13. Behavioural modalities of 'non-vector' biting Diptera: from olfaction to feeding

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Abstract

Though they are not considered to be important vectors, stable flies and related species can have direct economic impacts, and as well might influence pathogen transmission by affecting the feeding behaviour of flies such as tsetse. We discuss here the responses of Stomoxyinae to host-based kairomones. Further, we consider the interplay between these Diptera, other vectors and their common hosts as it relates to disease epidemiology and control strategies. In general, we find that the odour-evoked responses of stable flies in the laboratory are similar to other groups of fast moving Diptera that blood feed (e.g. tsetse), i.e. activity increases and flights are steered upwind. In field studies, several compounds have been associated with increased catch of Stomoxyinae from traps, but only CO_2 consistently achieved this effect. On the whole, results suggest that non-CO_2 kairomones are not that important to host finding by *Stomoxys* and, further, that synthetic host odours in general might not have a place in sampling or control schemes for this pest. We also describe the interplay between Stomoxyinae and their hosts, highlighting the role that their feeding behaviour might have as regards the epidemiology of animal trypanosomiasis. Finally, we discuss briefly some of the potential practical applications that might arise from a better understanding of the interrelationship between *Stomoxys* species and their hosts.

Keywords: behaviour, host odour, olfaction, *Stomoxys*, *Glossina*, stable fly, tsetse fly

Introduction

The blood feeding habit has arisen independently in several orders of insects (Lehane 2005) and, with this, has come a means of spreading a variety of human and veterinary pathogens. These range from those with a modest or focal impact on human population health (e.g. Murray Valley encephalitis, La Crosse encephalitis), to others that play a significant role more-or-less globally (e.g. malaria, dengue). It is our desire to manage the threats presented by vector-borne pathogens that, to a large extent, has driven research to understand the behavioural ecology underpinning haematophagy. Such efforts have aimed to provide novel means of controlling disease by, for example, luring vectors to lethal baits or developing means of preventing them from finding and biting a host. Unfortunately, and outside some notable exceptions, efforts in this area often have not contributed meaningfully to control efforts. There are many reasons for this, but it no doubt at least partly reflects a systematic failure on the part of investigators to appreciate and/or evaluate behaviour *sensu stricto*. Instead, the tendency has been to measure the final outcome of a complex repertoire of behaviours. To paraphrase, we have spent entirely too much time catching biting insects in traps (or huts, or on hosts, or in olfactometer ports) and not nearly enough time analysing, quantitatively, the behavioural processes that got them there in the first place. Why this should be the case is difficult to fathom, especially given that some of the earliest research (Kennedy 1939) in the field seemed to avoid this pitfall. Moreover, early workers did warn of the potential problems with taking a 'black-box' approach to behavioural research. One of the most vocal in this regard was John Kennedy, who often discussed the potential for bias in behavioural work, especially where experimental endpoints are framed through an anthropomorphic lens (Kennedy 1992). In any event, one result of not heeding these cautions is that our understanding

of the behavioural ecology of vectors is poorer than it ought to be. Whether or not this failing has impacted on the effectiveness of interventions that target vectors remains unclear.

On the upside, in recent years the need to analyse vector behaviour objectively and quantitatively seems to have been re-emphasised, and there has been technological improvement and new funding opportunities to support this research. The result has been a greater volume and quality of work on vector behaviour. Examples include other chapters in this book, as well as recent studies in the literature (Cooperband and Cardé 2006, Dekker *et al.* 2005, Torr *et al.* 2008).

Chapter scope

This book focuses on disease vectors, whether veterinary or medical. So the inclusion of a chapter on 'non-vector' biting flies seems odd. However, it is probably more precise to say that the main subjects of this chapter are not especially good or important vectors. By no means are we suggesting that they do not have direct or indirect impacts on health or productivity, because they might and do (Hansens 1951, Mullens *et al.* 2006, Pickard 1968, Schofield and Torr 2002, Schwinghammer *et al.* 1986, 1987, Zumpt 1973). We also feel that the information consolidated herein might have some relevance to vectors more generally, for the emphasis of this book is on behaviour rather than diseases or pathogens. In particular, it can be revealing to contrast the behaviours of non-vector biting flies with better-studied vectors such as the Glossinidae, not so much to make note of similarities, which abound, but rather to highlight intriguing differences.

For simplicity, and to allow a more in-depth treatment of the subject material, we decided to limit our coverage to taxa of non-vector biting flies that occur globally, and for which a reasonable body of laboratory and field-based behavioural data exists. The resulting list is very short indeed, i.e. it is arguably comprised of species that fall within a single group, i.e. the Stomoxyinae. Within this subfamily, our emphasis will be on the synanthropic and cosmopolitan species *Stomoxys calcitrans* (L.) (the stable fly).

In terms of chapter structure, our thoughts are presented along a continuum that starts with the insect as a sensory receptor, and then moves through events that might culminate in feeding on a vertebrate host. Hence, though much of the discussion focuses on the role of olfaction in distance orientation, visual cues are touched upon and near- or on-host feeding behaviours are discussed. We also consider laboratory data related to distance orientation in tsetse in this chapter. Finally, while we acknowledge that interesting olfactory work has been done in several areas of stable fly biology (e.g. see Carlson *et al.* 2000, Romero *et al.* 2006), we focus on behaviour related to haematophagy.

A framework for host orientation

Despite our above caution regarding the need to be aware of bias and anthropomorphisms, we begin our discussion with a model for host-seeking behaviour that is somewhat dated, oversimplified, perhaps biased and almost certainly anthropomorphic. Nevertheless, it provides a useful framework around which thoughts – and manuscripts – can be organised.

