11. Black fly interactions with their hosts

James F. Sutcliffe

Abstract

Haematophagous black flies (Diptera: Simuliidae) seek mammal and bird hosts as blood sources and are responsible for significant human and animal hardship and economic loss due to annoyance, effects of biting and disease transmission. Simuliids locate and choose their hosts by orienting to host-originating chemical, visual and temperature stimuli. Little is known about the nature of the stimuli involved as chemical mediators though carbon dioxide is influential and certain other breath and body odours also clearly play roles. Effects of visual stimuli are limited to the effective visual range of the host to the fly which is determined by host size and environment. Vision appears to take precedence over olfaction when the host-seeking fly makes visual contact with the host. Landing and biting site choices appear to depend on a combination of visual and skin-associated chemical and thermal stimuli. Black fly responses to potential host stimuli seem dependent on the context in which the stimuli are received. Some stimuli (e.g. warmth) induce landing or biting for flies in the host seeking mode (or context) but induce escape reactions for non-host-seeking flies. Host choice (or rejection) may be based on the ability of these host stimuli, encountered in an appropriate sequence, to sustain the host seeking mode up to its consummation (blood feeding). Research on black fly host seeking has been slowed by the relative difficulty of doing meaningful behavioural experiments in the lab with this group, by the dearth of lab colonies and the difficulties and costs of maintaining them and by the lack of adequate field tools and experimental approaches.

Keywords: attractants, blood feeding, host-seeking behaviour, sensory ecology, simuliids, visual cues

Introduction

General

Black flies (Diptera: Simuliidae) are a geographically widespread insect family found in almost all areas, including many oceanic islands, where there is running water. Crosskey (1990) includes 1,554 species[1] in his world list and notes that many more species will eventually be added as species complexes are resolved taxonomically.

Immature stages of simuliids occur in running water ranging, depending on species, from small streams such as those found in the highlands of Central America or on the Precambrian shield of Canada and Europe, to large rivers such as the Niger in West Africa or the North Saskatchewan that flows through the parklands of Alberta and Saskatchewan, Canada.

For the most part, black flies are pestiferous in numbers in rural and wilderness settings. Various factors, many of them not well-understood, appear to exclude simuliids from urban locations. In eastern Canada, black flies are a pest of humans in the spring and early summer months (late May, June, early July) while in western Canada the large river species (mainly *Simulium arcticum*

[1] According to Adler *et al.* (2003), Crosskey recognizes 1,774 valid black fly species in his 2002 update published by the British Museum of Natural History.

s.l. and *S. luggeri* Nicholson and Mickel) have multiple generations and are potential pests all summer long.

In their comprehensive treatment of the systematics of black flies, Adler *et al.* (2004) provide brief sketches of several aspects of general black fly biology (including behaviour, ecology, physiology) for North American species. Crosskey (1990) covers similar topics more extensively with a worldwide perspective.

Adler *et al.* (2003) provide an exhaustive revision of the taxonomic status of all North American black fly species which, because many are boreal, includes many Palearctic species as well. For the purposes of this review I use species names as reported in the original publications; however, the reader can determine the probable valid names for the species discussed by referring to the 'Taxonomic Accounts' section Adler *et al.*'s invaluable book.

Females of most simuliid species are blood feeders on mammals or birds though females of several species are autogenous in their first gonotrophic cycle and a few do not blood feed at any time having adapted to certain extreme conditions by adopting wholly autogenous reproductive strategies (Anderson 1986). While a few historical references have been made to black flies feeding on other insects, fish, frogs, etc., these are considered either mistaken or spurious (Crosskey 1990). The most credible report of black flies interacting with a non-mammal or non-bird vertebrate is by Smith (1969) who observed *S. venustum* Say swarming around a snapping turtle (*Chelydra serpentina* (Linnaeus)) in Algonquin Park, Ontario although none appeared to feed on it.

Black flies host seek only during the daylight hours (Crosskey 1990) suggesting that vision may be an important sensory modality in their host-seeking behaviour. While black flies are potentially active throughout the daylight hours, many species exhibit various peaks of activity in the early to mid-morning and during the late afternoon and evening. This is presumably to take advantage of the normally calmer conditions at these times and to avoid the desiccating heat of the mid-day and early afternoon hours.

Medical and veterinary significance of black fly blood feeding

Swarms of females can sometimes be massive around hosts and extremely annoying and stressful. Black fly bites can be painful and have, depending in the number of bites received and the sensitivity of the individual, consequences ranging from mild itching and irritation to severe illness and anaphylactic shock. Livestock in the parklands of western Canada have been killed by attacks of large numbers of *Simulium arcticum* (s.l.). Claims that this has been due to exsanguination are doubtless incorrect. Some combination airway blockage by fluid build-up around the tracheae in response to salivary antigens, allergic reactions to innumerable bites, and stress due to harassment and heat, especially in animals not previously exposed to black flies, is more credible as the cause of death (Beck 1980). Numerous examples of the pest significance of black flies are documented in many parts of the world (see Crosskey 1990 for several accounts).

Their blood feeding habit also makes black flies of significance as vectors of several important parasites and parasitic diseases of humans and animals. The most important parasite transmitted by black flies is the filarial nematode, *Onchocerca volvulus* (Leuckart), responsible for the condition called (among many other names) River Blindness. River Blindness is transmitted by various simuliid species in vast tracts of West and Central Africa and more focally in parts of Central and

South America. Thylefors and Alleman (2006) provide a brief history and up-to-date summary of onchocerciasis control efforts in Africa and in the New World.

Black fly species in many parts of the world are also vectors of various other microfilarial parasites of animals including a number of *Onchocerca* species of livestock (Lok *et al.* 2000) and *Dirofilaria ursi* Yamaguti (Addison 1980) of black bears (*Ursus americanus* Pallas) in North America.

A simuliid-vectored disease of great significance in birds is an 'avian malaria' caused by geographically widespread members of the sporozoan genus *Leucocytozoon*. *Leucocytozoon simondi* Mathis and Leger is a common cause of death especially for young birds on the nesting grounds in spring and *L. smithi* Laveran and Lucet affects geese, turkeys and domestic fowl (Cupp 1986).

The demonstration that Vesicular Stomatitis Virus (VSV) occurs at high levels in members of some black fly species (Francy *et al.* 1988) and that *S. vittatum* Zetterstedt is capable of transmitting VSV biologically in the lab (Cupp *et al.* 1992) has further established the vector significance of this family. VSV does not establish high viremias in its vertebrate hosts (e.g. cattle) making its transmission by black flies (and other blood feeders) something of a mystery that appears to have been solved by Mead *et al.* (2000) who showed that VSV transmission can occur between black flies as they feed on the same host.

