10. Understanding and exploiting olfaction for the surveillance and control of *Culicoides* biting midges

James G. Logan, James I. Cook, A. Jennifer Mordue (Luntz) and Dan L. Kline

Abstract

Culicoides midges (Diptera: Ceratopogonidae) are found worldwide with the exception of only a few countries including New Zealand, Patagonia, the Hawaiian Isles and Antarctica. They are a nuisance pest to human beings, but transmit a number of diseases that mainly affect livestock. Like many haematophagous insects, midges have evolved highly developed and sensitive olfactory mechanisms which allow them to locate a potential mate or a suitable vertebrate host. This chapter provides a brief overview of the *Culicoides* midge lifecycle, its status as a vector of disease and offers an extensive review of *Culicoides* chemical ecology research, which has led to the identification of semiochemicals that could be exploited to control them. Host location processes can be influenced by many factors including midge physiology, host kairomones, the complex interactions of other volatile chemicals that confer differential attractiveness of hosts and also physical cues such as light levels and climatic conditions. These factors are considered in detail and future areas of work are discussed.

Keywords: biting midges, Ceratopogonidae, behaviour, olfaction

Introduction

Of the five major sensory systems (olfaction, taste, touch, vision and sound) by which organisms perceive their environment olfaction can be said to be the most important for biting flies such as midges. This sensory modality provides adult female *Culicoides* (Diptera: Ceratopogonidae) with essential cues for the location and identification of hosts that are required for a blood meal in order to grow their eggs. The detection of small, volatile compounds borne on the wind provides the main cues for activation and upwind flight. This, coupled with other cues such as heat, moisture and visual cues at closer range and taste on landing drive the behavioural sequence known as 'host location'. Although some of these stimuli may be involved in other aspects of midge ecology, their role in the location of a suitable host has been mostly studied and has been exploited to manipulate the behaviour of host-seeking adult females. For example, odour-baited traps can be used to lure, capture and kill adults. Although, this method has been used with some success with other biting Diptera (e.g. *Glossina* spp. tsetse flies), research into the olfactory processes involved in midge-host interactions is not fully understood but certainly has the potential to provide a potential means of *Culicoides* control.

Few insects that attack man are more annoying during their peak of activity than *Culicoides* midges but previous control efforts have been met with limited success, with repellents not providing full protection and with insecticides failing to provide adequate control of populations. To this end, a thorough understanding of the host-location process of each *Culicoides* species of medical and veterinary importance is still needed. This chapter will review current knowledge of olfaction in midge-host interactions including knowledge of experimental and commercially available traps. Protective measures are essential not only for man but also for domestic animals against midges that vector diseases such as Bluetongue of sheep and cattle and African Horse sickness. Effective

control of targeted species is urgently required, particularly in light of the recent Bluetongue outbreak in Europe.

Culicoides midge biology and ecology

Lifecycle

Culicoides midges are members of the order Diptera, one of the largest orders within the class Insecta. They are one of four haematophagous genera within the Ceratopogonidae family and are considered to have the most medical and veterinary importance due to their biting action and ability to vector pathogens that cause disease in livestock (Kettle 1965, Linley *et al.* 1983). There are approximately 1,400 species of biting midge and they have a wide range of larval and adult biologies.

Midges have mostly subaquatic or subterrestrial immature stages, developing in/nearby pools, streams or bogs. However, some can also be found in a variety of terrestrial or semiterrestrial locations such as animal droppings and vegetation. The northern Palaearctic species complex *C. obsoletus*, implicated in Bluetongue virus (BTV) transmission in northern Europe, is fairly catholic in its breeding habitats occurring not only in dung but also a variety of habitats from woodland to open country and bogland to marshland (Hill 1947, Kettle and Lawson 1952). *Culicoides obsoletus* Meigen s.l. is strongly associated with shaded habitats and a higher green leaf density than the Old World BTV vector *C. imicola* Kieffer that is found in much drier, warmer habitats (e.g. straw/dung mix at dripping water pipes) in full sun (Conte *et al.* 2007). Many other species troublesome to humans breed in brackish, sandy habitats along coastlines.

Culicoides are holometabolous, having four larval instars followed by a pupal stage where the adult form develops before emergence, although some species can over-winter as a diapausing fourth instar (Figure 1). Climate change, and more specifically alterations in temperature, may affect larval stages of some *Culicoides* species. Allingham (1991) and Bishop *et al.* (1996) demonstrated that *Culicoides brevitarsis* Kieffer emerge between 17-36 °C, with most emergence occurring in the 25-36 °C range. They also showed that average temperature in the field prior to their experiment starting, i.e. when *C. brevitarsis* was allowed to oviposit, played a role in determining development time. This species can survive for periods outside the optimum temperature range, but only emerge if conditions become favourable again within 50 days. Additionally, a return to favourable temperatures is not necessarily problem free. When temperatures in one experiment were raised to 25 °C after an extended period at 12 °C the adults that emerged appeared too small to produce viable offspring. The effect of climate change on the interaction between adult *Culicoides* midges and their host is an important and topical issue which is discussed in more detail later in this chapter.

The adult midge has a body length of about 1-2 mm, a weight of approximately 0.5 µg and has two compound eyes. Most adult females require blood to develop eggs, although some species, e.g. *C. impunctatus* Goetghebuer, are autogenous and therefore do not require a blood meal to develop their first batch of eggs (Blackwell *et al.* 1992, Boorman and Goddard 1970). All female midges must locate hosts to obtain a blood meal and this complex process can be heavily influenced and, to some extent, controlled by various environmental factors such as solar radiation, wind velocity, temperature and humidity, all of which are thought to limit flight activity (Bhasin 1996, Blackwell *et al.* 1997). This aspect of their ecology is discussed in more detail later in this chapter.

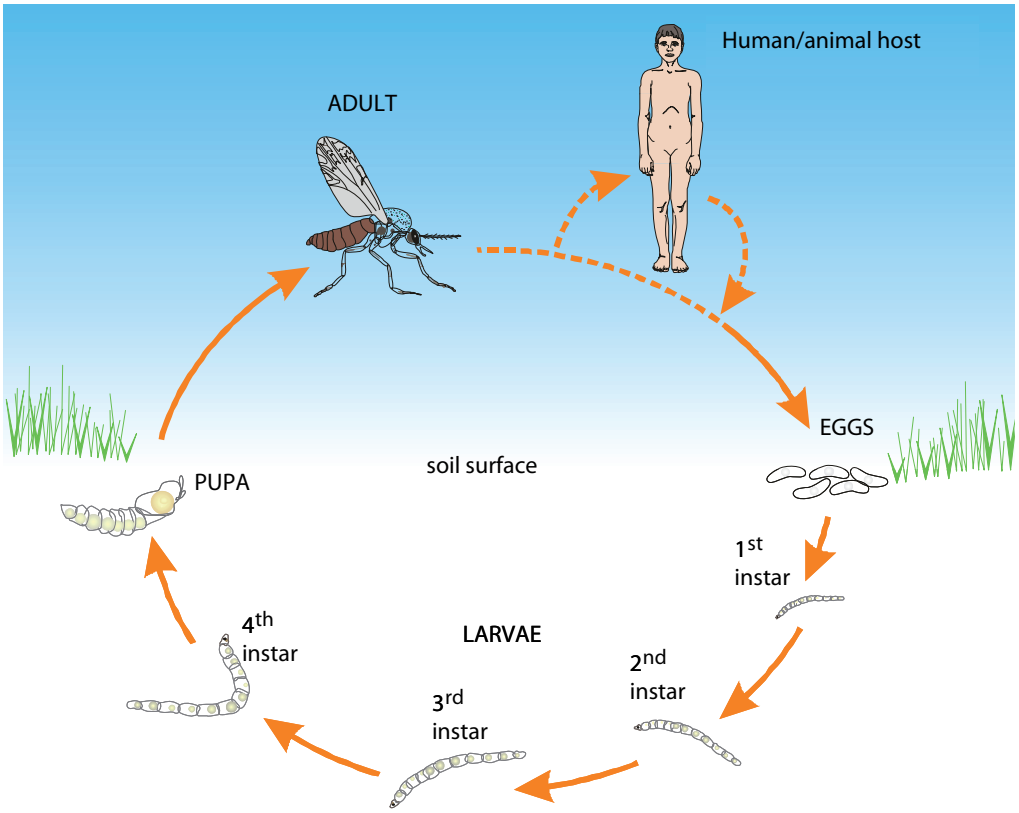

Figure 1. Illustrative representation of a female Culicoides *midge lifecycle (Lynda Castle, Rothamsted Research, Harpenden, UK).*

Blood meals are obtained preferentially from a variety of vertebrate hosts. For example, cattle, sheep and deer are among the predominant hosts of *C. impunctatus* although they will also readily feed on human beings (Blackwell *et al.* 1994b, Mands *et al.* 2004). ELISA tests on blood-fed *C. imicola* collected at farms in Ondersterpoort, RSA revealed that most midges had fed more than once on horse, cattle and sheep with horse being the most frequent (AWA Brown, AJ Mordue (Luntz) and GJ Venter, unpublished observation). The coat of cattle offers midges a greater surface area of accessible skin from which to feed than is available on sheep whose fleece they cannot penetrate so easily (Muller and Murray 1977). After blood feeding, as egg batches mature, females are thought to rest before seeking a suitable oviposition site (Boorman and Goddard 1970).

Vector status/ nuisance value and geographical distribution

Within the Ceratopogonidae, *Culicoides* is the most important genus regarding human and livestock comfort/welfare and health (Kettle 1965, Linley *et al.* 1983). Midges are found worldwide with the exception of only a few countries including New Zealand, Patagonia, the Hawaiian Isles and Antarctica. They have evolved to inhabit most temperate and tropical regions and therefore, in this chapter, we focus on those that are considered to be of high medical and veterinary importance.

With emerging vector-borne diseases in certain countries it is vital that we understand which species spread pathogens, the hosts that are most heavily afflicted and the midge geographical distribution and habitat.