Many species of haematophagous Diptera, including stable flies, must find their hosts from a distance. Elements of this process can be divided along spatial and temporal lines and, though it is impossible to attribute the logical framework to a single author, it is well summarised by Sutcliffe (1986, 1987). In this model, the receptivity of a given insect to host-based stimuli is

modulated by factors such as hunger (Klowden and Lea 1979, Klowden *et al.* 1987), competing behavioural priorities (e.g. oviposition) and endogenous activity cycles (Jones and Gubbins 1978, Nayar and Sauerman 1971). Presuming an insect that is receptive, host orientation *sensu stricto* is thought to be 'activated' by some type(s) of host cue. What follows are behavioural manoeuvres, often mediated by odour, that bias movement towards a host. For flying insects, environmental landmarks are critically important during this period, e.g. to steer flights. As the orienting insect approaches the blood source, host-based visual and/or other cues are thought to become increasingly relevant as behavioural modulators. Ultimately the searching insect might find and land upon the host and then feed to repletion.

Blood lust: the role of blood feeding in the biology of *Stomoxys*

Much, though not all (Kunz and Monty 1976, Mihok *et al.* 1995, 1996, 2007, Zumpt 1973) of our knowledge concerning *Stomoxys* biology comes from work done on stable flies. Like tsetse, stable flies are day-active and both sexes blood feed, which provides the materials necessary for males to develop reproductive competence (Anderson 1978, Venkatesh and Morrison 1980, 1982), and females to develop ovaries (Chia *et al.* 1982, Kuzina 1942, Spates *et al.* 1988, Venkatesh and Morrison 1980). In contrast to tsetse, stable flies also feed on plant-related sugar sources (Jones *et al.* 1992, Parr 1962) and blood feeding is often daily (Venkatesh and Morrison 1980) or even more frequent (S Schofield, unpublished data). In terms of endogenous activity, laboratory studies suggest a single daily peak, with a free running period of approximately 26 hours (Schofield and Brady 1996). Diel field activity is often bimodal (Beresford and Sutcliffe 2006, Charlwood and Lopes 1980, Kunz and Monty 1976, Schofield 1998), for example with morning and evening peaks, though unimodal patterns have also been described (Berry and Campbell 1985, Harley 1964). Regardless of the specific feeding periodicity or activity cycle, the reality for stable flies, especially females, is that they feed often. From a host location perspective, this presumably means that unless they have recently fed, they are likely to be receptive to host-based stimuli.

Stable flies as olfactory receptors

The molecular and physiological bases for insect olfaction are well covered elsewhere, including Chapters 2, 3 and 4 in this book. We therefore limit our discussion to a brief overview of electrophysiological studies that have evaluated stable fly responses to odour.

In this fly, olfactory receptors are concentrated on the third antennal segment (Lewis 1971, 1972), where single cell recordings by Lewis (1972) and later Bidgood (1980) demonstrated responses to the ubiquitous kairomone CO_2. Warnes and Finlayson (1986) similarly demonstrated responses to CO_2, but in this case it was through use of electroantennogram (EAG). With the same technique, they also recorded responses to acetone, 1-octen-3-ol, cattle odour, cattle odour filtered through soda-lime, human breath and the odour of cattle faeces. Schofield *et al.* (1995) were the first to screen a modest number of compounds using stable flies as electrophysiological receptors. Like Warnes and Finalyson, they used EAG and found that 1-octen-3-ol evoked substantial activity, whereas acetone did not. For saturated primary aliphatic alcohols, responses increased with carbon length to octan-1-ol; and for 1-octen-3-ol with dose over several orders of magnitude. Responses to a C_8 aldehyde, carboxylic acid and ketone were less that to C_8 alcohols. Several recent studies have expanded upon the number of compounds screened for electrophysiological activity. Birkett *et al.* (2004) worked with a number of biting and nuisance flies and discerned stable fly EAG responses to several types of compound including phenols (*m*- and *p*-cresol), alcohols (including 1-octen-3-ol), ketones, linalool and citronellol. These authors also tested the horn fly, *Haematobia irritans*

(L.), with responses being similar to those reported for stable flies, though with a few exceptions, notably, for example, an absence of response to phenols. Jeanbourquin and Guerin (2007a,b) focused on rumen and dung odours, and reported that 1-octen-3-ol and dimethyl trisulphide evoked the strongest EAG responses, but carboxylic acids, short chain alcohols and aldehydes, ketones, indoles, phenols and terpenes also elicited significant EAG responses. So what do the electrophysiological results tell us? For one, they indicate that some compounds consistently evoke responses (e.g. 1-octen-3-ol), whereas others do not (e.g. ketones, carboxylic acids). There are many possible explanations for such differences, some of which are explored by Schofield *et al.* (1995). Second, that stables flies act as receptors for a variety of host-derived compounds, and that these same compounds would be reasonable targets for further investigation. What electrophysiological outcomes do not address is whether or not active compounds have any behavioural (or biological) relevance – it is this question that comprises the subject matter of the next several chapter sections.

Starting the search engine: initiation of host-oriented behaviour

Activation is thought to represent a transition in behavioural state whereby the insect becomes (more) receptive to host-based cues. Hence, measurement of activation itself is not possible. Rather, early and observable events in the host-seeking process are used as indicators that activation has occurred. While one can debate as to whether activation is a useful concept, i.e. it is not testable, the behaviours that have been used as surrogate markers do provide insight into host-seeking processes. In this context, measurement of activation has traditionally employed frequency-based measures (e.g. flight periodicity) as behavioural indicators. As well, at least for stable flies, studies have been informed by results for other groups of blood feeding Diptera, in particular as regards the positive association between exposure to CO_2 and mosquito (Daykin *et al.* 1965) or tsetse (Bursell 1984, Turner 1971) activity.