Overview of black fly host preferences

Black flies are conventionally divided into ornithophilic and mammalophilic groups in terms of broad host preferences, though the gap between these two types of hosts is spanned by some species. Black flies are generally considered to be fairly host specific within these host ranges. At one extreme, *S. euryadminiculum* Davies apparently restricts itself to feeding from the common loon (Adler *et al.* 2004) and is the poster species for the supposed high host-specificity of black flies. Mammalophilic species also appear in some cases to draw some clear distinctions between potential hosts. For instance, *S. arcticum* Malloch in Alberta forms massive swarms around cattle through which humans can walk and within which humans can work and rarely, if ever, get bitten (Shemanchuk 1986). Many other mammalophilic species appear to have broader host preferences (see Crosskey 1990 for a more detailed review of known black fly host preferences and Adler *et al.* 2004 for more recent information for North American species). It would be of great interest to know what role black fly responses to host-originating stimuli play in the evolution and expression of these host preferences.

Aims of this review

Various authors have reviewed aspects of host orientation and responses to blood hosts by biting flies. These reviews have different scopes ranging from those that look at the roles of specific types of stimuli (e.g. olfaction in mosquitoes – Takken 1991; visual stimuli in biting flies – Allan *et al.* 1987) to those that focus on particular parts of the process (e.g. 'distance orientation' in biting flies – Sutcliffe 1987) to others that summarise the broad scope of hosting seeking behavioural control for a number of biting fly taxa (e.g. Gibson and Torr 1999). In the first part of this review, I will deal mainly with the part of the host-black fly interaction that corresponds to active orientation of the insect to the host up to and including landing on the host and seeking out a biting site. This represents a part of the overall host location-blood feeding process in black flies that was interpreted by Sutcliffe (1986) as beginning with activation, when the blood feeder first detects

the presence of the host, and as culminating in gorging followed by the departure of the blood fed insect out of the reach of the host's defensive reactions.

In the second part of this review I will discuss related topics including what the details of black fly responses to host stimuli may say about the evolution of host preferences, the nature of host seeking-blood feeding as a behavioural package and, finally, some of the challenges inherent in research on host seeking in simuliids.

Black fly – host interactions (outside of active orientation phase)

In the following paragraphs I provide a brief overview of the components of the black fly – host interaction outside of active orientation for purposes of setting active orientation (the main topic of this review) in its larger behaviourally meaningful context.

Activation

This larger context includes the activation step which precedes host seeking proper. In activation, the insect is stimulated out of some ground or resting state into an actively host-seeking mode by contact with host-originating stimuli (see section on 'Host seeking-blood feeding as a behavioural mode' for further discussion of this concept). In other biting flies, CO_2 and other host odours are widely considered to be activating (see reviews by Gibson and Torr 1999, Takken 1991). This is likely the case for most simuliids too since many species are caught in larger numbers at traps and targets baited with CO_2 only than at traps and targets without it (Sutcliffe 1986, 1987). This makes a logical case for CO_2 as an activating stimulus in these instances since for flies to be caught at a trap or target they first need to have been activated. Nonetheless, there are clear exceptions to the CO_2 as activator rule (if it is a rule) since certain black fly species have been caught in situations where only non-CO_2 host stimuli were present. For example, in Algonquin Park, Ontario *S. euryadminiculum* swarmed near sources of loon uropygial odour (e.g. filter papers bearing residues from ether extracts of uropygial gland – Lowther and Wood 1964) whether CO_2 was co-released or not and the inclusion of CO_2 did not greatly increase numbers swarming (Fallis and Smith 1964; JF Sutcliffe personal observations). In this case, it must be concluded that it was some of the odours from uropygial gland secretions that were the activating principal(s). Examples where CO_2 appears not to be necessary to catch black flies (though it may augment catches achieved with other odours) at traps or targets are also known. In field experiments in Cameroon, Thompson (1976b) found that traps baited with odours from human sweat would catch large numbers of *S. damnosum* Theobald s.l. even in the absence of CO_2. By the same logic, these substances must to possess odour components that have activating properties for this species. Uropygial secretions of ducks also appear capable of activating some duck-biting species such as *S. rugglesi* Nicholson and Mickel and *S. anatinum* Wood (Fallis and Smith 1964). While odours are often cited as activating stimuli for host seeking in black flies and other biting flies, species in open savanna or parkland habitats may also rely on visual stimuli because of the longer site lines available to them. Thompson (1976a) presents evidence that suggests the savanna form of *S. damnosum* may require no more than the sight of the human form to be activated while the same appears not to be the case for the purportedly less anthropophilic (Crosskey 1990) 'forest form' of *S. damnosum*.

Biting site selection

Post active orientation (i.e. post landing) components of the host-black fly interaction include several complex activities such as biting site selection, biting, gorging and disengagement from the host followed by escape.

Biting site selection in many black flies is characterised by rapid movement over the skin, fur or feathers accompanied by a patting or scanning action of the prothoracic legs. The prothoracic tarsi bear larger numbers of gustatory sensilla than the mesothoracic or metathoracic tarsi (Sutcliffe and McIver 1976, 1987). This suggests that chemical cues are involved in mediating the biting response though we still know very little about the nature of these cues or whether others (e.g. thermal cues) also play a role in biting site selection. See the discussion under 'Heat and moisture cues' and 'Non-breath odours (including natural repellents)' below for further consideration of this topic.

Biting proper is accomplished by a complex interaction of the mouthparts with the skin. This process is quite well understood from a mechanical – anatomical perspective. Various authors have examined simuliid mouthparts in detail (Sutcliffe 1985, Sutcliffe and McIver 1982, Wenk 1962) and Sutcliffe and McIver (1984) provided a detailed analysis of the mouthpart sensilla of *S. venustum* s.l. and their various possible roles during biting. The make-up and properties of black fly saliva has been investigated as well. Like other blood feeders' saliva, black fly saliva contains a range of substances many of which are suited to interrupting the haemostatic process (Cupp and Cupp 1997) thus allowing feeding to proceed as quickly and efficiently as possible. Studies by (Stallings *et al.* 2002) on *S. vittatum* further reveal that black fly saliva has properties that may help microfilariae in nearby sub-cutaneous tissues to move toward the black fly's mouthparts during biting.

Active feeding

The onset of active feeding (gorging) is known to be stimulated by certain substances (gorging stimulants, phagostimulants) in the blood. Cessation of feeding is probably due to inputs from gut stretch receptors though no specific information about this exists for black flies. Phagostimulants for *S. venustum* s.l. were studied using artificial feeding methods (Smith and Friend 1982, Sutcliffe and McIver 1979) and include various nucleotides (in particular ADP) which can stimulate full gorging at concentrations as low as 10^{-5} M in saline.

Of particular interest in further understanding how biting and feeding are controlled in black flies are electrophysiological studies of the tarsal, antennal, and mouthpart and food canal sensilla. Putative functions for these receptors are reviewed by McIver and Sutcliffe (1986) based on sensillar morphology and placement but until tested electrophysiologically, these proposed functions must be considered unconfirmed.

Active orientation to the host

Carbon dioxide

Carbon dioxide is a powerful mediator of host seeking for many biting flies including many black fly species. For this reason, CO_2 from a block of dry ice or a tank has been a standard method used to increase the black fly catch at many types of traps and targets. In such applications, CO_2

is often referred to as an 'attractant'. This is a term that has been used loosely by many authors (including this one, e.g. Sutcliffe 1986) over the years. For a chemical mediator of host seeking to qualify as a true attractant it must induce upwind anemotaxis. It is not possible to know simply by observing the accumulated swarm of flies around the trap, or by the increased catch of a sticky target or entry trap, whether the greater numbers are due to an attractant effect of CO_2 or whether they are, instead, due to elevated CO_2 levels simply stimulating increasing unoriented black fly flight activity that, in turn, causes more flies to encounter the arresting stimulus of the visually conspicuous trap or get caught on the sticky target.

