 2007, Noor *et al.* 2009), allowing for a massive surge in technological advances using a private/public partnership. The control of tsetse flies using semiochemicals has received commercial interest, but to date only on a limited scale. On a larger scale the control of nuisance mosquitoes using semiochemical technology was undertaken by American Biophysics Corp. The latter company, however, dissolved after only a few years of successful operation. Although the reasons for the breakup of this company remain elusive, the results of their activities show that there was a broad private and public interest in this method of pest control. Now that better and more advanced methods of semiochemical production have become available, it seems worthwhile to examine the possible integration of this technology into the control of nuisance insects and disease vectors.

The reviews in this book demonstrate the importance of chemical ecology in vector-host interactions. The rapid advances in this field made in recent years suggest that we can now begin to consider applying the collective knowledge gained for manipulation of these vectors, introducing a novel, sustainable tool for disease control. The example of the successful control of tsetse flies using semiochemicals remains the best proof that this strategy can be highly effective. Semiochemicals also provide a solid base for studies of population densities and regulation of disease vectors, so that more accurate and objective measurements of transmission risk as well as the impact of vector control can be obtained.

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