To our knowledge, the earliest work on behaviour initiation by stable flies was done by Gatehouse and Lewis (1973), who recorded the number of stable fly landings on targets situated within a wind tunnel. They observed more landings in the presence of CO_2, and suggested that this kairomone evoked flights and/or increased the probability that a flight would terminate on a target. Warnes and Finlayson (1985a) followed this up with a study using stable flies that were placed singly into a test chamber and exposed to various concentrations of CO_2, or to human breath. They found that flight activity generally increased with CO_2 concentration, was positively associated with hunger status, and that breath was associated with higher levels of activity than could be accounted for by CO_2 alone. Alzogaray and Carlson (2000) also suggested an activation-associated effect for CO_2, in this case based on net movement of stable flies away from one end of an olfactometer. In a more comprehensive study, Schofield *et al.* (1997) evaluated responses of groups of four stable flies that were placed into a test chamber situated in a wind tunnel and that were then exposed to CO_2. In line with the results of earlier studies, CO_2 was associated with increased flight frequency, decreased latency to take-off, and increased likelihood that flights would terminate on a visual target placed in the test chamber. Moreover, the authors pointed out that the response threshold to CO_2 was similar in their and Warnes and Finalyson's work, e.g. 0.006% compared to 0.01%. However, they did caution against reading too much into this similarity. First, because inter-study differences in plume structure could affect responses. Second, because these thresholds, if expressed as odour flux, were about an order of magnitude different. Finally, because the activity increase was manifest as a step function, whereas in Warnes and Finlayson's work there was a dose-response relationship.

Schofield *et al.* (1997) also evaluated responses to acetone and 1-octen-3-ol. For acetone, outcomes were more muted but followed a similar pattern to those for CO_2, i.e. flight activity increased with a response threshold of about 0.01 ug/l, and the proportion of flights terminating on a target increased in the presence of this compound. By contrast, the principle impact of 1-octen-3-ol was a decrease in flight activity at the highest concentrations. This latter finding matches well with at least some work on tsetse where high release rates of 1-octen-3-ol have been associated with reduced: trap catch (Vale and Hall 1985a), upwind turning response (Paynter and Brady 1993), and activity (Bursell 1984).

More recently, Jeanbourquin and Guerin (2007a,b) examined the behavioural responses of stable flies to rumen and dung-based volatiles. In one experiment, groups of five flies were placed into a release chamber situated in a wind tunnel, with 'activation' defined by a fly leaving the chamber. Increased rates of leaving were evident for raw rumen volatiles, as well as several individual chemicals (dimethyl trisulphide, p-cresol and butanoic acid). As with previous studies, 1-octen-3-ol was not associated with an increase in activity. Nor, as was documented in the Schofield *et al.* (1997) study, was it associated with a decrease in activity. In another experiment, single flies placed into a wind tunnel were exposed to raw dung volatiles, with activation being defined as three or more take-offs within the flight area. Both cow and horse dung were associated with increased 'activation' compared against the control group, e.g. about 70% of flies were activated compared against 30%. Unfortunately, the authors did not characterise responses over a range of compound exposures, nor did they use survivorship analyses to explore the data. As a result, potentially informative comparisons that might have been made with earlier studies regarding, for example, latency of response or nature of the dose-response curve, are not possible.

In-flight responses to odour: wind tunnel results

If the intent of behavioural work is to inform vector control, then research should ultimately be done in, or at least translate to, the field. As pointed out earlier in this chapter, much of the existing field research on haematophagous Diptera has not looked at behaviour *per se*, but rather has recorded behavioural endpoints, e.g. trap catch. Clearly, this partly reflects the difficulty of executing and interpreting behavioural assays in the field. It is for this reason that laboratory assays of behaviour can be especially useful, i.e. they can document behaviour under carefully controlled and relatively easy to set up conditions. Despite this, most such work has, just as for field studies, measured behavioural endpoints, e.g. position in an olfactometer, net displacement in a wind tunnel. For example, using the set-up described earlier, Gatehouse and Lewis (1973) suggested that while CO_2 increased flight activity, it was not associated with a landing bias on a baited versus unbaited target, and hence they concluded that it did not elicit directional responses. On the other hand, they found that emanations from a hand resulted in relatively more landings on a baited target and therefore inferred direction orientation. Of course, in the absence of actual measurements of behaviour *per se*, it is impossible to validate these inferences. Indeed, subsequent work on stable flies, as we shall see, contradicts their conclusion concerning CO_2. Warnes and Finlayson (1985b) evaluated responses of stable flies to CO_2 and acetone in a low speed wind tunnel, using net movement over a three-minute period to infer (or not) directed orientation. They found that various concentrations of CO_2 resulted in net upwind movement, as did acetone (0.8 g/min), expired breath and odour from guinea pigs. Certainly, a parsimonious explanation for these results is upwind anemotaxis, but by no means was this behavioural strategy specifically demonstrated. Indeed, it has been argued that the results for acetone might also reflect an arrestant artefact because cessation of upwind flight responses to acetone have been documented at a concentration/flux some 20 times less than was tested in this study (Schofield

and Brady 1997). In the last couple of years, several additional studies have evaluated orientation behaviours of stable flies in the laboratory. Jeanbourquin and Guerin (2007a,b) were interested in stable fly responses to rumen and dung volatiles. 'Attraction' in these experiments was determined on the basis of whether flies showed a minimum upwind displacement in a wind tunnel. As above, while these positive outcomes are suggestive of directional upwind movement, they do not establish causation. In any event, these authors reported that a number of compounds, including horse and cow dung, raw rumen volatiles, dimethyl trisulphide, p-cresol and butanoic acid were associated with attraction in a wind tunnel. Though flights were also classified as being oriented (or not) on the basis of 'sinuous flights in the direction of the odour source', the authors did not provide the detail necessary to validate this classification scheme, nor the related interpretations. Most recently, Beresford and Sutcliffe (2008), looked at the influence of male age on the responsiveness of stable flies to CO_2 in a wind tunnel. Interestingly, net upwind displacement at the end of a two-hour observation period was inversely correlated with age, which the authors suggest might reflect an age-based bias in the propensity of males to seek blood meals versus female conspecifics. However, inferring orientation on the basis of fly position after an extended period cannot be considered a reliable indicator of directional orientation, especially because a negative control was not employed, e.g. these results also could reflect age-based variability in activity levels or dispersal tendencies.