To address this and similar questions, a method of intercepting black flies in their host-seeking flight is needed. If interception traps can be deployed some distance away around a CO_2 source, it should be possible to differentiate between attraction and increased unoriented flight activity since the former will be characterised by high catches on the downwind sides of traps while the latter will be characterised by more or less equal catches on downwind and upwind sides. Working in Alberta on populations made up mostly of *S. arcticum* (IIS-10.11), Sutcliffe *et al.* (1995) deployed six Tangletrap-coated clear Lexan panels (upright sticky traps – USTs) as interception traps (see section entitled 'Challenges in the study of black fly host seeking behaviour' for further discussion of these traps) in a 12 m diameter ring around a CO_2-baited cow silhouette trap (CST). Approximately 82% of the black flies caught were on the downwind sides of the panels supporting the interpretation that they had flown upwind in the CO_2 plume. These results are similar to those of Golini and Davies (1971) for *S. venustum* Say in Algonquin Park, Ontario. Such results justify the use of the term 'attractant' with respect to CO_2 for these particular black fly species and, they may also be taken as some indication of CO_2 acting as an attractant for other black fly species. Nonetheless, this is a term that should always be used with due consideration.

In general in simuliids there appears to be good evidence for an increased response (in the form of increased trap catches) with increased release of CO_2, i.e. a dose-effect. However, the shape of the dose-response curve, whether it is different for different species and what affects the curve's shape is poorly known. Part of this is due to the fact that much of the field work that reports on the effects of CO_2 dose on numbers of black flies caught has been based on very rough methods of controlling and measuring CO_2 release rate. To be most worthwhile, such work should employ precision release valves and accurate rotameter type flow gauges to regulate and measure release rates from CO_2 tanks fitted with two stage regulators.

Fallis *et al.* (1967) measured the CO_2 dose-response for black flies in Algonquin Park, Ontario, Canada and found that catch rate at CO_2-baited fan traps increased in decreasing proportion to CO_2 release rates from 0 to 800 ml/min. Sutcliffe and Schofield (unpublished results) have also found a clear dose-response of CO_2 for UST catches for several black fly species in Alberta and Ontario. As in Fallis *et al.* (1967), these dose-effects 'top out' at higher, though still physiologically relevant for cattle, CO_2 release rates. It is not clear whether this is due to the plume simply recruiting more and more black flies in its active space, until there are no more flies left to recruit, or if it is due to an ever-increasing recruitment by the plume coupled with a fall off in numbers that succeed in navigating the greater distances to the USTs. Torr (1990) found that CO_2 plumes were not readily navigable for *Glossina pallidipes* Austen but became much more so when acetone was co-released. It would be interesting to see the effects of other chemical mediators of black fly host-seeking on the shape of the CO_2 dose-response curve.

Livestock under black fly attack can often be observed to bunch up apparently for the same reasons other herd animals bunch up when under threat of attack from a predator – there is

safety in numbers. What this means in the context of biting fly attack is not clear. The 'topping out' of the CO_2 dose-response in black flies may provide the explanation for this behaviour in animals under black fly attack since it suggests that though a large group of animals will produce a great deal of CO_2, the per unit return in terms of black flies attacking will not be linear, i.e. the ratio of flies to individual cattle should drop and so, therefore, should individual host biting rate. Ratti *et al.* (2006) present quantitative data showing a dilution effect on black fly biting on black grouse proportional to group size. They do not discuss why this occurs but the shape of CO_2 dose-response curve may be part of the explanation.

Schofield and Sutcliffe (1996) show that the amount of CO_2 produced by human individuals also appears to account for individual host 'attractiveness' for human biting black flies in Algonquin Park. Black fly catch on sticky Lexan panels mounted above the heads of seated, similarly dressed human subjects proved to be significantly and consistently different over two years of experiments. Removal of breath from the vicinity of the subjects (by having them breathe out through long tubes with their openings many meters away at right angles to the wind) eliminated individual differences and reduced catches on the panels by about 85%. This was shown to have been due to the removal of the CO_2 component, and not the non-CO_2 breath odours, by the fact that release of tanked CO_2 from a tube at the subjects' head levels at subject-specific rates restored individual panel catches to about 90% of their previous levels and completely restored individual differences.

Non-CO_2 breath odours

To date there is very little known about the chemical identities of non-CO_2 breath odours that mediate black fly host seeking though there is a variety of evidence that such substances exist for at least some species (Sutcliffe 1986, 1987). It may be significant, for instance, that Schofield and Sutcliffe (1996) were only able to restore about 90% of the whole breath catch on sticky panels mounted above the heads human subjects in field experiments in Algonquin Park (see experiment description above) when subject-specific amounts of tanked CO_2 were released. This could mean that non-CO_2 components of breath are responsible for larger numbers of black flies responding to the plume or that non-CO_2 breath components play a role in 'focussing' the responders so that more of them were intercepted by the sticky panels. The latter explanation is supported by results from field work in Algonquin Park by Dean (2004). In these experiments, a yellow square (46×46 cm) or cylinder (20 cm high×15 cm diameter) target was baited either with nothing, CO_2 at 300 ml/min or with whole breath (adjusted to contain 300 ml/min of CO_2) of a single adult male subject. At the same time, a black cloth target or cylinder of the same configuration as its yellow counterpart, was placed across the wind from the yellow target at separations of 0 m, 1 m or 3 m. Total number of flies caught on sticky interception screens placed immediately in front of the yellow and black targets were not significantly different for CO_2 and breath-baited trials but, for any given separation, yellow targets baited with whole breath captured a higher proportion of the total than did CO_2 baited yellow targets. This suggests that whole breath contained substances that 'focussed' the responding flies more tightly in the odour plume (perhaps by making the plume more navigable) but that these substances did not recruit more flies to host seeking. Similar properties have been found in tsetse flies for acetone and octenol which Torr (1990) found improve the navigability of a CO_2 plume for *Glossina pallidipes*.

Sutcliffe *et al.* (1994) tested several substances found in cattle breath for their abilities to enhance catches of black flies in Athabasca, Alberta. These included acetone and 1-octen-3-ol (hereafter termed octenol), 1- and 2-octanol and octanoic (caprylic) acid each at 'high' and 'low' release rates and each accompanied by CO_2 released from a pressurised tank. Only acetone at the high rate

produced significantly higher catches than the CO_2-only control. Unfortunately, this work was plagued by inadequate CO_2 flow control problems and the release rates of the chemicals was not quantified. This, and the fact that the octenol effect in those host seekers it is known to occur in is dose dependent (Takken and Knols 1999, Vale *et al.* 1985) means the lack of an octenol effect in these experiments should not be taken for the lack of an octenol effect. Subsequent work done in Alberta incorporating more precise CO_2 flow control and using UST catching methods (JF Sutcliffe unpublished results) failed to reproduce an acetone effect and further failed to show any effect of octenol.