Diseases transmitted by midges mainly affect livestock. For example, *Culicoides* are well documented as vectors of livestock arboviruses (Kettle 1984, Purse *et al.* 2005), including bluetongue virus of sheep and cattle (BTV), African horse sickness virus (AHSV) and epizootic haemorrhagic disease (EHD) of deer (Mellor 1990, 1993, 1994, Sellers and Maarouf 1991). Some species also vector the filarial nematode *Onchocerca cervicalis* Railliet and Henry, which affects horses (Mellor 1972), or cause 'sweet itch', which is not a disease, but a distressing skin condition of horses caused by midge saliva (Mellor and McCaig 1974, Townley *et al.* 1984).

Bluetongue virus infects domestic and wild ruminants and can cause more than 70% mortality in sheep (Jennings and Mellor 1988). It is prevalent in Central, West and South America, Africa, Asia, the Middle East and Australia (Holbrook and Tabachnick 1995, Mellor 1990). More recently it has spread throughout Europe. *Culicoides imicola* is believed to be one of the main vectors of BTV. Following the introduction of *C. imicola* to Italy and the subsequent establishment of BTV (Baylis *et al.* 2001) several studies have been conducted to predict the likely spread of BTV (Tatem *et al.* 2003, Wittmann *et al.* 2001). In 2005-2006, Takken *et al.* (2008) investigated the presence and distribution of *Culicoides* spp. at different habitats in the Netherlands to identify where potential BTV outbreaks may occur. This information was coupled with climate data to develop a BTV risk model (De Koeijer and Elbers 2007). A mixture of habitats comprising wetland areas, peat land areas, floodplains and livestock farms was examined. In total 15 species were collected and identified with variations in abundance for each habitat type. *Culicoides impunctatus* was most prevalent in wetland and peat bogs/moors. Nine other species were found in the wetland areas while the diversity was much lower in the peat land environments, with only *C. obsoletus* and *C. pulicaris* species complexes being identified. *Culicoides obsoletus* were more prevalent in floodplains and livestock farms than *C. impunctatus*. *Culicoides scoticus* Downes and Kettle, *C. dewulfi* Goetghebuer and *C. chiopterus* Meigen were also present at the livestock farms.

In August 2006, BTV spread throughout northern Belgium, France, Germany, Luxembourg and the Netherlands. This extremely worrying outbreak occurred further north than the occurrence of *C. imicola*, the main vector of the southern Europe outbreaks. However, *Culicoides dewulfi*, which breeds in the dung of cattle and horses, was found RT-PCR positive to the BTV-8 serotype that had caused the northern European outbreak (Meiswinkel *et al.* 2007). This may present further problems as it may be possible for this species to also transmit AHSV as the virus is closely related to BTV (Meiswinkel *et al.* 2008). Similarly, *C. obsoletus sensu stricto* and *C. scoticus* (which comprise two of the four species in the *obsoletus* complex), were found to be RT-PCR positive to BTV-8. *Culicoides pulicaris* Linnaeus *s.s.* has previously been incriminated in the field transmission of BTV (Caracappa *et al.* 2003), and *C. impunctatus* (another member of the *Pulicaris* complex) has been implicated in the laboratory (Carpenter *et al.* 2006, Jennings and Mellor 1988). Despite this, the information available suggests that *C. obsoletus* played a significant role in the European outbreak (Meiswinkel *et al.* 2008). In June 2007 it was reported that an animal from a holding in Germany had displayed evidence of BTV-8 infection in April. This represented the first indication that the virus had successfully overwintered in the region (IAH 2008a). In September 2007 the first case of BTV was identified in the UK (IAH 2008b). There are currently 136 premises affected by BTV (DEFRA 2008). At the time of the European outbreak, temperatures soared to a record high and this appeared to promote the replication and transmission of BTV by a number of *Culicoides* spp. (Meiswinkel *et al.* 2008). It is also possible that other viruses such as AHSV, Akabane virus (AKAV),

epizootic haemorrhagic disease virus (EHDV) and equine enchephalosis virus (EEV) may also be circulated in northern Europe under favourable climatic conditions (Meiswinkel *et al.* 2008). AHSV is currently prevalent in sub-Saharan Africa and causes mortality rates that exceed 90% in horses (Mellor 1994).

One of the best studied midge species is *C. impunctatus*, which is found throughout northern Europe, Russia and northern China but is particularly prevalent on the west coast of Scotland (Campbell and Pellham-Clinton 1960, Hill 1947). Although at least seventeen other species exist in this area, *C. impunctatus* outnumbers the others considerably (Blackwell *et al.* 1992). *Culicoides impunctatus* is among the most numerous of haematophagous flies in the UK where it can reach huge population densities (Hendry and Godwin 1988). Although *C. impunctatus* does not vector diseases in the UK presently, it causes significant losses to the economy, especially in terms of reduced tourism and outdoor activities in Scotland, where it is responsible for more than 90% of the biting attacks on humans (Blackwell *et al.* 1997). This is mainly due to the painful reactions caused by their bites, which discourage tourists and disrupts outdoor industries including agriculture and forestry. An estimated 20% loss in working hours has been reported in the forestry industry each year due to midge bites alone (Hendry and Godwin 1988). Their intense numbers during such attacks was recently highlighted by Mordue (Luntz) and Mordue (2003) who reported a rate of around 40,000 landings per hour on the forearms of volunteers. Similarly high numbers are found attacking people in coastal areas of Australia and the United States. In these countries several species of biting midges breed in brackish salt marshes and mangrove swamps. The encroachment of residential developments and recreational facilities into areas close to these breeding locations and the manmade extensions of breeding habitats has resulted in biting midges becoming an increasing problem.

Human pestilence by *Culicoides* biting midges is an acute problem along Australia's tropical and temperate shores and significant attack in the sublittoral and hinterland environs is confined to the southern parts of the country. Of the three littoral species groups involved the *C. immaculatus* group is of lesser importance than the other two. Both the *C. ornatus* group and the *C. molestus* group include the most seriously offending species. The former group is usually associated with mangrove habitats and the latter with the sandier reaches of open estuaries subject to tidal influence. The *C. ornatus* group is very large. It includes 50 species overall. A number of undescribed species in this group may ultimately be shown to contribute significantly towards the pestilence problem of the tropical coastline. *Culicoides ornatus* Taylor, *C. longior* Hagan and Reye and *C. marmoratus* (Skuse) are well known as serious pest species. Biting midges (e.g. *Culicoides ornatus*) are also serious pests around the coast of the Northern Territory. *Culicoides ornatus* is the dominant species in the Darwin area and numbers collected at certain locations reflect a relatively intense pest problem (Reye and Lee 1963). Prior to 1963 little was known of the biting midges on the Gold Coast of Australia as their larval habitat was isolated and they were of little consequence to man. However, this changed with the development of residential areas in close proximity to creek estuaries. *Culicoides subimmaculatus* Lee and Reye is the dominant pest species in these areas. With the construction of residential canal estates on the Gold Coast a new breeding habitat was created for the species *C. molestus* (Skuse). Here man interfered with the environment and created a vast new breeding ground for what was once a minor species. *Culicoides molestus* became the dominant midge species in the Gold Coast and interfered with the idyllic outdoor lifestyle for which the Gold Coast is famous. As residential development spread further to the north of the Gold Coast *C. marmratus* and *C. henryi* became the most prevalent midge species. On the Gold Coast biting midges rapidly replaced mosquitoes as the most prevalent nuisance or public health pest insect. The *C. molestus* group is smaller, with 11 recognised species overall.

Perhaps the supreme pest species of the Queensland coastline is included in this group. Ironically it remains undescribed and has been regularly misidentified as *C. subimmaculatus* Lee and Reye from which it differs in morphological detail and most likely in its biology.

The *C. immaculatus* group contains a single significant human pest species in Australia, namely *C. immaculatus* Lee and Reye. Its preferred habitat is along open shores and its larval habitat remains unknown.

In the United States the tremendous numbers of *C. furens* (Poey), *C. barbosai* Wirth and Blanton, *C. hollensis* (Melander and Brues), *C. melleus* (Coquillett) and *C. mississippiensis* Hoffman make living in coastal areas almost unbearable at certain times of the year. Abundance of these species varies geographically along the extensive USA coastline, and spatially and temporally at different geographic locations. Tourist business is greatly affected by biting midge abundance at these locations. Kettle (1962) succinctly puts it, 'One midge is an entomological curiosity, a thousand can be hell'. However, the population level or midge density at which female midges must reach before affecting people is dependent upon personal tolerance. Established residents of a midge-infested area will tolerate high midge densities whilst new residents a low density is too much to tolerate. The tourist business is greatly affected by midge (also called sand fly in this area) problems along many Florida beaches where there are significant populations of *C. furens*, *C. barbosai*, *C. hollensis* (Melander and Brues), *C. melleus* and *C. mississippiensis*. In some inland localities, particularly in the 'Highland' and 'Crystal Springs' counties, *C. floridensis* Beck and *C. tissoti* Wirth and Blanton may become a nuisance. Linley and Davies (1971) presented an excellent review of the problem of midges versus tourism, a summary of the biology of the most important species, and recommendations for control.

Culicoides furens makes life miserable in the Caribbean and on the eastern seaboard of America from New York in the north to Sao Paulo in Brazil in the south. With the aid of the wind this species may disperse 3 or 4 miles (5 to 7 km) (Linley and Davies 1971). Another species, *C. barbosai*, had previously been confused with *C. furens* (Wirth and Blanton 1956). This was an important discovery because, as the local Research Unit showed later, the breeding sites of *C. furens* and *C. barbosai* were quite different. The existing measures which were directed against *C. furens* were less effective against *C. barbosai*. *Culicoides furens* bred in the open and in areas shaded by the white and black mangroves (*Languncularia racemosa* L. and *Avicennia nitida* Jacq., respectively) while *C. barbosai* bred in wetter areas in the shade of the red mangrove (*Rhizophora mangle* L.). Removal of the mangrove cover had no effect on the breeding of *C. furens* but a year later *C. barbosai* had almost disappeared from the cleared area (Davies 1969).

Nuisance biting and the ability to transmit diseases, as described above, along with the recent spread of *Culicoides* populations mean that the need for effective control is extremely important. However, the olfactory processes and behaviours associated with host location and host preference is still poorly understood and requires much research.