To our knowledge, the only laboratory experiment that has recorded and critically evaluated in-flight manoeuvres of stable flies was done by Schofield and Brady (1997). This work has much in common previous work on tsetse flies where upwind anemotaxis (Colvin *et al.* 1989, Paynter 1991, Paynter and Brady 1993, 1996) and ortho- and klinokinetic responses (Warnes 1990, Paynter and Brady 1993, 1996) to host-based odour were observed. All of these studies analysed, quantitatively, the actual flight tracks of test insects. In the interest of efficiency, we will discuss the tsetse work here (instead of in Chapter 12, this volume), with particular attention being paid to studies (Colvin *et al.* 1989, Paynter and Brady 1993, 1996) that used a set-up very similar to that employed for the stable fly experiments. Bursell (1984) was one of the first to evaluate carefully tsetse behaviour in the laboratory, and suggested that resting flies took off and flew in an upwind direction when exposed to CO_2. Following this, Colvin *et al.* (1989) released tsetse flies (*Glossina morsitans morsitans* Westwood) from a crosswind position into a wind tunnel and analysed their responses to: (1) the olfactory stimulus of a CO_2 odour plume; (2) the visual stimulus of a moving floor with a strong visual pattern and (3) the physical stimulus generated by wind moving at 0.25 m/s. By using clever combinations of these three stimuli, the authors demonstrated that flights were indeed steered into the wind in the presence of this compound, and that this was guided by visual flow, i.e. that upwind anemotaxis was occurring. This reinforced results from field work where upwind turning had been observed (see Chapter 13), but more importantly provided strong evidence for the mechanism of in-flight steering. Paynter and Brady (1996) expanded upon this work by examining tsetse responses to CO_2 at various wind speeds. In addition to upwind anemotaxis, they described kinetic responses to CO_2, specifically that tsetse slowed down and turned more in its presence. However, these effects and upwind anemotaxis were not evident at a low wind speed (e.g. 0.1m/s). The authors suggested that this might reflect a limit on the part of tsetse to detect wind-induced drift. Not surprisingly, stable flies tested using a similar apparatus (Schofield *et al.* 1997) also moved upwind when exposed to CO_2. In this case, responses were dose-dependent with flies showing a substantial propensity to move and exit and turn upwind, and to execute a major turn over three orders of magnitude of CO_2 concentration (Table 1). Moreover, flight kinetics differed between the negative control and the odour treatments, and between different concentrations of CO_2 (Table 2). In other words, and as with tsetse, stable flies slowed down, turned more, and headed upwind in the presence of CO_2. However, they differed from

Table 1. Percentage of flights exiting upwind, exhibiting major turns and turning upwind in CO_2, acetone, and 1-octen-3-ol. Controls consisted of clean air. CO_2 concentration is given as percent above ambient (from Schofield and Brady 1997).

Concentration	Exit up	Major turn	Turn up
CO_2 (%)			
Control	25	38	54
0.0012	47	42	63
0.006	58	65	74
0.012	69	68	73
0.06	70	80	84
0.12	84	80	88
Acetone (µg/l)			
Control	30	33	43
0.001	34	32	50
0.01	59	61	79
0.1	50	57	73
1.0	43	79	40
10.0	20	72	34
1-octen-3-ol (µg/l)			
Control	30	39	48
0.0002	58	61	64
0.002	58	60	62
0.02	57	60	76
0.2	26	66	36
2.0	19	61	21

n for each row: CO_2 = 73-92, acetone = 43-74, 1-octen-3-ol = 57-79.

tsetse by displaying substantially slower flight speeds and higher turning rates in clean air, and in plumes containing CO_2.

In-flight responses of tsetse and stable flies to acetone and 1-octen-3-ol have also been evaluated in the laboratory. For tsetse and over a range of doses, both of these compounds were associated with an increased probability of upwind flight (Paynter and Brady 1993). As regards kineses, tsetse exposed to 1-octen-3-ol slowed down and turned more at the highest doses tested, whereas acetone did not produce consistent concentration-dependent kinetic effects. For stable flies, exposure to acetone or 1-octen-3-ol yielded modest upwind responses with more flies flying upwind and/or executing a major turn over several concentration increments. However, the highest 1-octen-3-ol exposures were associated with a reduction in upwind flight compared against the negative control (Table 1).

Overall, the results of laboratory studies implicate a number of kairomones as being of potential importance to host-orientation for stable flies (and tsetse flies). These include CO_2, acetone and 1-octen-3-ol. Perhaps more importantly, they also highlight specific behavioural strategies (taxes and kineses) that occur in the presence of odour that reasonably can be expected to increase the probability of host finding. Indeed, it has been postulated that taxes and kinesis, depending on

Table 2. Mean (±SE) values for angular velocity, sinuosity and velocity of video-recorded stable fly flights in CO_2, acetone, and 1-octen-3-ol. Controls consisted of clean air (from Schofield and Brady 1997).

Concentration	Angular velocity (°/s)	Sinuosity (°/cm)	Velocity (cm/s)
CO_2 (%)			
Control	114.6±10.8	0.7±0.11	170.9±5.3
0.0012	129.1±10.5	0.8±0.09	188.4±5.1
0.006	162.8±10.1	1.1±0.09	172.2±4.9
0.012	192.2±10.9	1.5±0.12	151.3±5.3
0.06	191.5±9.4	1.5±0.08	139.4±4.6
0.12	212.9±9.9	1.9±0.09	133.3±4.8
Acetone (µg/l)			
Control	102.2±12.4	0.6±0.11	169.8±5.6
0.001	124.7±11.5	0.8±0.10	160.3±5.2
0.01	122.9±9.7	0.9±0.08	149.8±4.4
0.1	151.9±9.5	1.1±0.08	157.1±4.3
1.0	167.0±9.4	1.3±0.12	145.5±4.3
10.0	156.3±8.4	1.2±0.07	140.3±3.8
1-octen-3-ol (µg/l)			
Control	114.2±9.3	0.7±0.08	170.2±3.7
0.0002	151.4±10.9	1.1±0.10	142.0±4.3
0.002	148.4±11.2	1.3±0.10	136.9±4.4
0.02	152.7±10.4	1.1±0.08	148.6±4.1
0.2	151.5±9.4	1.2±0.08	139.5±3.7
2.0	137.7±11.4	1.2±0.10	123.5±4.8

n for each row: CO_2 = 73-92, acetone = 43-74, 1-octen-3-ol = 57-79.