Atwood and Meisch (1993) also investigated the effects of octenol odours (presented with and without CO_2) on black fly catches in tent traps in Arkansas. Results were mixed though octenol (released from pipe cleaner wicks inserted into vials) plus CO_2 resulted in significantly larger catches of *Cnephia pecuarum* Riley than CO_2 alone in one trial of three. In the single trial in which *S. meridionale* Riley catches are reported, octenol plus CO_2 produced much smaller catches than CO_2 alone.

Results from experiments such as those reported in this section reveal some of the problems that must be addressed for field experiments on odour mediation of black fly host seeking to be maximally valuable. For instance, neither study could report precise or even estimated dose rates for chemicals tested in the field because release from wicks (Atwood and Meisch 1993) and from vials with different sized holes in the caps (Sutcliffe *et al.* 1994) would have varied through the day depending on environmental conditions. The importance of such information in the interpretation of results like these is difficult to over-state. Other features of these studies, such as inadequate control of CO_2 release rate and collecting devices (tent trap and CST, respectively) that require landing and or trap entry for the fly to become part of the sample are also problematic. The former is easily done with high precision valves and flow meters attached to a CO_2 tank with a two-stage regulator. The latter can best be addressed by using simple interception traps such as USTs (Sutcliffe *et al.* 1995, see 'Challenges in the study of black fly host seeking behaviour' section for further discussion of this).

Non-breath odours

One of the most dramatic examples of non-breath chemical mediation of black fly host seeking is the effect of secretions of the uropygial glands of certain waterfowl on some ornithophilic species. Lowther and Wood (1964) first reported that *S. euryadminiculum* is strongly and apparently exclusively 'attracted' to loon uropygial gland extracts with or without a visual stimulus and with or without CO_2 (Fallis and Smith 1964). In this same study, catches of other waterfowl biters (e.g. *S. rugglesi, S. anatinum*) in sweep net collections over filter papers impregnated with various extracts of duck uropygial glands were also greater than control sweeps though, in this case, the addition of CO_2 significantly increased catch of these species. The active specifics in loon and duck uropygial secretions have not been isolated and identified though we have unpublished data (SW Schofield and JF Sutcliffe) that indicates that long chain alcohols in loon uropygial gland extracts may play a role.

Sutcliffe *et al.* (1994) examined the ability of crude and fractionated 'whole cow odour' (WCO) and cow urine to enhance CST catches of black flies (largely *S. arcticum* IIS-10.11) in Alberta. While crude WCO plus CO_2 resulted in catches significantly higher than the control (CO_2 only), none of the fractions did so suggesting a possible synergism between two or more components of the WCO. Cow urine in combination with CO_2, while it resulted in higher-than-control catches in CSTs

than CO_2 alone, did not have a significant effect though it was close to significance ($P<0.12$). In work done subsequently in Alberta (JF Sutcliffe, unpublished results) with better CO_2 flow control and using USTs instead of CSTs, cow urine (stored for no more than 3 weeks in a 4 °C refrigerator) plus CO_2 was found to result in larger catches than CO_2 alone.

Information on human odours other than breath as mediators of black fly host orientation is scant. The first evidence that such substances influence human biters was provided by Thompson (1976a,b) who found that clothing that had been worn for some time enhanced trap catches of the forest form of *S. damnosum* s.l. in Cameroon even in the absence of CO_2. Thompson (1976b) makes a circumstantial case for the source of this substance being apocrine sweat though this has not been investigated further.

Schofield and Sutcliffe (1997) provide the only other evidence to date for an effect of human skin secretions on biting responses of black flies. In field-lab experiments with human biting species (*S. venustum* s.l.) in Algonquin Park, they first established that human test subjects had different 'bitabilities' (defined as each subject's likelihood of being bitten by a given black fly) by placing black flies caught individually in vials off a CO_2-baited cloth target, directly onto a subject's skin and observing for a biting response for three minutes. Different subjects had significantly different bitabilities that were maintained over several weeks. To isolate a possible skin substance effect from other subject-specific characteristics that may have been influencing biting, similarly caught black flies were given the opportunity to bite artificial latex rubber membranes that had been treated with subject skin secretions and then stretched over a warmed (approx 32-37 °C) aluminum block. The hierarchy of individual bitability and subject-membrane bitability corresponded closely although in an unexpected manner. Treated membranes were bitten less frequently than control membranes and membranes of more bitable individuals were so because they were less 'repellent' than membranes corresponding to less bitable individuals. Nothing is known of the chemical properties of these apparent biting inhibitors though the observation that flies in vials on treated membranes stayed on the sides and top of the vials could suggest that the effect was, at least in part, due to volatiles. This work raises the possibility of what Schofield and Sutcliffe (1997) refer to as 'antibitants' in human skin. Many a black fly field worker has experienced severe black fly attack immediately after bathing and hair washing. The temporary removal of antibitants from the skin and hair brought about by bathing and shampooing may help explain this phenomenon. While the notion of antibitants or natural 'repellents' has not been pursued further for simuliids, there is mounting evidence for host-derived compounds that variously interfere with aspects of host seeking and blood feeding in other haematophagous arthropods (e.g. Logan *et al.* 2008).

While on the face of it the treatment effect described above looks like repellence, interpretations of such experiments must be done carefully. Schofield and Sutcliffe (1997) recognise this and propose alternatively that what is an apparent repellence could actually be the effect of skin substances that stimulate walking activity that normally occurs when the insect lands on the skin (see description of 'patting' behaviour in 'Biting site selection' section). Confinement of the black fly in the vial might make this look like an attempt to escape a repellent substance.

A final, indirect, mode of chemical interaction between the host and the blood feeding black fly may be what McCall and Lemoh (1997) refer to as the 'invitation effect' observed in certain members of the *S. damnosum* complex. This is suggested to be a pheromone effect between black flies in the act of biting the host and black flies that have not yet selected a biting site. The effect is to increase the number of flies biting on a given area of the host's skin. The sensory basis of this effect has not been elucidated nor has it been determined who (the sender or the receiver)

benefits from the message though the term 'invitation effect' implies the sender. It is arguable, however, that by biting where saliva from previous bites has already increased blood flow to the area, the receiving individual may benefit by being able to feed rapidly at that site. If this is the case, it might be better to re-name this the 'party crasher effect'.

Visual interactions with hosts

In Sutcliffe's (1986) framework for host seeking in simuliids, chemically-mediated orientation to the host eventually brings the fly into visual contact with the host. This contact would first be with the host animal as a whole but with closer approach, the fly would be able to see and respond to particular physical and textural features of the host.

All other things being equal, the range at which the host first becomes visible for different species would be determined by the physical setting. Visual obstructions and the complexity in woodland surroundings may make it more difficult for the host seeker to see a host animal of a given size, shape and colouration than in relatively less complex, more open settings such as savanna and parkland. However, the relative ambiguity of odour information about the location of an odour source (such as a host animal – Brady *et al.* 1990) should mean that the host-seeking fly will give priority to much less ambiguous visual information when it becomes available. This, in turn, suggests that woodland species may rely on olfactory cues for longer than open landscape species which may rely more on visual cues. Relatively little information on this question is available though some support for this in the savanna form versus the forest form of *S. damnosum* is presented in the section titled 'Activation'.