Midge/host interactions and influencual factors

Host location is essential for *Culicoides* midges to find a suitable blood meal. Midges are known to detect visual, olfactory and gustatory stimuli, which aid in their location of a host. External stimuli provided by vertebrate hosts induce a series of behaviours that ultimately lead to the female finding a host and obtaining a blood meal. Host-derived chemical odours are considered to be important as they can be detected by the insects at considerable distances and act as flight

activators or attractants (kairomones). As midges approach their host convective heat (Brown *et al.* 1951), humidity, and visual cues such as shape, size, colour and contrast may also play a role (Bernier *et al.* 1999, Bidlingmayer 1994, Khan 1977, Muir *et al.* 1992, Takken 1991, Chapter 6 in this Volume). Bishop *et al.* (2008) used two-dimensional plastic cattle shapes and simulated stimuli to investigate host location for *C. brevitarsis*. They subsequently proposed that initial attraction was to visual stimuli, whilst host odours such as CO_2 and 1-octen-3-ol were secondary and probably occurred closer to the host. Landing is suggested to be a visual response to the interface between the backline of the host and the background. Additionally, this work also revealed information on the preferential biting sites of this vector. Sticky traps placed over the replica cattle shape showed that preference is for the ridge line of the back, extending from the neck to the tail. Interestingly, attraction of the mosquito *Culex annulirostris* Skuse was also investigated, and the visual element was found to play no role at all. For *C. impunctatus,* landing responses on targets painted with black and white vertical or horizontal stripes showed that females exhibited a clear preference for black stripes irrespective of the presence of host odour or whether the target was moving (rotating) or stationary. The target was shown to be unattractive to females at a distance. Striped targets were however important at inducing a landing response with horizontal black stripes, perhaps related to edge perception, being an effective landing stimulus (A Bhasin, unpublished observation).

Most adult *Culicoides* are \crepuscular, with peak activity around dawn and dusk, and to a lesser extent through the night, although a few species bite during the day (Blackwell 1997, Mellor *et al.* 2000). *Culicoides impunctatus* follows this crepuscular pattern (Blackwell *et al.* 1997) with the endogenous rhythm of diel flight periodicity greatly enhanced in the presence of host odour (CO_2) (A Bhasin, unpublished observation). Although for *Culicoides* species the onset of activity is usually triggered by falling light intensity (Mellor *et al.* 2000), meteorological variables other than light intensity appear to trigger activity in *C. impunctatus* (Blackwell 1997). Light levels are thought to accentuate an underlying rhythmically determined periodicity (Blackwell *et al.* 1997). This theory corresponds with that of Marsh (1986) who predicted a bimodal circadian rhythm for *C. impunctatus*.

Like other arthropods, *Culicoides* females employ semiochemicals for host-location (Mordue (Luntz) 2003). Host-related kairomones for *C. impunctatus* include CO_2, acetone, butanone and L-(+)-lactic acid (Bhasin *et al.* 2000a,b, 2001). However, the full set of olfactory stimuli has not yet been identified. To achieve this, we must examine the response of the insects to host odours and then identify the components that are physiologically relevant within those stimuli. Recently, Mands *et al.* (2004) investigated the response of *C. impunctatus* to animal extracts in a Y-tube olfactometer. They showed that water buffalo extract was the most active (threshold 0.22 g/ml), similar to deer extract, whereas other host extracts including pony, calf and sheep were more than ten times less active. The effects of CO_2 and 1-octen-3-ol on three species of *Culicoides* were investigated in salt marshes, Georgia, USA (Kline *et al.* 1994). It was found that CO_2 was an effective attractant for all three species: *C. furens, C. hollensis* and *C. melleus*. However, each species did possess a different response pattern. Contrastingly, 1-octen-3-ol was only an effective attractant for *C. furens*, and was tested both singly and in combination with CO_2, where it was synergistic. Repellent effects were reported for *C. hollensis* and *C. melleus*. 1-Octen-3-ol has also been shown to be attractive to *C. impunctatus* (Blackwell *et al.* 1996, Mands *et al.* 2004) and *C. vexans, C. delta C. pulicaris, C. lupicaris and C. albicans* (Mands *et al.* 2004) in the field.

Bhasin *et al.* (2000b) investigated the behavioural responses of *C. impunctatus* to nine synthetic compounds that had been identified as components of mammalian host odour: 1-octen-3-ol, acetone, butanone, L-(+)-lactic acid, phenol, 3-n-propylphenol, 3-methylphenol, 4-methylphenol

and 4-ethylphenol. The non-phenolic compounds all induced an attractive dose-dependent response over the range tested. As doses of butanone increased to supra-optimal levels arrestment of responses was noted. Furthermore, for phenol and lactic acid, supra-optimal concentrations actually appeared repellent. 1-Octen-3-ol has also been shown to arrest responses or even repel at high concentrations (Blackwell *et al.* 1996). *Culicoides nubeculosus* (Meigen) behavioural responses were tested for 1-octen-3-ol and acetone, and were shown to perform in a similar fashion to *C. impunctatus* but with lower thresholds of response (Bhasin *et al.* 2000b). Trapping experiments in southeastern Queensland, Australia, showed that the addition of CO_2 to traps increased catches of *C. histrio* and *C. subimmaculatus* significantly. Addition of 1-octen-3-ol and CO_2 increased catches of *C. molestus* only (Ritchie *et al.* 1994).

Pheromones

An interesting aspect of the host-location process is that it may also include the detection of pheromones that act synergistically with host odours. Mating in *C. nubeculosus* occurs either when a female flies into a swarm of males, with copulation taking place during descent to the ground, or when a female is already feeding upon a host (Pomerantzev 1932, Downes 1950, Downes 1955). In the latter case, volatiles from the blood of a vertebrate host (produced either at the wound itself, or from the excretion products resulting from concentration of the blood meal) could stimulate sex pheromone production and grooming behaviour, contributing to dispersal of the pheromone and attracting males whilst on the host. Virgin female *C. nubeculosus* perform a greater amount of grooming behaviour in response to blood volatiles, than to controls (Figure 2). It is hypothesised that this grooming behaviour is directly associated with the release of the sex pheromone, n-heptadecane, and is initiated by the presence of blood volatiles (Figure 3; Mordue (Luntz) and Mordue 2003). The behaviour could be a way of transferring the pheromone from the abdomen to the wings, with wing flutter aiding pheromone dispersal. An effective trapping system for this species, therefore, may involve the combination of host-derived olfactory stimuli as well as the sex pheromone. Similarly, during feeding, *C. impunctatus* females are thought to produce an aggregation or 'recruitment' pheromone (Blackwell *et al.* 1994a). This is believed to work synergistically with host-derived kairomones such as 1-octen-3-ol and cause aggregation of female midges on a vertebrate host. This is suggested to be a means of protection from host defence by using the 'safety in numbers' strategy or a strategy that enables a midge to find a rare but non-resource-limiting host (Blackwell *et al.* 1994a, 1996). A contact sex pheromone, which stimulates copulation, has been identified for *C. melleus* and comprises 2-methyldocosane, 8-methyldocosane and 9-methyltricosane (Linley and Carlson 1978).

Indoors/outdoors

Culicoides midges were believed to be purely exophagic (i.e. feed on animals outdoors) and exophilic (i.e. they will not enter inside buildings). Indeed, anecdotal evidence by Muller (1991) showed that covered areas (yards, stables and sheds) significantly reduced numbers of *C. brevitarsis* reaching host cattle but had little effect on mosquitoes. However, Bishop (2002) proposed the lack of response by the midge species to be due to blocked vision, rather than an aversion to enclosed spaces. Baldet *et al.* (2008) recently found *Culicoides* indoors albeit with a lower species diversity (six species) than outdoors (nine species). Similar results have been obtained with *C. impunctatus* that will readily enter through a window or door of a tent or a chalet containing a human volunteer or with a light left on (JG Logan, unpublished observation).

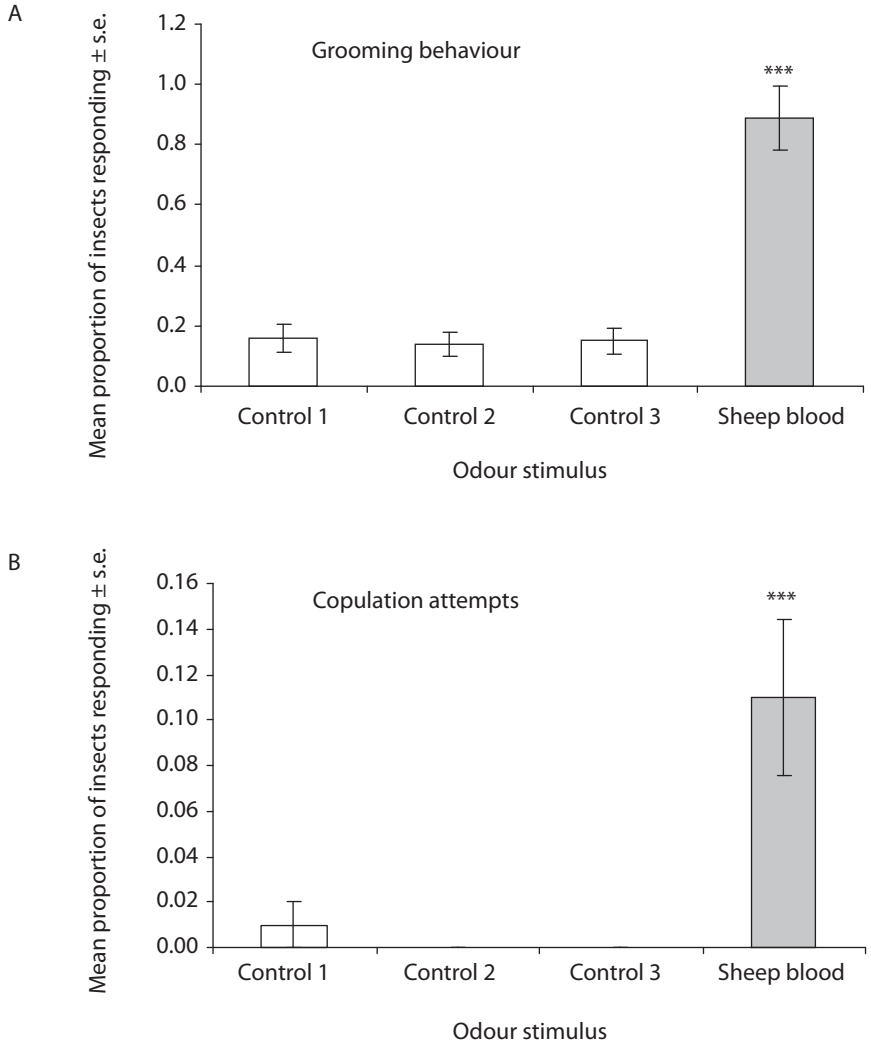

Figure 2. Behavioural responses of 24-48 h old adult Culicoides nubeculosus *females to sheep blood volatiles in Alsevers solution. (A) Grooming behaviour, (B) Copulation attempts.*
*Control 1: empty flask; Control 2: distilled water; Control 3: Alsevers solution. Mean proportions ±s.e. are shown. Asterisks indicate statistically significant differences from the controls (ANOVA *** P<0.001; n=100 replicates and 6 insects (3 males and 3 female) per replicate).*