the environment, might play a more or less important role in the orientation response. This idea was explored by Williams (1994), who suggested that the consistency of wind direction could be an important determinant of the specific strategy deployed by foraging tsetse. In this model, if wind-generated directional information was reliable at the time of odour detection, then upwind anemotaxis with various odour sampling frequencies should optimise the probability of host encounter. By contrast, if directional information was very poor then kinetic responses to odour detection should be preferred, i.e. the fly should slow down and turn more. Supporting this model, there is field evidence to suggest that tsetse can move upwind very quickly to an odour source (Griffiths *et al.* 1995). While the model was developed for tsetse, the general approach and its conclusions are applicable to any fast-flying insect responding to an odour source.

Of course, as with all laboratory studies, the important uncertainty is their relevance to the field situation. Aside from the usual litany of issues such as the use of laboratory-reared insects and artificial test conditions, we make note here of several additional points for consideration. First, stable flies and tsetse move quickly relative to some other groups of blood feeding Diptera (e.g. mosquitoes), or other insects for which orientation behaviours have been well documented (e.g. moths). The upshot is that laboratory observations of *Stomoxys* and tsetse are, by default, usually limited to relatively few behavioural events, at least compared to the types of records that can be obtained for moths and mosquitoes. The consequence is that we have not been

able to observe directly how sequences of behaviours are put together for these groups, the comparator being the elegant work that has been done on moths as they fly into and out of odour plumes (Murlis *et al.* 1992, Chapter 6 in this volume). This limitation does come with some analytic benefit. In particular, 'less' behaviour allows for easier analyses, and the rapid horizontal movement displayed by these groups makes two-dimensional analyses more tractable. Second, analyses have focused on concentration as a measure of exposure, with limited consideration of odour flux (i.e. amount of material moving through area per unit time, often expressed as: moles/s/cm^2). It remains uncertain which, if either, of these measures is more appropriate. Is this a critical issue? Perhaps not, but certainly it can affect inter-study validation, e.g. as pointed out above in our discussion of the threshold at which CO_2 evokes stable fly flight activity. However, because flux loses its distinction from concentration where flights are substantially faster (e.g. stable flies, tsetse) or slower than wind speed (Paynter and Brady 1996, Schofield 1996), and because it is more challenging computationally, it seems prudent to express exposure intensity based on concentration. However, this comes with the caveat that impacts of test conditions on odour presentation should be considered when interpreting results.

Field work on the responses of Stomoxyinae to host odour

Before discussing the specifics of field studies evaluating responses of Stomoxyinae to host kairomones, we wish to present a conclusion from our review. As far as we can tell, there is little evidence to indicate that trap-out strategies have broad-based utility as a control intervention for stable flies, and even less data to suggest that host-odour might contribute significantly to any such approach.

While we cannot say whether this conclusion will hold into the future, the current lack of evidence does beg a question – what is the practical aim of carrying out such work? For one, it might allow for heightened sensitivity of surveillance systems. Of course this needs to be weighed against the need for a surveillance system itself (i.e. for a non-vector), and the net benefit accrued by adding host odour into the surveillance equation. Regarding the former, the nuisance and consequent economic costs associated with Stomoxyinae seem to validate the need for at least targeted surveillance, though the need to employ host odour as part of a surveillance system has not been convincingly demonstrated. Another area where this work has proved to be useful, albeit tangentially, is the investigation of host-stable fly interactions. Work in this area, which partly originated due to interest in responses of flies to host odour, has yielded useful insights into intra- and interspecific host-parasite relationships (see below)

In any event, visual cues, as well as traps and targets based on various combinations of these have long been used as surveillance tools for stable flies (Schofield 1996). It has been around these technologies that most odour-based research has been carried out. With the exception of a few studies where trap efficiency was also considered (Mihok *et al.* 2007, Schofield 1998, Vale and Hall 1985b), the bulk of the field work on Stomoxyinae has used a behavioural endpoint, i.e. trap catch, as the outcome measure. As regards specific kairomones, the only compound that has yielded consistent and positive results from the field is CO_2. Hoy (1970) reported that Malaise traps releasing CO_2 at 3 l/min caught about three times more stable flies than unbaited traps, and Roberts (1972) reported that catches from a Malaise trap baited with 3.5 l/min CO_2 were similar to those from a trap baited with odour from a single steer. More recently, Mihok *et al.* (1996) found that Vavoua traps baited with CO_2 at 2 l/min caught about twice as many Stomoxyinae flies as unbaited traps. Some of the early work on tsetse responses to odour also yielded useful information for Stomoxyinae. Vale (1980), while investigating the responses of tsetse to host

odour, also collected Stomoxyinae; later work (Schofield 1998) suggests that the *Stomoxys* spp. collected by Vale comprised a mixture of three species: *S. niger niger* Macquart, *S. sitiens* Rhodani and *S. calcitrans*. Flies were intercepted using electric nets that were positioned downwind of a stationary target. When CO_2 was released at 2.5 or 15 l/min from near to the target, catch was increased by more than an order of magnitude. For the variety of other compounds tested in this study, which included ketones, aldehydes, alcohols and acids, the only one that was observed to affect the behaviour of Stomoxyinae was acetic acid (at 0.7 g/hr), which reduced the catch. Vale and Hall (1985a) found that catches of Stomoxyinae at beta traps or electrified targets increased with CO_2 release rate and was some 6 or 16 times higher, respectively, at the maximum tested (20 l/min) when compared against unbaited controls. The potency of CO_2 was again demonstrated by Vale and Hall (1985b) with catches at baited targets (2 l/min) being >10× greater than at an unbaited trap. Several *de facto* dose responses to CO_2 also have been obtained from Zimbabwe. In these experiments, Stomoxyinae were collected from electric targets baited with the odour from various sized groups of cattle (Hargrove *et al.* 1995, Torr *et al.* 2007). For both studies, catch rose with bait mass in a logarithmic fashion (Figure 1). Schofield (1998) also deployed electric targets in Zimbabwe to evaluate the influence of CO_2 (2 l/min CO_2) and found that it more than doubled catches when compared against an unbaited target. Target efficiency, estimated by positioning an electric screen next to the target, was > 50% in this study and was not affected by CO_2.