Based on field experiments, Sutcliffe *et al.* (1995) estimated that host seeking *S. arcticum* (IIS-10.11) responded visually to a host-mimicking CST (110 cm long by 46 cm wide) from about 8 m. At this distance, they calculated that the image of the CST would subtend an angle of about 8° (in the horizontal plane) and would cover all or parts of up 15 ommatidia. If other species of black flies have a similar visual resolution capability for their hosts, it would mean that something the size of a human would be resolved at a distance of approximately 20 m while a host the size of a loon would not become apparent until it was within about 100 cm. Black fly species that bite smaller birds in leafed out, relatively dimly lit wooded areas might not make effective visual contact until within a few centimetres.

When visual and chemical stimuli are both 'in play', which take precedence? The reasoning presented at the beginning of this section indicates that the less ambiguous visual signal should. In field experiments in Algonquin Park, Ontario, Canada, Dean (2004) undertook to examine this question for host-seeking mammalophilic black flies. In these experiments a yellow square (46×46 cm) or cylindrical (20 cm high × 15 cm diameter) target was baited either with nothing, CO_2 at 300 ml/min or with whole breath of a single adult male adjusted to 300 ml per minute CO_2. At the same time, a black cloth target or cylinder of the same configuration as its yellow counterpart, was placed either beside the yellow target (0 m separation) or at separations of 1 or 3 m. When at 0 m separation, the sticky screens in front of black target always caught significantly more than half of the flies irrespective of the bait. However, when the targets were separated by 1 or 3 m, the sticky screens in front of the baited (yellow) targets collected relatively more flies than those in front of the dark targets. Clearly, our understanding of how responses to different modalities of stimulus are ordered during host seeking in simuliids requires further examination and probably needs to integrate elements such host/target movement.

Crosskey (1990) provides a detailed discussion of the preferred feeding sites for different groups of mammal-biting black flies. While details vary from host to host, he notes that most species biting large mammals do so on either on the muzzle and ears or on the belly, chest and front legs. The adaptive reasons for black flies having preferred biting areas are poorly researched but presumably relate to some degree to avoidance of host defensive behaviours. Other reasons for certain areas of the body being preferred for biting may also relate to avoiding competition with other black fly or biting fly species. In ornithophilic species such as *S. euryadminiculum* and *S. rugglesi*, preferred biting sites are on the head and neck Bennett *et al.* (1972) again presumably because the animal is less able to defend itself from flies in such places.

Visual cues play an important role in determining where on the host the black fly will land, i.e. in leading the fly to its preferred biting area on the host. Extensive study of colour and shape preferences of a number of black fly species (reviewed by Sutcliffe 1986, 1987) have established that black flies generally prefer to land on darker colours and matte surfaces. While black flies do not seem to assess overall shape prior to landing, some species do exhibit a preference for landing near edges and on prominences. Choice of landing site appears to be largely independent of odour stimuli. Yee and Anderson (1995) showed that females of several black fly species they studied in California would land on the head ends of deer-mimicking three dimensional models irrespective of whether CO_2 used to bait the models was released from the head end of the model or from beneath it. Similarly, the black fly *Wilhelmia equina* L. will orient to the head end of a host model even of the odour bait is at the tail end (Wenk and Schlorer 1963). When not given a head or ears to indicate which end of a CST is which, *S. arcticum* landed equally on both ends (Sutcliffe *et al.* 1995). These and similar findings suggest that though there is a range over which the host seeking black fly is guided by host odours, at very close range, visual stimuli take over and guide landing/biting site selection.

Heat and moisture cues

The importance of heat/thermal stimuli for host-seeking black flies is implied by the fact that simuliids bite only warm-blooded hosts (birds and mammals). The role of thermal stimuli in mediating landing and biting in human biting black flies in Algonquin Park Canada was examined by Sutcliffe and McIver (1979), who showed that female flies collected individually in small vials after landing on a human host and subsequently transferred onto a warmed latex membrane, would bite the membrane more readily when the temperature differential between the membrane and the air just above it was greater. They argued that this means that skin (or membrane) temperature alone is not the key stimulus and suggested that the thermal differential may have served as a short range landing cue. Schofield and Sutcliffe (1997) in their experiments on the bitability of latex membranes treated with human skin secretions also measured the effect of the temperature differential between the air and the artificial biting surface. Again, black flies that experienced a greater temperature differential (14-17 °C) between the air and the membrane were much more likely to bite the membrane than flies that experienced a lesser differential (11-14 °C). The question of whether the temperature differential is a short range orientation/landing stimulus (Sutcliffe and McIver 1979) or a biting stimulus (Smith and Friend 1982) remains an open one.

Virtually no information exists for the effects of water vapour either in breath or from the skin on black fly host seeking.

Discussion

Host seeking-blood feeding as a behavioural mode

The concept of 'activation' is used extensively in the discussion of host seeking in biting Diptera though what is meant by the term is not always clear. Sutcliffe (1986, 1987) suggested that activation is the event that causes a 'switching over' from some ground state of behaviour into a host seeking 'behavioural package' or mode. While in the host seeking mode the host seeker would be biased to respond to host originating stimuli in a manner appropriate to the host seeking circumstances but, under different circumstances (e.g. in an oviposition behavioural mode), the insect might respond to the same stimulus differently or not at all. This suggests that the activation and continuation of black fly host seeking may fit the 'rolling fulcrum' model (Miller and Strickler 1984) developed as a theoretical framework to explain host-seeking behaviour in plant feeding insects.

During artificial feeding experiments in Algonquin Park, Sutcliffe and McIver (1979) observed that black flies captured individually in vials after landing on a host would only bite the skin-simulating artificially-warmed membrane if given the opportunity to do so within a few minutes of collection. Flies held for too long would often not bite when placed on the membrane and many appeared agitated and to be trying to get away from the warmth of the membrane. They interpreted this as the result of the flies having switched out of the host-seeking mode during the holding period. In the framework of the rolling fulcrum model, the host-related stimuli that served to establish (activate) and sustain host seeking-appropriate responses had been interrupted long enough for some of the flies (those that would not bite) to revert to some more basic behavioural mode in which sources of heat might be interpreted as representing and increased desiccation risk. When black flies were held in vials for different lengths of time after being collected from a host, we found that there was a gradual decrease in the proportion of the sample that would bite the artificial membrane such that the 'half life' of biting persistence was only a few minutes (JF Sutcliffe, unpublished results). Dean (2004) investigated this phenomenon in human biting black fly species in Algonquin Park and tried to determine factors that account for some flies switching out of the host seeking mode before others and to see if biting persistence could be extended in captive female black flies by providing flies with continued contact with simulated host stimuli (in this case, slightly elevated levels of CO_2 in an air stream) during the holding period. The treatment had no effect and has not been followed up though it would be interesting to do so with more complex and complete mixtures of host-odour stimuli.