In northern France, adult parous female *C. obsoletus/C. scoticus* activity is greater indoors than outdoors with a high proportion being freshly blood fed, but only during the autumn months. Similarly, *C. bolitinos* Meiswinkel, *C. dewulfi* and *C. chiopterus* are thought to be strongly endophagic whereas *C. pulicaris* and *C. punctatus* Meigen are more exophagic. However, it could be that the housing of all farm animals may cause exophagic/exophilic species to enter buildings to feed (Meiswinkel *et al.* 2000). The olfactory processes and other behaviours associated with *Culicoides*

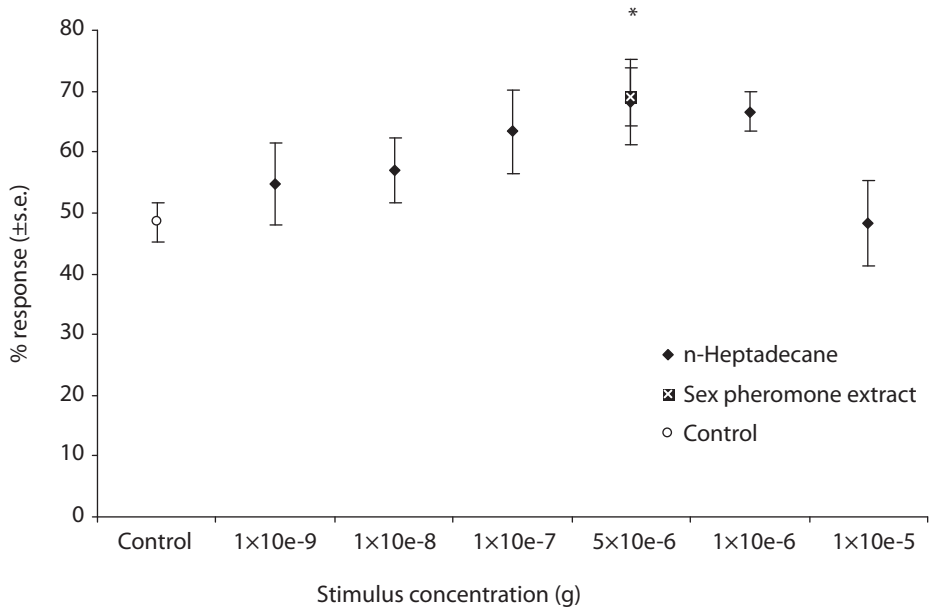

Figure 3. Behavioural responses of Culicoides nubeculosus *females to a pheromone air entrainment extract and six doses of n-heptadecane in hexane, in a Y-tube olfactometer.*
*Mean percentages of midges in the treated arm of the Y-tube ± s.e. are shown. Asterisks indicate statistically significant differences from the control (ANOVA, * P<0.05; n=17, except pheromone extract where n=6).*

midges entering buildings are not fully understood and may be quite different to those associated with finding a host in an open environment and requires further investigation. This is particularly important since some authorities recommend that livestock should be housed at night to reduce *Culicoides* attack rates.

Climatic conditions

The interaction between *Culicoides* midges and their host can be heavily influenced by climatic conditions. Midges display a preference for feeding in still, warm and humid conditions. Wind speeds of 1-2 m/s are enough to inhibit flight and subsequent host-location activities (Lehane 2005). Mellor *et al.* (2000) reviewed information on the suppression of flight for various *Culicoides* species, ranging from 3 m/s for *C. imicola* in Kenya (Walker 1977) to 2.2 m/s for *C. brevitarsis* in Australia (Murray 1987). It has also been noted that limits are lower for mating swarm formation by certain species. In addition to the negative effects of wind on activity, it can also affect the abundance of *Culicoides* through dispersal of the adults (Baylis and Rawlings 1998). Similarly, lower temperature limits for flight range from 10 °C for *C. variipennis* (Coquillett) (Nelson and Bellamy 1971) to 18 °C for *C. brevitarsis* (Murray 1987).

In our own landing catch experiments on the west coast of Scotland, the effect of weather on host-seeking *C. impunctatus* females can clearly be seen (Figure 4). Although this species is active in light rain and heavy cloud cover, it is less so during heavy rain. Also, as one might expect, fewer midges land on the arms of human volunteers with increasing wind speed. In contrast to

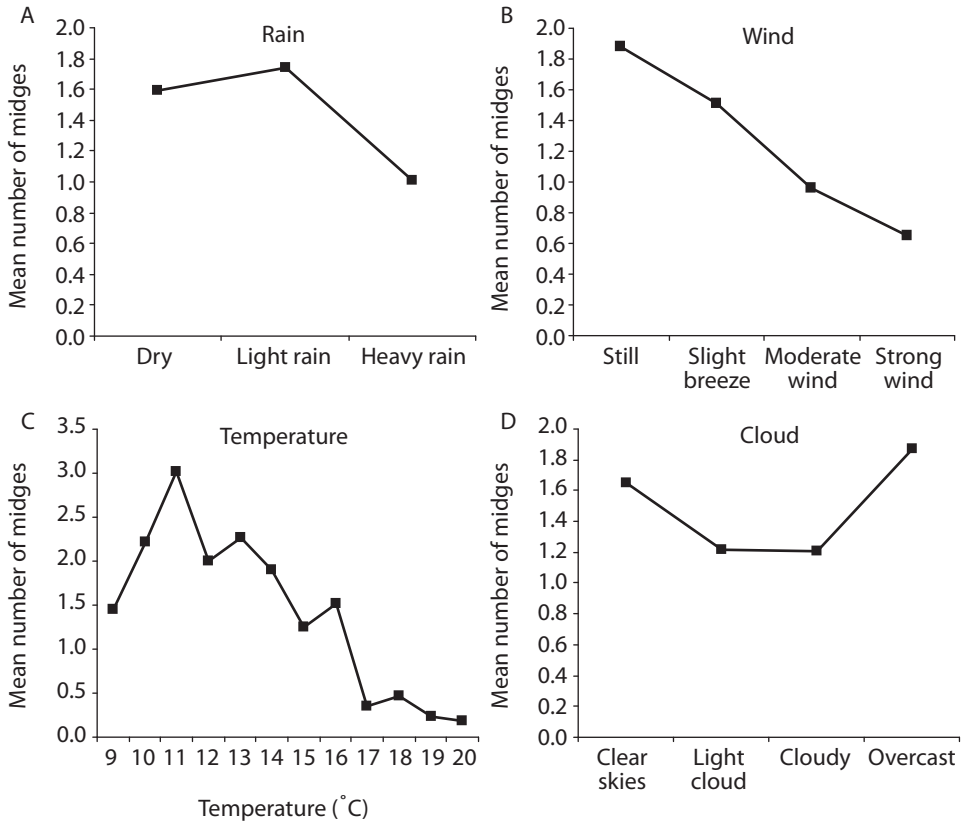

Figure 4. Mean number of Culicoides impunctatus *midges landing on the forearms of volunteers under different weather conditions on the west coast of Scotland during a 3 min repellency trial.*

other species, their host-seeking behaviour is high above 9 °C and optimum at around 11 °C, but decreases with increasing temperature and with very little activity above temperatures of 17 °C. (JG Logan, unpublished observation)

Transmission of BTV and AHSV by *C. variipennis sonorensis* Wirth and Jones is favoured by high temperatures (27-30 °C) as the reduction in longevity is compensated for by the shorter viral extrinsic incubation period (EIP). Contrastingly, at lower temperatures where adult longevity was extended, the EIP was disproportionately prolonged, meaning few midges could survive long enough to transmit the virus (Wittmann 2000).

Intra and interspecific differential responses to host

Midges are differentially attracted to their vertebrate hosts. For example, odours collected from different host species such as waterbuck, pony, red deer, cattle and sheep result in differential trap catches of *C. impunctatus* females (Mands *et al.* 2004). Interestingly, there is anecdotal evidence that individual human hosts also show variation in their attractiveness, and it is hypothesised that more unattractive individuals produce 'masking compounds' that might indicate host unsuitability

to midges (Mordue (Luntz) and Mordue 2003). Much evidence exists that individual human beings are differentially attractive to mosquitoes (Brady *et al.* 1997, Brouwer 1960, Burkot 1988, Khan *et al.* 1966, Lindsay *et al.* 1993, Mayer and James 1969, Qiu *et al.* 2004, Schreck *et al.* 1990) and there is much anecdotal evidence for biting midges. Recently, *C. impunctatus* has been shown to respond differentially to odours collected from individual human volunteers (Logan *et al.* 2009). Certain chemicals that were found in greater amounts in extracts from volunteers that caused low attractiveness, elicit a repellent effect in laboratory assays and repellency trials in the field. It is suggested that differences in the production of these natural human-derived compounds could help to explain differential 'attractiveness' between different human hosts to *C. impunctatus*. A mixture of two compounds in particular, 6-methyl-5-hepten-2-one and geranylacetone ((*E*)-6,10-dimethylundeca-5,9-dien-2-one), showed significant repellency in the field and have the potential to be developed as novel repellents. These types of semiochemicals may interfere with olfactory mechanisms from a distance, preventing the insects from being attracted to their hosts, as has also been demonstrated for *Ae. aegypti* L. (Logan *et al.* 2008). Similar studies have shown that some individual cattle produce masking compounds such as the isoprenoid derivative, 6-methyl-5-hepten-2-one, that make them less attractive to the fly, *Hydrotaea irritans* (Fallen), than others within a herd (Birkett *et al.* 2004).