Can non-CO_2 odours derived from hosts increase the catch of stable flies in the field? Some of the early studies in Zimbabwe suggested not (Vale 1980, Vale and Hall 1985a,b). However, since this work, evidence has emerged to indicate that other kairomones, especially 1-octen-3-ol, can sometimes affect trap catch. Holloway and Phelps (1991) found that F3 traps baited with 1-octen-3-ol (0.7 mg/hr) caught about twice as many *Stomoxys* spp. as unbaited traps, though subsequent work carried out in the same area (Schofield 1996) with the same type of trap was not able to

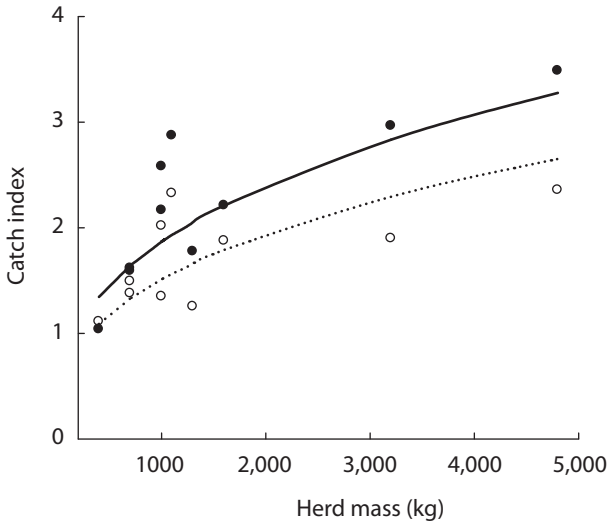

Figure 1. Catch indices of tsetse (solid circles) and Stomoxys *(open circles) attracted to herds of cattle of various size. Indices are calculated as the detransformed mean catch from a herd divided by that from a single ox weighing 400 kg. Lines fitted by regression of* log_{10} *(herd mass) against* log_{10} *(catch index); regression co-efficients for tsetse and* Stomoxys *were 0.41 (±0.070, SE) and 0.25 (±0.093), respectively (adapted from Torr* et al. *2007).*

duplicate this result, and earlier studies, also in this area (Vale and Hall 1985a,b), were only able to show a 'repellent' effect for 1-octen-3-ol (5-500 mg/hr) when it was released with ox odour. Of importance, most of the above studies only classified catch to the genus level, and hence inter-species variability might have affected outcomes.

In Kenya, Mihok *et al.* (1995) evaluated the influence of acetone, 1-octen-3-ol and various urines and dungs on the catch of *Stomoxys* spp. and other Stomoxyinae (e.g. *Haematobosca, Prostomoxys, Rhinomusca, Stygeromyia*) using Vavoua traps. Most of the test compounds did not affect trap catch, though 1-octen-3-ol yielded mixed results and sometimes was associated with increased (about 3-fold) catch, especially at high doses (2 mg/hr). In a similar study, Mihok *et al.* (1996) did not find a consistent effect for 1-octen-3-ol. In two experiments it did not enhance catch of *Stomoxys* compared to CO_2 alone, and when released by itself caught less than half the number of flies as a CO_2-baited trap, which is similar to the outcome (Mihok *et al*. 1995) for a CO_2-baited Vavoua trap against an unbaited trap. However, catch of *Haematobosca* spp. were about 2-3 times higher for one of the experiments in which CO_2 was compared against CO_2 and 1-octen-3-ol. In work carried out in North America (Cilek 1999), release of 1-octen-3-ol from alsynite cylinders was not associated with increased stable fly catch, though acetone (50 mg/hr) or a mixture of phenols were. Finally, a series of studies carried out in North America and Africa (Mihok *et al.* 2007) using Nzi traps yielded similarly mixed results, i.e. 1-octenol sometimes was associated with modest increases in catch, and sometimes not. These same authors also reported that traps baited with acetone (890 mg/hr) in Ethiopia caught about twice as many flies as unbaited traps, but increases were not evident when acetone was combined with 1-octen-3-ol and/or cattle urine.

Mihok *et al.* (2007) put forward an interesting hypothesis to explain the variable results for 1-octen-3-ol. Specifically that it is not efficacious when presented in combination baits or in areas where livestock are near. We refer the reader to the paper for a fuller explanation, but do point out that in addition to the exception noted by the authors (Cilek 1999), Vale and Hall (1985b) and Schofield (1996) tested 1-octen-3-ol without cattle in the immediate vicinity, and did not observe increased trap catch. Albeit, substantial numbers of wild ungulates were present around the test sites. Moreover, even though they might not have been immediately adjacent to the test area, there was a large (>100) herd of cattle at the research station in Zimbabwe, as well as wild ungulates, when Holloway and Phelps (1991) carried out their studies. Several alternative, though not necessarily mutually exclusive, explanations for this inconsistent response are possible. For one, even where 1-octen-3-ol has had demonstrable effects, catch increases have been rather modest (e.g. about 2-3 fold). Assuming a substantial random error term and a small expected difference between treatment and control, experiments might have been underpowered for detecting differences. Second, 1-octen-3-ol has been shown to have both positive (e.g. upwind anemotaxis) and negative (activation, reduction in upwind movement, reduced trap catches) effects in the laboratory and field, and exposure to high levels of 1-octen-3-ol have been associated with physiological refractoriness in stable flies (Schofield *et al.* 1995). Given this variability, it would seem that there is a fairly narrow window to capture 'positive' responses to 1-octen-3-ol, which might be manifest as highly variable results for field work. Finally, we suggest that non-CO_2 kairomones simply might not be that important to host orientation in stable flies. Yes, they are active in the laboratory and sometimes in the field, but their relative importance seems small compared to CO_2. This last perspective is supported by the work of Torr *et al.* (2006) who found that differences between cattle in terms of 'attractiveness' to *Stomoxys* were reduced by standardising CO_2 release.