The existence of a behavioural mode that appears to establish a behavioural context for the host seeking stimulus-responses has implications for the interpretation of results from experiments designed to study the host seeker-host interaction and for the circumstances under which such work can be done meaningfully. In my experience, it is simply not possible to get female black flies that have been held after being collected in the field to respond 'appropriately' to host stimuli in the lab. This makes behavioural work on these problems 'out of season' and anywhere but in the field problematic. Thus, lab-based olfactometer and wind tunnel experiments, odour choice tests, artificial feeding experiments using previously collected flies or using flies reared from pupae and so-on are exceedingly difficult to perform for this group. Even if it were possible to get black flies to 'behave' in the lab, the fidelity with which lab responses reflect what would happen in a natural setting would be open to question. Indeed, research on host-seeking responses in biting flies in general should be carefully interpreted since the concept of host seeking as a context-sensitive behavioural mode is not unique to black flies.

Host preferences

While the range of potential black fly host preferences is determined at one level by ecological and temporal factors (i.e. the fly and the prospective host species must occur in the same habitat at the same time), whether a given black fly species actually prefers a particular host species is presumably determined by subtleties of sensory cues given off by hosts and by the responses of the host seeker to those stimuli.

The precise dimensions of the host preferences for most black fly species are not well known partly because many black fly species are very difficult to tell apart in the field and can often only be differentiated at the species level with cytogenetic or molecular methods. Another reason for our poor understanding of black fly host preferences is that is very difficult to field-collect black flies with appreciable amounts leftover blood meals in the field. Thus, it has been difficult to apply ELISA and other immunological methods to the analysis of black fly blood meal sources. However, the current widespread availability of very sensitive molecular methods may make it more feasible than ever to determine the origins of blood meal remnants in flies even several days after feeding. Though this opportunity has not been exploited widely yet, some researchers have taken advantage (e.g. Malmqvist *et al.* 2004) and the approach has already yielded information that has implications for black fly interactions with their hosts. Hellgren *et al.* (2008) found that three of four specimens of *S. annulus* (Lundstrom) collected with a car top trap at points 200 km apart in northern Sweden had all fed from Common Cranes (*Grus grus* (Linnaeus)) despite the fact this bird is not numerous in the study area. This suggests that *S. annulus* may orient preferentially to *G. grus* because of specific odour or visual stimuli the bird possesses. Results like these when investigated further may lead to many more examples of specific, host stimulus-based preferences in black flies like those that exist between, for instance, *S. euryadminiculum* and loons and between *S. rugglesi* and waterfowl.

A better understanding of the stimuli that the host seeking black fly encounters and how the fly reacts to these stimuli may explain much about the sensory and behavioural mechanisms that result in host preferences. For instance, where in the host-seeking process does host the preference exert itself (i.e. where does host discrimination occur)? Is it from the start of active orientation or does the insect begin host seeking based on initial minimal information about the prospective host and make decisions about whether to continue further host seeking when subsequent cues fail to, or succeed in, satisfying certain 'expected' criteria (e.g. host-specific body or breath odours, visual host qualities that the insect will respond to in the host seeking behavioural context) that confirm that the prospective host is indeed suitable. That the insect could have enough information about the prospective host from initial contact seems highly unlikely unless the stimulus is highly specialised such as in the case of loon uropygial odours for *S. euryadminiculum*. For many more generalist species, first contact may be with more generalised stimuli such as elevated levels of CO_2. In the latter situation, host discrimination must be ongoing as the insect continues to move closer to the host coming into contact with more and more host-related stimuli that ultimately allow the insect to determine whether it should continue to invest effort and invoke risk both of which host seeking and the act of blood feeding entail.

The range of host preferences exhibited in black flies, and in biting flies in general, must reflect evolutionary 'choices' made by different species or lineages. Different host-seeking strategies would come with their own sets of advantages and disadvantages. A high degree of host species specialisation could be combined with a narrow range of specific host-related stimuli that the insect would require to initiate and engage in sustained host seeking. This strategy would narrow

the number of host animals available for the specialist to feed on but would help ensure that its investment of energy in host seeking would be most likely to result in a blood meal with minimum risk. Alternatively, for biting flies with broader host ranges, a better strategy might be to respond initially to more general host-related stimuli (e.g. CO_2) and discriminate as more information, in the form of host-related stimuli, becomes available. This greatly enlarges the number of potential host animals available but it also increases the probability of investing energy and undertaking additional risk for no return.

Members of the *Anopheles gambiae* Giles mosquito species complex are host specialists and appear to conform to this pattern. In behavioural experiments in Burkina Faso, Costantini *et al.* (2001) showed that *An. gambiae* s.l. females relied less on CO_2 cues and more on human-specific odours to mediate host seeking. Perhaps *An. gambiae* made the evolutionary 'choice' to specialise on humans long ago. This is plausible for an African species since it could have co-evolved with humans over several hundred thousand years. The human biting black fly counterparts to *An. gambiae* could be the savannah and forest forms of African species *S. damnosum* s.l. Thompson (1976a,b) working in Cameroon showed that substances in human skin and sweat (with or without CO_2) result in large swarms of the forest form around traps while the human profile without any skin or breath odours, is swarmed heavily by savannah form. Certain black flies that bite non-humans are also highly host specific and also fit the pattern. *Simulium euryadminiculum* appears to specialise on loons and swarms en masse to odours of extracts of loon uropygial gland. The addition of CO_2 to uropygial gland odour appears not to increase numbers. Species such as *S. rugglesi* and *S. anatinum* with reportedly somewhat broader host preferences (various ducks and to a limited extent other birds) only respond to duck uropygial gland secretions if accompanied by CO_2 and to a lesser extent to CO_2 alone (Fallis and Smith 1964). Other ornithophilic species such as *S. aureum* s.l. and *Prosimulium decemarticulatum* Twinn which have even broader host preferences among waterfowl and passerines, respond in large numbers to CO_2 alone but not to uropygial gland secretions.

Given the broad occurrence of CO_2 as a sensory cue for insects of many types including for many non-biting Diptera (Guerenstein and Hildebrand 2008), perhaps CO_2 represents the ancestral host odour for black flies and sensitivity to other host odours evolved subsequently. This process could have resulted in the various degrees of host specificity that are represented in the family today and to a gradual de-emphasis of the CO_2 cue for those with the greatest degree of host-specificity.

Challenges in the study of black fly host-seeking behaviour

Notwithstanding the concerns about the interpretation of lab-evoked 'host seeking' responses (above), work with many mosquito species and with certain other biting flies (e.g. stable flies, tsetse) from lab colonies has helped elucidate various features of host seeking in these groups. However, to date, virtually all work that sheds light on aspects of host seeking in black flies has been field-based because black fly colonies are few and far between because they are difficult to establish and labour-intensive and costly to maintain. Edman and Simmons (1986) summarise the challenges, which include the infrastructure requirements associated with maintaining an insect that oviposits and whose larval stages occur in flowing water as well as the particular solutions that must be found to inducing mating, blood feeding, and oviposition by adults. According to Adler *et al.* (2004), only four North American black fly species have been successfully colonised on a continuing basis and only a handful of colonies currently exist.