Most chemicals that are described as causing positive behavioural responses in *Culicoides* and other haematophagous insects, such as CO_2, 1-octen-3-ol, lactic acid, ammonia, acetone and fatty acids are found ubiquitously in all humans and in many other vertebrates. Logan and Birkett (2007), in their review of semiochemicals for biting fly control, proposed such chemicals may comprise a basic 'core' suite of olfactory signals that, when present, convey information to an insect that a vertebrate is nearby. Alteration of this 'core' by addition, or increase, of certain other chemicals that can 'mask' activity of these attractants, or act as repellents (Pickett *et al.* 1998), could be a way by which inappropriate or unsuitable vertebrate hosts are avoided by host-seeking insects.

There is some evidence that the production of repellents in some vertebrates is genetically determined (Thomas *et al.* 1987). If this is true for *Culicoides* hosts, it could be exploited to develop breeding programmes to select for unattractive individuals. However, our knowledge of *Culicoides* host preference to date is very poor for many *Culicoides* species and particularly for the vectors of BTV and therefore, this requires further investigation.

Investigating host-derived semiochemicals for the control of midges

The exploitation of host-derived semiochemicals in control technologies first requires an in-depth knowledge and understanding of ecologically relevant compounds that are likely to be of practical use. It is therefore vital that we appreciate how target insects interact with such compounds at the behavioural and physiological level by investigating the ecology of the insect-host interaction. Various chemical ecology-based techniques can facilitate this. Semiochemicals can be isolated from relevant odour sources by air entrainment, solvent washings, solid phase micro extraction (SPME) and vacuum distillation. Behavioural and electrophysiological assays can then be performed to locate and identify biologically and ecologically relevant semiochemicals. Subsequent analytical chemistry techniques, such as gas chromatography (GC) and GC-mass spectrometry (GC-MS) can identify the chemicals accurately. Fractionation has been used in the past although this can be a laborious task with compounds being missed. A more efficient method is to use gas chromatography combined with electroantennography (GC-EAG) or single-sensillum recordings (GC-SSR), which is a unique and effective way of locating relevant components

within a complex array of chemicals in natural extracts. Such techniques are used to identify semiochemicals that can be integrated into methods for the management and control of insect pests as well as enhancing our knowledge and understanding of the behaviour and evolution of mosquitoes and midges.

Olfaction and electrophysiology

Midges, like most haematophagous insects, have a well developed olfactory system and use their antenna and maxillary palps to detect semiochemicals from vertebrates, giving them information about the location and suitability of a host. A full understanding of how *Culicoides* detect olfactory signals from their host to date is a poorly studied area, but it is vital to the development of strategies aimed at controlling these insects (Grant and Kline 2003). Such an understanding of the olfactory process can provide important information about the way *Culicoides* midges detect and respond to host odour stimuli.

Insects detect odour molecules (kairomones) using olfactory receptors known as sensilla. The olfactory sensilla of different arthropods show a high degree of similarity (Hallberg and Hannson 1999). This suggests that there may consequently be a degree of similarity in odour responses of different arthropod species. Although this is the case for ubiquitous compounds such as CO_2, it is often not the case for other olfactory stimuli. The sensilla are porous hairs located on the antennae and maxillary palps of the insect. Branched dendrites are found extending up into the hair and these dendrites join to a sheathed neurone, which has axons leading to the central nervous system. The sensilla are well designed for odour capture with the whole of the surface area involved in the process. An odour molecule impacting upon a sensillum diffuses laterally through the surface layers. These include an outer layer of polymerised lipid with a thin outer membrane composed of electron transport material, and an electron dense layer. After diffusing through these layers, the odour molecule reaches the receptor lymph where it is thought to be bound to an odourant binding protein (OBP) resulting in the formation of a complex. This complex is then bound by a receptor binding protein present in the membrane of the dendrite, which subsequently causes a change in membrane potential. The altered membrane potential allows an ion flux across it creating a receptor potential. The receptor potential may be large or small depending on the magnitude of the original stimulus. From the dendrite, the receptor potential spreads to the cell body and action potentials are produced. These action potentials are very rapid, lasting only a few milliseconds, and fire at different frequencies proportional to the magnitude of the receptor potential. Therefore, it can be seen how an impacting odour molecule is converted into electrical signals that can be processed by the central nervous system of the insect (Zwiebel and Takken 2004, see also Chapters 2 and 4 in this Volume). By connecting microelectrodes to an antennal preparation of an insect, the detection of a compound can be recorded as an electroantennogram (EAG), which is the electrical potential from the insect's antennae.

Culicoides antennae and maxillary palps have several olfactory sensilla types including trichodea which have multiporous walls and a number of neurones which are typical of olfactory receptors. They also possess basiconic and coeloconic sensilla which have double walls and unbranched dendrites which could be olfactory, thermo-, or hygroreceptors (Blackwell *et al.* 1992, Chu-Wang *et al.* 1975). Morphological characteristics of the olfactory apparatus, such as the size and shape of the subapical labral sensilla (also known to contain olfactory receptors), the size and depth of the palpal sensory pit, and the number and shape of heads of the palpal sensilla, can be used to classify and identify *Culicoides* species (Blackwell 2004, Braverman and Hulley 1979, Jamnback 1965, Lane 1984). In some cases, it is believed that differences in the number of sensilla coeloconica and

palpal sensilla can even determine host preferences. For example, antennae having few antennal flagellomeres with pits and less coleloconic sensilla might be associated with the need to detect large amounts of odours and thus could be characteristic of mammalophilic species (Blackwell 2004, Jamnback 1965). Blackwell (2004) noted that some species found in the West Indies, such as *C. darlingtonae* Wirth and Blantone, *C. glabellus* Wirth and Blantone, *C. insinuates* Ortiz, *C. paraensis* (Goeldi), and *C. pseudodiabolicus* Fox are morphologically characteristic of mammalian feeders when compared to other species such as *C. heliconiae* Fox and Hoffman and *C. flavivenula* Lima whose host preferences were unknown. Although such morphological studies can be useful to identify certain *Culicoides* species, they may also provide some information about host preference and may even indicate the types of compounds they can detect. However, to fully understand the olfactory process, a physiological investigation is usually required.

To date, electrophysiological studies on *Culicoides* midges are scarce. Most work has been performed on the European species, *C. nubeculosus*, to look at the response of host odour extracts and individual compounds. Whilst laboratory models are useful, where possible, working with the insect of interest is preferable. It is also preferable to work with field caught insects as it has been shown for some insects, such as the tsetse fly, that responses can diminish within a few generations. The better studied (at the behavioural level) *C. impunctatus* has also been investigated using electrophysiology, but with more logistical difficulties due to the fact that this species is only available from field populations. *Culicoides impunctatus* females have a range of olfactory chemosensilla on their antennae that respond to host-derived chemicals (Bhasin *et al.* 2000b, Blackwell *et al.* 1996, Pappenberger 1996, Sutcliffe 1994) and on the maxillary palps that contain the receptors for CO_2 (Bhasin *et al.* 2001). *Culicoides impunctatus* has antennal receptors that respond to a variety of other host derived kairomones including 1-octen-3-ol and other plant-derived compounds including methyl salicylate (Blackwell *et al.* 1996, 1997). Blackwell *et al.* (1995, 1996) were the first authors to describe the olfactory basis of host seeking in *C. impunctatus* and since then, other authors have demonstrated EAG activity for other host-derived kairomones, including CO_2, 1-octen-3-ol, phenols and acetone (Bhasin *et al.* 2000a, Blackwell *et al.* 1997, Grant and Kline 2003). Most recently, fifteen EAG-active compounds were found in human odour extracts and some new compounds for *Culicoides* midges were identified including benzaldehyde, 6-methyl-5-hepten-2-one, octanal, 2-ethylhexanol, limonene, dihydromyrcenol, nonanal, linalool, (*E*)-2-nonenal, menthol, naphthalene, decanal, geranylacetone and alpha-*iso*methylionone (Logan *et al.* 2009).

It is also thought that measuring the electrophysiological response of the receptors on the antennae can determine host preferences. For example host-odour extracts were assayed on parous female *C. impunctatus* response in a Y-tube olfactometer and by EAG on *C. nubeculosus* laboratory-reared parous females. Positive behavioural responses to host odours were dose-dependent with a water buffalo extract being most active (threshold 0.22 g/ml), similar to deer, whereas other host extracts were less active. Correspondingly, the EAG threshold was lowest for water buffalo, 10-fold greater for deer, calf and pony, but not detected for sheep (Mands *et al.* 2004).

Recordings can also be made from single olfactory receptor neurones (ORNs) by inserting a microelectrode into the sensilla to detect which receptor is responding to specific compounds. In *Culicoides*, this has not been done for receptors on the antenna, but has for receptors on the maxillary palps. The sensilla basiconica, which are located on the maxillary palps of *Culicoides*, contain an ORN that responds to CO_2 (Grant and Kline 2003) and shares physiological characteristics with mosquitoes (Grant *et al.* 1995). The CO_2 receptor in *Culicoides* midges is thought to be extremely

sensitive with the threshold of detection being significantly lower than that of mosquitoes. For example, stimulation with 150 ppm CO_2 elicits a response in *C. furens,* whereas in *Ae. aegypti,* higher concentrations are required to elicit a response (Grant *et al.* 1995, Grant and O'Connell, this Volume). This suggests that *Culicoides* ORNs to CO_2 are likely to be more active at ambient levels.