Hence, the story for *Stomoxys*, though heavily influenced by tsetse work, turns out to be somewhat different as regards the diversity of host-based kairomones that consistently evoke field responses.

For tsetse, there are several kairomones (Chapter 12, this volume), for *Stomoxys* there is just one. Of course, additional compounds might be identified in the future, and novel uses for these compounds could be developed. However, as it stands, and likely for at least the near future, there seems to be limited operational advantage to deploying non-CO_2 odours, or arguably even CO_2, for the purpose of stable fly surveillance or control.

Feeding by *Stomoxyinae*: strategies and host selection

The work of Torr *et al.* (2006) described above highlights that conspecific hosts can vary in their ability to draw *Stomoxys* to their vicinity, something that also has been demonstrated for other blood feeding insects (Mukubana *et al.* 2002, Schofield and Sutcliffe 1996). Of course, once an insect gets near to a host a variety of cues, perhaps including host odours, influence whether or not feeding occurs. As it turns out, several groups of Stomoxyinae have received attention in this regard. For example, the load of *Haematobia irritans* (L.) on cattle has been shown to be affected by a variety of factors (e.g. breed, hair density, sebum, host size, host colour) (Jensen *et al.* 2004). Similarly and as pointed out by Mullens *et al.* (2006), it has long been known that cattle show substantial variation in the stable fly load that they support (see Cheng 1958, Mullens and Meyer 1987). Most of the work that critically examines this relationship has been published in the last decade or so. The exception is Warnes and Finlayson (1987). These authors carried out experiments in a laboratory arena and found that calves that displayed higher rates of defensive behaviour had lower fly roads. Subsequent to this, and often in concert with studies examining tsetse, several authors have considered the interrelationship between stable flies, their hosts and other insects. Torr and Mangwiro (2000) evaluated interactions between tsetse feeding success, host, and populations of other flies by observing individual cattle placed within an incomplete ring of electric nets. Though not intended to focus on *Stomoxys* behaviour, the study was relevant because in addition to documenting that tsetse feeding 'success' varied between hosts, related largely to the host's age, it linked this effect to the intensity of host defensive behaviour. Further, rather than defensive behaviour primarily being regulated by tsetse load, the strongest association was with the numbers of *Stomoxys* attacking the host. Hence, their suggestion that interspecific interaction (driven by *Stomoxys* populations) might have important effects on feeding outcomes for tsetse, and by extension could affect trypanosomiasis epidemiology. Of note, while this study considered stable fly populations, it did not collect detailed information on their feeding behaviour. The interplay suggested by Torr and Mangwiro (2002) found substantial support in a follow-up study (Torr *et al.* 2007). In this case, *per capita* load of *Stomoxys* was associated with a significant and negative reduction in tsetse feeding success, from about 80% at a *per capita* load of 20 flies to about 40% at a *per capita* load of 1000 flies (Figure 2). Though not assessed for *Stomoxys* specifically, results from this study are also intriguing because they demonstrate the substantial heterogeneity that is possible with respect to host selection, something that can have interesting and potentially important implications for disease epidemiology. A more specific evaluation of *Stomoxys* feeding behaviour was made by Schofield and Torr (2002) who carried out two experiments in which host-defensive behaviour and feeding success was directly observed. Interestingly, most *Stomoxys* were disturbed while feeding (73%), whether by other flies (65%) or by host-defensive behaviour (35%). Moreover, feeding success varied with host (Figure 3) and, somewhat paradoxically, there was a positive (though not significant) trend between *Stomoxys* density and increased feeding success. This last observation runs contrary to the conventional wisdom that this interaction, if anything, should be in the negative direction. The plausible explanation for this, which is somewhat unique as regards the extant literature, is that the majority of *Stomoxys* were dislodged by non-biting Diptera versus host-based defensive behaviour. Hence, increasing the number of *Stomoxys* on the host would presumably reduce their *per capita* exposure to non-biting Diptera, and in turn might

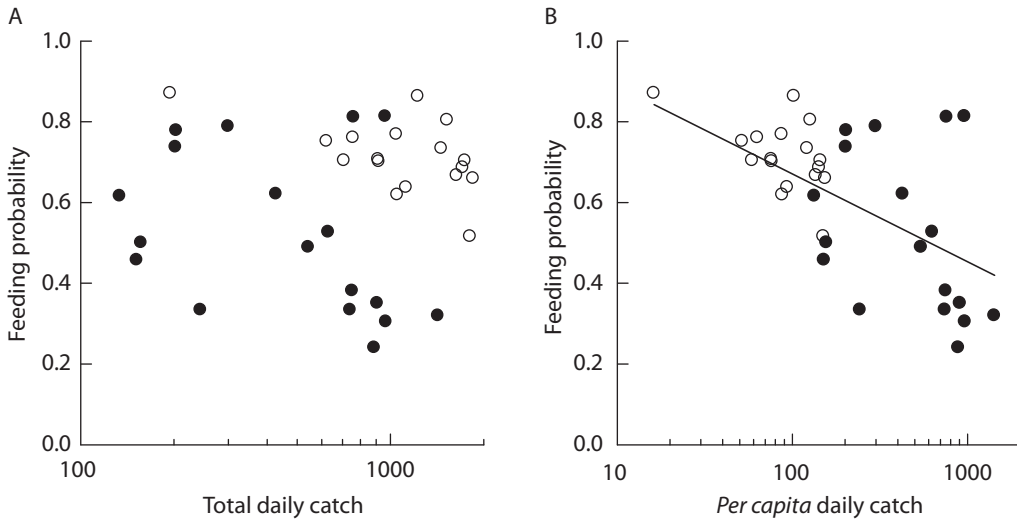

Figure 2. Feeding probability of Glossina *plotted against (A) total daily catch of* Stomoxys *or (B)* per capita *catch of* Stomoxys *for a single ox (solid dots) or a herd of 12 cattle (open circles). Line fitted by logistic regression (adapted from Torr* et al. *2007).*