The usefulness of colony reared black flies for host seeking work, if they were available, would probably also be limited for reasons already discussed. Indeed, many attempts to colonise black flies have involved autogenous species since they do not have to blood feed (or to be induced to perform some lab equivalent of host seeking) to produce eggs. Though inducing meaningful host-seeking responses with colonised black flies is problematic, there are other related constructive uses that black flies from a lab colony could be put to. For instance, colonised black flies could be used for electroantennogram-screening of candidate host-mediating chemicals thus helping focus efforts in the field.

Tsetse fly trap and target responses are relatively well worked out (Gibson and Torr 1999; Chapter 12, this volume) making it possible to design field experiments appropriately for the purposes intended. Black fly host-seeking research over the years has relied on a variety of host mimicking traps and targets of various shapes and sizes but, unlike in the tsetse world, how black fly traps and targets work is not well understood nor do we know in detail the responses they evoke from the host-seeking black fly. Electric nets in various configurations and applications have been one of the key tools in the analysis of tsetse host seeking behaviour because they can be used to intercept host orienting flies in a non-interactive way. Black fly work has not had an equivalent of the electric net. In an attempt to address this, we have used both sticky Lexan squares and sticky neutral coloured fibreglass window screening in such applications (e.g. Schofield and Sutcliffe 1996, Sutcliffe *et al.* 1996). In experiments where the desire is to assess the effects of candidate attractants without the introduction of other variables such colour preferences, trap entry behavior, and so-on, these methods are superior to more complex systems such as CSTs. However, sticky Lexan sheets and screens still have not been assessed for their ability to truly intercept host seeking black flies. This would be a worthwhile undertaking as would an assessment of the applicability of electric net technology to black fly host seeking research.

Conclusions

As we attempt to come to grips with the most basic of questions relating to host-seeking behaviour in black flies and biting flies in general, the landscape itself is changing. The most recent ingredient added to the host-insect interaction picture is the growing appreciation that the parasites that blood feeding Diptera transmit manipulate many aspects of their vertebrate and insect hosts' physiologies including those aspects that mediate host-seeking. The ability of certain malaria parasites to manipulate their mosquito vectors during blood feeding has been known for many years (e.g. Rossignol *et al.* 1984, 1986) but evidence is now mounting that parasite-infected host seekers may respond differently than non-infected host seekers during host seeking. There is also considerable evidence that parasite-infected vertebrate hosts may be more attractive for vector species (see reviews by Hurd 2003, Lefevre and Thomas 2008; Chapter 16, this volume). Kruppa and Burchard (1999) compared the ability of *Onchocerca volvulus*-infected individuals to 'attract' filarial-carrying and non-carrying black flies but found no differences. Nonetheless, there is no doubt that there are parasite manipulations of hosts that influence the host-biting fly interaction (indeed, that make this a three-way interaction) and that they will also have to be accounted for before a full understanding of biting fly host seeking behaviours can be achieved.

References

Addison EM (1980) Transmission of *Dirofilaria ursi* Yamaguti, 1941 (Nematoda: Onchocercidae) of black bears (*Ursus americanus*) by blackflies (Simuliidae). Can J Zool 58: 1913-1922.

Adler PH, Currie DC and Wood DM (2003) The black flies (Simuliidae) of North America. Comstock Publishing, Cornell, USA.

Allan SA, Day JF and Edman JD (1987) Visual ecology of biting flies. Annu Rev Entomol 32: 297-316.

Anderson, JR (1986) Reproductive strategies and gonotrophic cycles of black flies. In: Kim KC and Merritt RW (eds) Black flies. Ecology, population management and annotated world list. Pennsylvania State University, University Park, PA, USA, pp 63-76.

Atwood DW and Meisch MV (1993) Evaluation of 1-octen-3-ol and carbon dioxide as black fly (Diptera, Simuliidae) attractants in Arkansas. J Amer Mosq Contr Assoc 9: 143-146.

Beck BE (1980) Clinical studies of the effect of the black fly *Simulium arcticum* on cattle. In: Haufe WO and Croome GCR (eds) Control of black flies in the Athabasca River. Alberta Environment, Lethbridge, Alberta, Canada, pp 233-237.

Bennett GF, Fallis AM and Campbell AG (1972) The response of *Simulium* (*Eusimulium*) *euryadminiculum* Davies (Diptera: Simuliidae) to some olfactory and visual stimuli. Can J Zool 50: 793-800.

Brady J, Packer MJ and Gibson G (1990) Odor plume shape and host finding by tsetse. Ins Sci Applic 11: 377-384.

Costantini C, Birkett MA, Gibson G, Ziesmann J, Sagnon N'F, Mohammed HA, Coluzzi M and Pickett JA (2001) Electroantennogram and behavioural responses of the malaria vector *Anopheles gambiae* to human-specific sweat components. Med Vet Entomol 15: 259-266.

Crosskey RW (1990) The natural history of blackflies. Wiley, Chichester, UK.

Cupp EW (1986) The epizootiology of livestock and poultry diseases associated with black flies. In: Kim KC and Merritt RW (eds) Black flies. Ecology, population management and annotated world list. Pennsylvania State University, University Park, PA, USA, pp 387-395.

Cupp EW and Cupp MS (1997) Black fly (Diptera: Simuliidae) salivary secretions: Importance in vector competence and disease. J Med Entomol 34: 87-94.

Cupp EW, Mare CJ, Cupp MS and Rowley FB (1992) Biological transmission of Vesicular Stomatitis Virus (New Jersey) by *Simulium vittatum* (Diptera, Simuliidae). J Med Entomol 29: 137-140.

Dean BL (2004) Factors affecting host location behaviour in a field population of the black fly *Simulium venustum* (sensu lato) (Diptera: Simuliidae). MSc Dissertation, Trent University, Peterborough, Canada.

Edman JD and Simmons KR (1986) Maintaining black flies in the laboratory. In: Kim KC, Merritt RW (eds) Black flies. Ecology, population management and annotated world list. Pennsylvania State University, University Park, PA, USA, pp 305-312.

Fallis AM and Smith SM (1964) Ether extracts from birds and CO_2 as attractants for some ornithophilic simuliids. Can J Zool 42: 723-730.

Fallis AM, Bennett GF, Griggs G and Allen T (1967) Collecting *Simulium venustum* females in fan traps and on silhouettes with the aid of carbon dioxide. Can J Zool 45: 1011-1017.

Francy DB, Moore CG, Smith GC, Jacob WL, Taylor SH and Calisher CH (1988) Epizootic vesicular stomatitis in Colorado, 1982 – isolation of virus from insects collected along the northern Colorado Rocky Mountain front range. J Med Entomol 25: 343-347.

Gibson G and Torr SJ (1999) Visual and olfactory responses of haematophagous Diptera to host stimuli. Med Vet Entomol 13: 2-23.

Golini VI and Davies DM (1971) Upwind orientation of female *Simulium venustum* Say (Diptera) in Algonquin Park, Ontario. Proc Entomol Soc Ont 101: 49-54.

Guerenstein PG and Hildebrand JG (2008) Roles and effects of environmental carbon dioxide on insect life. Annu Rev Entomol 53: 161-178.