A study involving EAG is useful to screen whole extracts or single compounds that are likely to have ecological relevance to *Culicoides* midges. However, to fully understand how they find their hosts by way of olfaction and to identify novel semiochemicals, natural host odour, which comprises a complex mixture of relevant and irrelevant compounds, must be separated and tested. Screening all host-derived compounds (often in excess of 400 volatile chemicals) by EAG and in behavioural assays would be a time-consuming process and is highly impractical. A more efficient solution is to analyse human odour extracts by coupled gas chromatography-electroantennography (GC-EAG). This technique allows the location of EAG-active chemicals within complex extracts by taking advantage of the high-resolution of the GC while simultaneously recording the response of an antennal preparation of a female midge. This technique was first reported in 1969 (Moorhouse *et al.* 1969) and has been used for many insects (Logan *et al.* 2008, Pickett and Woodcock 1996). However, due to the difficulties with rearing *Culicoides* midges in the laboratory, electrophysiological techniques are complicated. Although portable EAG kits are available (see Syntech, http://www.syntech.nl/), they can be difficult to use. When searching for novel host-derived semiochemicals, it is essential to combine the electrophysiology techniques with that of gas chromatography. This poses a massive logistical problem with such high-tech equipment. However, in our laboratory, we are able to send *C. impunctatus* adult females (in a moist box), previously caught in Argyllshire in Scotland using a sweep net to our laboratories at Rothamsted in Hertfordshire (a distance of ~400 miles and taking around 24-48 hours). The coupled GC-EAG has been advanced to possess the capabilities of also detecting responses of single olfactory cells (GC-SCR) (Wadhams 1982), but this has never been performed on *Culicoides* midges.

Generally, insect olfactory systems are not only sensitive to specific molecular structures, but also to ubiquitous compounds. In the later case, sophisticated mechanisms such as coincidence detection, whereby blends of volatiles in specific ratios from a host plant are detected by insects within a complex background of volatiles from non-host plants, may be used. This might be facilitated by paired or clustered ORNs that allow fine scale resolution of such complex signals. Odours from a host are likely to occur in 'pockets' emanating from a host and therefore receptor cells that respond simultaneously may indicate the presence of a host, whereas the same receptor cells being stimulated with a delay would not (Bruce *et al.* 2005). Although this hypothesis has only been described for phytophagous (plant-feeding) insects thus far, coincidence detection might also occur in biting flies including *Culicoides* midges considering the complexity of odours within their environments (Chapter 6, this Volume).

Laboratory-based experimentation

To understand how an insect responds to natural semiochemicals, laboratory bioassays are commonly used as a first step. These behavioural experiments can be useful to determine the effect of semiochemicals on midge behaviour whilst minimising other environmental variables and provide a simple and rapid means of quantifying behavioural responses to many types of stimuli. Some of the earliest behavioural studies with haematophagous insects were conducted using host-seeking mosquitoes and used simple bioassays such as enclosed chambers or arenas to examine responses to temperature and moisture stimuli (Bar Zeeve 1960, Howlett 1910, Parker 1948, Thomson 1938). Such bioassays were only able to measure short range responses

(Bowen 1992). More recently olfactometers that allow and encourage flight responses to odour stimuli have been developed, particularly for mosquitoes, and provide crucial information about responses of insects to olfactory stimuli in many investigations. Dual choice olfactometers, such as Y-tubes, have also been used with midges and mosquitoes and allow insects to respond to two air streams containing different odour stimuli, often comprising one test air stream containing a stimulus and one control (blank) air stream (Bhasin *et al.* 2000b, Blackwell *et al.* 1996, Brown *et al.* 1951, Dekker *et al.* 2001, Geier *et al.* 1999, Logan *et al.* 2008, 2009, Mands *et al.* 2004, Price *et al.* 1979, Roessler and Brown 1964, Smart and Brown 1957, Qiu *et al.* 2005).

For *Culicoides* midges, most laboratory-based olfactometer studies have been conducted with *C. impunctatus*. Due to the problems associated with rearing this and many other *Culicoides* species in the lab, these assays can be difficult. However, where possible, taking portable equipment to a field laboratory can overcome this. For example, on the West coast of Scotland, a portable Perspex wind tunnel and small glass Y-tube olfactometers (Blackwell *et al.* 1996, Bhasin *et al.* 2000b) have been used to examine the response of wild populations of *Culicoides* to odour stimuli. The end of the wind tunnel is placed outside a window of the field lab and the number of midges that fly upwind from outside can be counted by the number of flashes on a killing grid. For other experiments, insects can be caught by a sweep net and used in the olfactometer inside field laboratory (Logan *et al.* 2009). In these smaller Y-tubes, the insects walk towards a light and enter one of two arms which can contain an odour stimulus. Logan *et al.* (2009) showed that *Culicoides* midges respond to certain chemicals in the Y-tube olfactometer including benzaldehyde, 6-methyl-5-hepten-2one, octanal, 2-ethylhexanol, limonene, dihydromyrcenol, nonanal, linalool, (E)-2-nonenal, menthol, naphthalene, decanal, geranylacetone and alpha-*iso*methylionone.

In this Y-tube assay light is used to stimulate upwind movement towards the arms of the Y-tube, thus 'forcing' the midges to make a preferential movement to either arm. The Y-tube allows for walking and some attempts at flight but not sustained flight and as such is not entirely representative of a natural situation where *C. impunctatus* females would normally fly toward their host by way of positive odour mediated anemotaxis. Insects may not respond the same to odour stimuli when walking as opposed to flying (Kennedy 1977) and it might allow chemotactic responses to odour stimuli to occur. Additionally, there can be steep odour gradients at the interface between airstreams and this may allow chemotactic and chemokinetic responses to occur which might result in insects remaining within one air stream (Kennedy 1977). Most olfactometers may allow several behaviours to occur simultaneously such as orthokinesis, klinokinesis, chemotaxis and anemotaxis and do not allow the discrimination between close and long range responses. Therefore, caution must be taken when interpreting behavioural results. Despite such complications, olfactometric experiments are a useful tool designed to give an indication of the response of biting insects to odour stimuli under controlled conditions and are thus useful to test host odours without the host suffering the discomfort of being bitten. This is particularly important for *Culicoides* research where midge populations can be so immense that field trials become ethically unacceptable. Encouragingly, previous studies have demonstrated corresponding results between such laboratory trials and the field for biting midges. For example *C. impunctatus* show positive responses to 1-octen-3-ol in a Y-tube olfactometer in the laboratory and trap catches are enhanced by the addition of this compound to traps in the field (Bhasin *et al.* 2000b, Blackwell *et al.* 1996, Mands *et al.* 2004). With this in mind, it is important that studies are also conducted in the field to test semiochemicals in a more realistic and natural setting.

Control and field based experimentation

Insecticide applications against adult biting midges have had a limited effectiveness especially when long-term relief from pestiferous populations is desired (Linley and Davies 1971). Furthermore, reduction of midge populations via larval control by the application of insecticides to the soil, or physical modification of wetland developmental sites, is not an option in many locations such as coastal wetlands and acidic bogs because immature developmental sites are extremely vast. Insecticide use in these areas is often forbidden due to environmental regulatory issues. Repellents only provide minimal and temporary relief and are not considered a long-term solution (Carpenter *et al.* 2005, Schreck and Kline 1981, Trigg 1996). Apart from altering one's life-style when midges are presented by staying indoors, repellents are presently the only means of self-protection. These provide an inexpensive reasonably efficient protection method against biting midges. DEET (*N,N*-Diethyl-*m*-toluamide), available since 1957, is still the most widely used repellent against insects and arthropods of medical importance such as mosquitoes and ticks and can show some efficacy against midges.

Ultimately, more effective control of *Culicoides* will need to rely on the development of integrated pest management programs using multiple control strategies to relieve the biting pressure of these pestiferous biting midges. New techniques are being investigated due in part to environmental concerns and desires to reduce the use of chemical insecticides. One technique that has received increased attention is the use of traps for control (Day and Sjogren 1994). The feasibility of reducing local biting midge populations through the use of removal trapping has recently been investigated by Kline and Lemire (1998; DL Kline unpublished data) in Florida, USA. They demonstrated that a single line barrier of either attractant-baited traps (CDC-type, Model 512, John Hock Company, Gainesville FL, USA) or cloth targets impregnated with an insecticide (lambdacyhalothrin) reduced mosquito and biting midge populations on a barrier island resort in Southwest Florida. Traps and targets were baited with CO_2 and 1-octen-3-ol. Subsequently, Day *et al.* (2001) reported the reduction of *C. furens* populations in two delimited areas along the Atlantic coast in South Florida and on an island in the Bahamas, using insecticide-treated fabric targets baited with carbon dioxide and 1-octen-3-ol. This mass trapping programme against *Culicoides* resulted in a significant reduction in the number of *Culicoides* throughout the island. Cilek *et al.* (2003) and Cilek and Hallmon (2005) attempted to reduce *Culicoides* populations around individual homes in north-western Florida using either one Mosquito Magnet® Pro (American Biophysics Corporation, North Kingston RI, USA[1]) or one ABC PRO suction trap (Clarke Mosquito Products Inc., Roselle IL, USA) per backyard. The ABC PRO traps were baited with CO_2 (500 ml/min) and a 4:1:8 1-octen-3-ol/phenol mixture of 1 octen-3-ol:3-n-propylphenol:4-methylphenol, released from 16 ml screw capped glass vials via a single wick (Dills TM pipe cleaner, United States Tobacco Sales and Marketing, Greenwich, CT, USA). Overall release rates during the study averaged 5.39±0.54 mg/h. Population reduction, however, was very inconsistent (ranged from 2.3% to 70.6%) over time and these researchers concluded that 1 trap per backyard was insufficient for consistent *Culicoides* population reduction. They suggested that using more than one trap per backyard, or a perimeter of traps, might be the key to achieving consistent population reduction. At certain times during the study it was obvious that the attractant-baited traps were 'overwhelmed' by the sheer volume of adult blood seeking midges moving into the targeted control zone. Another possibility to achieve consistent population reduction was tested by Lloyd *et al.* (2008) by placing traps in all the adjacent neighbouring yards, which in effect, created a barrier along the perimeter of their backyards. Each trap was placed in the centre of a 0.30-0.40-ha area, which resulted in full