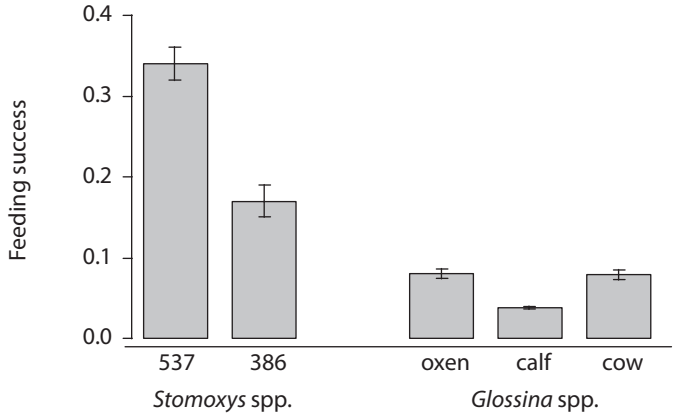

Figure 3. Feeding success (±SE) of Stomoxys *on two different adult oxen, designated by ear tags 537 and 386, and feeding success of tsetse on different types of cattle in Zimbabwe (adapted from Schofield and Torr 2002).*

increase the probability of a complete feed. In terms of the comparison of *Stomoxys* and tsetse, the principal differences observed were that the former showed a higher rate of feeding success, a lower relative rate of host-induced disturbance, a longer average period on the host (whether or not disturbed) and a non-constant rate of leaving the host. The authors suggest that these differences are compatible with the life attributes of these groups. On the one hand, *Stomoxys* are rather short lived (daily survival has been estimated to be about 0.75-0.9 (Berry *et al.* 1981, Scholl 1986)), and feed daily or even more often. Hence, expectation is for a more persistent, 'riskier'

feeding strategy. On the other hand, tsetse are longer lived (daily survival >0.95; Hargrove 1988) and likely feed only every ~3 days. Thus, at least compared to stable flies, they should be 'choosier' feeders. So, just as for kairomones, while some of the gross patterns of behaviour for these groups are similar (e.g. host-selection heterogeneities), they show marked differences at a finer scale.

One of the most ambitious studies of interactions between stable flies and their hosts was undertaken in California by Mullens *et al.* (2006). In this work, individuals in four herds of 25 cows were observed 8-10 times per week for 12 weeks. For each observation period, the numbers of flies and defensive behaviours were recorded. In addition to a very strong correlation (r^2 >0.8) between the weekly mean estimates for defensive behaviour and fly populations, the authors were able to demonstrate that ranked fly load per animal was consistent over the experiment, as was ranked intensity of defensive behaviour. Consistent with work for tsetse, older cows tended to maintain higher fly loads and demonstrated a lower rate of effective defensive manoeuvres (i.e. leg stamps) than younger animals. Finally, the *per capita* rate of defensive behaviour against stable flies decreased with time, suggesting that animals became habituated to stable fly attack. Though the authors were unable to characterise economically relevant impacts of fly attack (e.g. decreased milk yield), they make the valid point that more work is required to relate economic impact – to host behaviour – to biting fly pressure. Finally, they suggest that monitoring defensive behaviour (instead of fly population) might provide a simpler, more efficient and sensitive indictor for decision-making around pest management.

In contrast to the situation for distance-orientation kairomones, results from studies evaluating close-range or on-host behaviours/interactions would seem to have direct operational relevance to stable fly management. This might simply be in terms of developing efficient surveillance indicators (Mullens *et al.* 2006), or more ambitiously could be articulated in the context of developing more sustainable and/or cost-effective control strategies (see Mullens *et al.* 2006, Prior 2003, Torr *et al.* 2001, 2007).

Conclusion

While odour-based orientation to host animals is necessary to stable flies, it might not be readily exploited from a control perspective. This is substantially different to the situation for at least some species of tsetse, where research on and eventual use of host odour has powered successful control programs (see Chapter 12 in this volume). The example of tsetse research also shows how a better appreciation of the host-orientation behaviour of flies can enhance our understanding of host-parasite interactions, which in turn might lead to improved methods of control (e.g. insecticide-treated cattle to control tsetse, see Torr *et al.* 2007). Whether or not this also will be the case for stable flies remains to be seen.

Aside from these practical considerations, the odour-oriented behaviour of *Stomoxys* and tsetse provide many examples of where host selection is governed more by its defensive behaviour than its kairomone profile. By contrast, much research on identifying 'attractants' and 'repellents' for mosquitoes and midges has focussed on comparing the odour profiles of individuals that are bitten more or less (see other chapters in this book). The relative importance of odours in host selection by different species of biting fly may be related, in part, to their respective activity periods: day-active species can make better use of visual cues such as host -colour, -movement and -shape whereas odour is, perhaps, the only cue that can be relied on to distinguish one sleeping host from another. However, we also suggest that this might reflect an underlying assumption that odours alone are responsible for inter-host variability in attractiveness. The cases of *Stomoxys*

and tsetse provide numerous examples where this is not the case. Perhaps we should be checking that the same does not apply to other vectors?

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