[1] American Biophysics Corp went out of business in 2006

coverage of this residential area. In this study two commercially available attractant-baited traps were compared (two models of Mosquito Magnet™ [MM], American Biophysics Corporation, North Kingston RI, USA) on the island of Rye Key in Cedar Key, FL, USA to determine which model is more successful at capturing *Culicoides* spp. Functionally, the two trap models are similar with some difference in their bait-plume temperature ranges and CO_2 output. The plume temperature for the MM-Freedom® (Freedom) ranges from 33.34 °C to 36.67 °C compared to 33.34 °C to 40.56 °C for the MM-Liberty Plus® (Liberty Plus) (K McKenzie, personal communication). Both traps utilised the counterflow geometry design (Kline 2002) and their suction fans were powered thermoelectrically by the combustion of propane, which also resulted in the production of the attractants CO_2, moisture and heat. The Freedom produces ca. 420 ml/min CO_2, compared to ca. 550 ml/min CO_2, for the Liberty Plus. Both traps caught *Culicoides* spp. very well. Operationally, the Liberty Plus was the more reliable trap. There was an apparent species preference for the traps. The overall mean daily collection of *C. furens* in Freedom traps was significantly higher than the number collected in the Liberty Plus traps. In contrast *C. mississippiensis* selected the MM-Liberty Plus over the MM-Freedom. This could be due to temperature and CO_2 differences between the traps. *Culicoides furens* is attracted to heat (Kline and Lemire 1995), CO_2 (Kline *et al.* 1990) and 1-octen-3-ol (Takken and Kline 1989). It is possible that the Freedom and Liberty Plus are presenting the attractants differently (Cooperband and Cardé 2006), which could affect a trap's ability to attract and capture host-seeking insects. Kline *et al.* (1994) reported that *C. furens* was the only species of three major coastal *Culicoides* species that were attracted to a combination of 1-octen-3-ol bait and CO_2 in Sea Island, GA, USA. They reported that all *Culicoides* spp. were attracted to CO_2. The combination of heat, 1-octen-3-ol and CO_2 may be why *C. furens* was captured in such high numbers when compared with *C. mississippiensis* in this study. It is not certain what kairomones are used by *C. mississippiensis* during host location. Minimal work has been done with this species. What this means is that, for each target species, the trap type and attractant combination needs to be determined. This will be key to adult biting midge reduction using removal trap technology. For many *Culicoides* spp, especially those associated with livestock, this will require additional basic studies (such as those discussed earlier) to address this complex and challenging problem. The problem of protecting livestock on farms where a bite from a single viraemic midge is sufficient for transmission of BTV or AHSV remains a challenge (Mordue (Luntz) and Mordue 2006). Trap number and placement are also important points to consider. Just as important, adequate funding needs to be made available for such evaluations in order that innovative alternative control technologies can be transferred to the public in a timely manner.

Push-pull control strategy

Several ongoing research projects have evolved from these removal trapping studies to evaluating the feasibility of developing and utilising push-pull systems (DL Kline, unpublished data). Push-pull strategies involve the behavioural manipulation of pest species via the integration of stimuli that act to make the protected resource hard to locate, unattractive or unsuitable to the pests (push) while luring them toward an attractive source, such as an attractant-baited trap (pull) from where the pests are subsequently removed. Push-pull strategies maximise efficacy of behaviour-manipulating stimuli through the additive and synergistic effects of integrating their use (Cook *et al.* 2007). Development and use of push-pull strategies requires a thorough knowledge of a combination of behaviour-modifying stimuli that can be used to manipulate the distribution and abundance of pest insects for pest management. It requires a clear scientific understanding of the pest's biology and the behavioural/chemical ecology of the interactions with its hosts and its environment. The specific combinations of components differ in each strategy according to the pest species to be controlled (its specificity, sensory abilities, and mobility) and the resource

targeted for protection, for example people or farm animals. The pests are repelled or deterred away from this resource (push) by using stimuli that mask host apparency or are repellents or deterrents. These stimuli may act over the long or short range and ultimately lead to the pest being repelled or deterred from the resource or not even approaching it. Long range stimuli represent the first line of defence, preventing or reducing infestation in the first place. Stimuli that act over the short range, however, can be powerful tools in preventing specific pestiferous behaviours (Cook *et al.* 2007). In pull components of push-pull strategies, attractive stimuli are used to divert pests from the protected resource to a trap or target. The stimuli used to achieve this act mostly over a long distance. However, short-range stimuli can be useful additions to arrest and retain the pests in a predetermined place to facilitate the concentration of their populations and to prevent them from returning to the protected resource. The stimuli can be delivered in a variety of ways. Strategies primarily include visual and chemical cues or signals. Manipulation of colour, shape, or size has been studied in a limited way in Australia. Chemical stimuli, in particular semiochemicals, have the most versatility and potential for use in pest management. The principles of the push-pull strategy are to maximise control efficacy, efficiency, sustainability, and output, while minimising negative environmental effects.

Development of effective 'push-pull' systems for biting midge population management has been hindered by a lack of resources to develop the necessary components. Limited research at several locations worldwide has been dedicated to studies necessary to reveal the details of biting midge biology, life cycles and chemical ecology that could be applied to their control by behavioural means. Studies at these locations have mainly focused on nuisance pests of people. Very little work has been conducted on the 'push' component. Some studies are underway in the U.S.A. to determine the 'masking' effect of human volatiles and compounds such as linalool and dehydrolinalool (DL Kline, unpublished data) on host attraction of *Culicoides* to humans and horses, and by exploiting compounds discovered during investigations on the natural differential attractiveness within a host species (U. Bernier, personal communication). In the UK, repellents such as 6-methyl-5-hepten-2-one and geranylacetone that were identified from odours of 'unattractive' volunteers are now under development as commercially available topical repellents. Other studies have focused on the effectiveness of synthetic repellent products with either DEET or Picaridin as the active ingredient. Several chemicals related to DEET have been tested against midges and shown to be similar or more effective than DEET with respect to length of and degree of protection. One such compound, Bayrepel®, tested by Carpenter *et al.* (2005) on the Scottish biting midge proved to be effective for up to 8 hours post application. Plant essential oils such as citronella and eucalyptus, PMD (p-menthane-3,8-diol), isolated from lemon eucalyptus oil of *Eucalyptus citriodora* Hook. have been found to be effective against *C. impunctatus* (Trigg 1996). Eucalyptus and other plant-derived repellents lose effectiveness more quickly with time than synthetic compounds such as DEET. Blackwell *et al.* (2004) demonstrated the effectiveness of neem against *C. impunctatus* and *C. nubeculosus*. Recently, spatial repellents, which repel from a distance under field conditions, have been identified for *C. impunctatus* (JG Logan, unpublished observation).

Most of the research efforts have been invested in determining what kinds of semiochemicals and traps/targets can be used in the 'pull' component. A limited number of the well known host kairomones that mediate behavioural responses in other haematophagous insects (Bursell *et al.* 1988, Hall *et al.* 1984, Hassanali *et al.* 1986) have been shown to be active in the field against biting midges. Kairomones such as those found in breath, skin emanations and urine are the main sensory cues used by haematophagous insects to find their hosts. The emphasis has been on kairomones associated with humans and livestock including mammalian-associated volatiles such

as CO_2, 1-octen-3-ol and acetone from the breath, and a mix of body odours (Bhasin *et al.* 2001, Gibson and Torr 1999, Logan and Birkett 2007). Carbon dioxide (CO_2) was first shown by Nelson (1965) to be an attractant for members of the *C. variipennis* complex and, more recently, 1-octen-3-ol has been used to trap *C. furens* (Kline *et al.* 1990, Takken and Kline 1989). Carbon dioxide has a particularly important role in the attraction of haematophagous Diptera to their hosts (Gillies 1980). Stimulation by above-threshold levels of the gas, downwind of the host, activates them to respond to other kairomones and elicits upwind orientation (Bursell 1984, Healy and Copland 1995, Schofield and Brady 1997). It is released periodically from a mammalian host at an expired concentration of 4-5% above background (0.03%), and forms a filamentous plume, the exact structure of which is determined by factors such as source size and wind speed (Brady *et al.* 1989, Hargrove *et al.* 1995, Chapter 5 in this Volume). Response thresholds for activation by CO_2 are typically low: 0.006-0.01% for *Stomoxys calcitrans* (L.) (Schofield *et al.* 1997, Warnes and Finlayson 1985) and 0.01% for tsetse (Bursell 1984). Combination baits used have typically utilised CO_2 and 1-octen-3-ol (Ritchie *et al.* 1994, Takken and Kline 1989). Field studies have shown that CO_2 and 1-octen-3-ol act together to exert a synergistic effect on catches of *C. furens* (Kline *et al.* 1994). Maximal upwind responses occur to CO_2 plus 1-octen-3-ol or CO_2 plus acetone but only at host emission concentrations with higher concentrations causing arrestment (Bhasin *et al.* 2001). A combination of 1-octen-3-ol and a pheromone produced by a host-seeking female *C. impunctatus* has shown activity, both in the field and in the laboratory (Blackwell *et al.* 1996). For the Scottish biting midge traps containing host kairomones such as CO_2 with 1-octen-3-ol, acetone, a mixture of alkyl phenols, or human sweat components have been tested in the field (Bhasin *et al.* 2001, W Takken and AJ Mordue (Luntz), unpublished data) and promising combinations of attractant cues are being defined. Our work and that of others is beginning to reveal that certain human sweat components such as 3-methyl-2-hexanoic acid or 7-octenoic acid are either attractive to, or ignored by, midges in their repertoire of host attractants (AJ Mordue (Luntz) and J Pickett, unpublished data). Using knowledge of host specificity and preferences, the attractiveness of synthetic host odour blends can be maximised.

One difficulty with the current emphasis on host-seeking kairomones is that some species like *C. impunctatus* (Blackwell *et al.* 1992) and *C. mississippiensis* (Davis 1981) are autogenous species (Blackwell *et al.* 2004), which produce a significantly larger egg-batch in their first, host-free, period of oogenesis (Blackwell *et al.* 1992, Boorman and Goddard 1970). This, together with the paucity of blood meals in most areas of the highlands, would suggest that strategies acting upon the parous, biting proportion of the *C. impunctatus* population are unlikely to lead to more than short-term changes in the biting rates experienced, without constant, high intensity trapping. Thus research efforts should also be made to discover potential nectar sources. Both sexes utilise nectar and sugar feeding for various life cycle activities. Nectar sources are of particular importance because nectar provides energy for sustained flight in mating swarms (Downes 1969), increases adult longevity (Linley 1966a), and may play a role in egg maturation (Linley 1966b). The females of some species are not able to survive to the time of the first oviposition without nectar (Downes 1958). Volatiles from flowers may be utilised by adult biting midges to locate a nectar source. If these volatiles can be isolated and identified they can be used as attractants with traps or targets. Possible nectar sources have been determined for adult *C. mississippiensis* near Yankeetown, Levy County, Florida. Large numbers of adults have been found in early spring on flowering shrubs such as *Ilex vomitoria* Aiton. Other habitat related cues (apneumones) may be used by females to locate suitable oviposition or resting sites. Carpenter *et al.* (2001) identified the odour of *Juncus* spp. infusions together with the visual cue of upper layer photosynthetic sphagnum to be important in the induction of oviposition of *C. impunctatus*.

Numerous types of traps are available for use in the 'pull' component. Only the Midgeater and Midg-IT were developed specifically for biting midges. Their design is very similar to the Mosquito Magnet™ series of traps. A plethora of traps with various killing mechanisms including electrocution, drowning, sticky boards, live catching and desiccation, originally developed for mosquito control have been evaluated for their efficacy in capturing biting midges (DL Kline, unpublished data). The most widely used trap is the MM-Liberty Plus. New advances in trap technology continually need to be identified and subsequently evaluated. Various commercially available lures (several manufacturers of 1-octen-3-ol lures, Lurex [lactic acid], Lurex3 [lactic acid + ammonium bicarbonate] and BG Mesh lure [lactic acid + ammonia + propionic acid] as well as proprietary experimental lures, are currently being evaluated at Cedar Key, FL, USA. Visual aspects of trap design on biting midge attraction have not been evaluated very much, but need to be. Colour combinations, size and shape may be very important. Visual factors may enhance the effectiveness of the olfactory stimuli. For example, blue and black traps, approximating the size of a mammalian host, are used to control cattle tsetse fly (*Glossina* spp.). Crucial to the development of efficient traps was the finding that black stimulates landing (Gibson and Torr 1999). Traps need to be designed with visual (A Bhasin, unpublished observation) and olfactory 'pull' stimuli that help them compete effectively with the hosts that they are trying to protect and with other surrounding environmental stimuli. Trap design and positioning are important and can be maximised by adopting a systematic approach in which the behaviour of the insect is closely observed.

Culcioides and climate change

Changes in distribution and abundance of insects are likely to be amongst the most important and immediate effects of climate change (Wittmann and Baylis 2000). In addition to increases in temperature, changes in precipitation, wind patterns and climate variability are also predicted to occur (Houghton 1997). The global atmospheric concentration of CO_2 is rising markedly as a result of human activity. The average level has increased from about 280 ppm immediately before the industrial revolution to a daily average of 380 ppm in 2005 and is increasing at a rate of 2 ppm per year (Guerenstein and Hildebrand 2008). Such an increase in CO_2 is expected to affect the biology of a number of living organisms including insects and therefore is stimulating research on what those effects could be. Mondor *et al.* (2004) quoted an Intergovernmental Panel on Climate Change (IPCC) report from 2001 which states CO_2 and ozone (O_3) have risen 31% and 35% respectively since the mid-1800s. As well as contributing to effects of temperature, and the aforementioned effects on insects, these increasing levels of CO_2 and O_3 affect plants considerably. Elevated concentrations of these gases alter the nutritional and defensive characteristics of plants, and subsequently these effects can cascade through ecosystems and in turn impact upon higher trophic levels, such as insect herbivores and their natural enemies (Percy *et al.* 2002). All these changes may help create novel vector species by removing some of the barriers that render many *Culicoides* species refractory to infection. It is worth noting that it is not just the adult insects that may be affected. Borkent (2005) states that a variety of environmental factors have been studied for a few pest species, and the results indicate that temperature, moisture levels, chemical composition of the substrate, and population density all affect the rate of development of immatures.

Climate and weather have dramatic effects on *Culicoides* populations and, consequently, the epidemiologies of midge-borne viral diseases are similarly affected (Mellor *et al.* 2000). Due to their small size, adult *Culicoides* are particularly susceptible to desiccation and even brief periods at low humidity can reduce longevity (Murray 1991). However, changing meteorological factors

are not necessarily detrimental to the insects. While there are limits to vector competence, for example BTV and AHSV are unable to develop in *C. variipennis sonorensis* at temperatures below 14-15 °C (Mullens 1995, Wellby *et al.* 1996, Wittmann 2000), as stated previously, suitable changes in temperature may allow an increase in viable vectors.

Arndt (1995) investigated whether air pollutants react with pheromones, which would have potential ecological and economic consequences. Specifically, he studied the effects of ozone (O_3) on the pheromone-producing insect, *Drosophila melanogaster* Meigen. It was found that total pheromone containing extracts as well as commercially available pheromones lost their biological activity after short-term ozone fumigation at environmentally realistic levels. At the time the authors could find only one other reference relating to the subject. These findings, if true for other insects, suggest that climate change may have great impact on intraspecific and feasibly interspecific communication, particularly with the involvement of pheromones such as those for *C. impunctatus* and *C. nubeculosus*.

More recently Mondor *et al.* (2004) showed the existence of divergent pheromone-mediated behaviours in insects under conditions of global atmospheric change. Elevating CO_2 and O_3 levels, they investigated effects on a common aphid, *Chaitophorus stevensis* Sanborn. They showed that the aphids principle means of defence, dispersal response to alarm pheromone, decreased under elevated CO_2, however, increased under elevated O_3. Although the mechanism behind this differential dispersal response remains unknown, it is clear that elevated greenhouse gases may impact greatly upon intraspecific olfactory communication.

A further impact of climate change may be upon the voltinism of a species. *Culicoides impunctatus*, for example, is bivoltine, with two distinct emergence periods (Blackwell 1997) and has recently been shown to be orally susceptible to BTV in a laboratory study (Carpenter *et al.* 2006). However, alterations to temperature may create favourable conditions that allow additional generations within a given year. Tobin *et al.* (2008) investigated such effects using a phenology model of the grape berry moth, *Paralobesia viteana* (Clemens). It was designed to incorporate temperature-dependent development and diapause termination, as well as photoperiod-dependent diapause induction. They found that increases in mean surface temperature of greater than 2 °C can cause shifts in the ovipositional period resulting in dramatic effects on insect voltinism. Interestingly, they mention anecdotal evidence from grape growers that already suggests a shift, with increases in late summer populations. This will affect hosts and susceptibility and could even alter host preferences with changing farm animals and farming practices.

Such effects may have substantial implications not just from an ecological perspective, but also for how insect-derived compounds are used in pest management programmes. Different trapping methods can suggest different activity patterns and some meteorological conditions can affect the efficiency of trapping as well as the activity of the midge (Mellor *et al.* 2000).

Grant and Kline (2003) suggest that that a larger change in stimulus concentration is required to elicit a larger change in impulse activity than *Aedes* spp. mosquitoes indicating that *Culicoides* spp. perceive larger changes in carbon dioxide concentration than *Aedes* spp. It is possible that CO_2 at low concentrations is similar to that produced by photosynthesising plants and therefore, in conjunction with other plant-derived volatiles, may provide information enabling location of nectar for sugar feeding. Additionally, higher concentrations may serve as a host-seeking attractant for blood feeding. Ambient levels of CO_2 are higher now than ever and therefore the CO_2-sensitive neurons are possibly adapted to lower, pre-industrialisation levels of carbon dioxide

(see also Chapter 5, this Volume). This could simply reflect an earlier sensitivity, which has not genetically adapted to the dramatic CO_2 increase caused by accelerated human-based emissions of carbon dioxide (Grant and Kline 2003). With a higher level of ambient CO_2 this could be altering the ability of *Culicoides* spp. midges to respond to CO_2. Because ambient concentrations are much higher, more subtle (or smaller) changes in CO_2 need to be detected when host-seeking. This could hamper the ability of *Culicoides* spp. midges to host-seek and mosquitoes may have adapted to be able to do this. This demonstrates that specific information about *Culicoides* spp. midges is vital for the development of odour baited traps using CO_2 and other semiochemicals as the responses at the physiological and behavioural level differs to that of other haematophagous insects. However, this sensitivity to low concentrations of CO_2 may not have any effect on behaviour.

Conclusions and future prospects

For many *Culicoides* species, most aspects of their ecology and behaviour, and in particular the olfactory processes, remain undefined. This is in part due to the fact that *Culicoides* research is hindered by the difficulties associated with rearing these insects. Although *C. sonorensis* and *C. nubeculosus* have been successfully colonised, many species, including some other vectors of BTV, have not. This means that most studies are carried out with the 'laboratory models' or have to rely on field populations. For *C. impunctatus*, work has been carried out leading towards their successful colonisation. Suitable conditions for adult blood feeding, oogenesis, oviposition and adult survival (Carpenter *et al.* 2001, 2006), in the laboratory have been established but culture of the larvae remains a challenge for this species. Additional colonies of *Culicoides* species would allow much needed basic studies under controlled laboratory conditions to be carried out.

There are many other aspects of midge ecology that need to be elucidated. For example, climatic conditions heavily influence host location behaviour. Although there is some knowledge for species like *C. impunctatus*, other species are unstudied. This lack of knowledge, and particularly in light of climate change, is likely to affect and potentially hamper future control efforts. Additionally, oviposition site cues, those associated with the location of nectar, and pheromones involved in mate recognition and/or aggregation behaviour could provide new semiochemical tools. Investigating host preferences (at the intra and interspecific level) has already revealed potential new semiochemicals that could be exploited to develop better attractants for traps and new repellents for protection. Taking this further and understanding how unattractive odours are produced by vertebrates at the molecular/genetic level could lead to new breeding programmes whereby the trait for being unattractive is selected for. All of these areas of research warrant further investigation.

The combination of attractants and repellents could provide a push-pull control strategy, but further research is required on how this could be achieved for the many different *Culicoides* species. Additionally, formulation and delivery systems studies are needed to provide optimum technologies for specific species in specific geographical regions. Synthetic production of semiochemicals, and their formulation as sprays or in slow-release dispensers, will ensure standardisation and will contribute to the robustness of such strategies. GIS technologies could also be incorporated into biting midge control programs. An improved understanding of the spatial-scale effects of pest population dynamics and potential host interactions, coupled with increased capability of spatially explicit computer models, will enable us to deploy more accurate components of the various management strategies in terms of the quantities needed and their spatial distribution. Ultimately, a better understanding of the behaviour of *Culicoides* species midges, enabled by advances in analytical techniques, synthesis procedures, and formulation

science, may provide us with a larger and more effective armoury of semiochemicals and other stimuli for future use.

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