Hellgren O, Bensch S and Malmqvist B (2008) Bird hosts, blood parasites and their vectors – association uncovered by molecular analyses of blackfly blood meals. Molecular Ecol 17: 1605-1613.

Hurd H (2003) Manipulation of medically important insect vectors by their parasites. Annu Rev Entomol 48: 141-61.

Kruppa TF and Burchard GD (1999) Similar attraction by onchocerciasis patients and individuals putatively immune to *Onchocerca volvulus*. Trans R Soc Trop Med Hyg 93: 365-367.

Lefèvre T and Thomas F (2008) Behind the scene, something else is pulling the strings: Emphasizing parasitic manipulation in vector-borne diseases. Infection, Genetics and Evolution 8: 504-519.

Logan LG, Birkett MA, Clark SJ, Powers S, Seal NJ, Wadhams LJ, Mordue Luntz AJ and Pickett JA (2008) Identification of human-derived volatile chemicals that interfere with attraction of *Aedes aegypti* mosquitoes. J Chem Ecol 34: 308-322.

Lok JB, Walker ED and Scoles GA (2000) Filariasis. In: Eldridge BF, Edman JD (eds) Medical entomology. A textbook on public health and veterinary problems caused by arthropods. Kluwer, Dordrecht, the Netherlands, pp 299-375.

Lowther JK and Wood. DM (1964) Specificity of a black fly, *Simulium euryadminiculum* Davies, towards its host, the common loon. Can Entomol 96: 911-913.

Malmqvist B, Strasevicius D, Hellgren O, Adler PH, Bensch S (2004) Vertebrate host specificity of wild-caught blackflies revealed by mitochondrial DNA in blood. Proc R Soc Lond B 271: S152-S155.

McCall PJ and Lemoh PA (1997) Evidence for the "invitation effect" during bloodfeeding by blackflies of the *Simulium damnosum* complex (Diptera: Simuliidae). J Insect Behav 10: 299-303.

McIver SB and Sutcliffe JF (1986) Sensory basis of behavior and structural adaptations for feeding in black flies. In: Kim KC, Merritt RW (eds) Black flies. Ecology, population management and annotated world list. Pennsylvania State University, University Park, PA, USA.

Mead DG, Ramberg, FB, Besselsen DG and Mare CJ (2000) Transmission of vesicular stomatitis virus from infected to noninfected black flies co-feeding on nonviremic deer mice. Science 287: 485-487.

Miller JR and Strickler KS (1984) Finding and accepting host plants. In: Bell WJ, Cardé TR (eds) Chemical ecology of insects. Chapman Hall, London, UK, pp 127-157.

Ratti O, Ojanen U and Helle P (2006) Increasing group size dilutes black fly attack rate in Black Grouse. Ornis Fennica 83: 86-90.

Rossignol PA, Ribeiro JMC and Spielman A (1984) Increased intradermal probing time in sporozoite-infected mosquitoes. Am J Trop Med Hyg 33: 17-20.

Rossignol PA, Ribeiro JMC and Spielman A (1986) Increased biting rate and reduced fertility in sporozoite-infected mosquitoes. Am J Trop Med Hyg 35: 277-279.

Schofield SW and Sutcliffe JF (1996) Human individuals vary in attractiveness for host-seeking black flies (Diptera: Simuliidae) based on exhaled carbon dioxide. J Med Entomol 33: 102-108.

Schofield SW and Sutcliffe JF (1997) Humans vary in their ability to elicit biting responses from *Simulium venustum* (Diptera: Simuliidae). J Med Entomol 34: 64-67.

Shemanchuk JA (1986) Host-seeking behavior and host preference of *Simulium arcticum*. In: Kim KC and Merritt RW (eds) Black flies. Ecology, Population Management and Annotated World List. Pennsylvania State University, University Park, PA, USA, pp 250-260.

Smith SM (1969) The black fly, *Simulium venustum*, attracted to the turtle, *Chelydra serpentina*. Entomol News 80: 107-108.

Smith JJB and Friend WG (1982) Feeding behaviour in response to blood fractions and chemical phagostimulants in the black-fly, *Simulium venustum*. Physiol Entomol 7: 219-226.

Stallings T, Cupp MS and Cupp EW (2002) Orientation of *Onchocerca linealis* Stiles (Filaroidea: Onchocercidae) microfilariae to black fly saliva. J Med Entomol 39: 908-914.

Sutcliffe JF (1985) Anatomy of membranous mouthpart cuticles and their roles in feeding in black flies. J Morphol 186: 53-68.

Sutcliffe JF (1986) Black fly host location: a review. Can J Zool 64: 1041-1053.

Sutcliffe JF (1987). Distance orientation of biting flies to their hosts. Insect Sci Appl 8: 611-616.

Sutcliffe JF and McIver SB (1976) External morphology of the sensilla on the legs of selected black fly species (Diptera: Simuliidae). Can J Zool 54: 1779-1787.

Sutcliffe JF and McIver SB (1979) Experiments on biting and gorging behaviour in the black fly, *Simulium venustum*. Physiol Entomol 4: 393-400.

Sutcliffe JF and McIver SB (1982) Innervation and structure of mouthpart sensilla in females of the black fly, *Simulium venustum* (Diptera: Simuliidae). J Morphol 171: 245-258.

Sutcliffe JF and McIver SB (1984) Mechanics of blood feeding in black flies (Diptera: Simuliidae). J Morphol 180: 124-144.

Sutcliffe JF and McIver SB (1987) Fine structure of tarsal sensilla of male and female *Simulium vittatum* (Diptera: Simuliidae). J Morphol 192: 13-26.

Sutcliffe JF, Shemanchuk JA and McKeown DB (1994) Preliminary survey of odours that attract the black fly, *Simulium arcticum* (IIS-10.11)(Diptera: Simuliidae) to its cattle hosts in the Athabasca region of Alberta. Insect Sci and Its Application 15: 487-494.

Sutcliffe JF, Steer DJ and Beardsall D (1995) Studies of host location behaviour in the black fly, *Simulium arcticum* Malloch (IIS-10.11)(Diptera: Simuliidae): Aspects of close-range trap orientation. Bull Entomol Res 85: 415-424.

Takken W (1991) The role of olfaction in host-seeking of mosquitoes: a review. Insect Sci Applic 12: 297-295.

Takken W and Knols BG (1999) Odor-mediated behavior of Afrotropical malaria mosquitoes. Annu Rev Entomol 44: 131-157.

Thompson BH (1976a) Studies on the attraction of *Simulium damnosum* s.l. (Diptera: Simuliidae) to its hosts. I. The relative importance of sight, exhaled breath, and smell. Tropenmed. Parasitol 27: 355-473.

Thompson BH (1976b) Studies on the attraction of *Simulium damnosum* s.l. (Diptera: Simuliidae) to its hosts. II. The nature of substances of the human skin responsible for attractant olfactory stimuli. Tropenmed Parasitol 27: 83-90.

Thylefors B and Alleman M (2006) Towards the elimination of onchocerciasis. Ann Trop Med Parasitol 100: 733-746.

Torr SJ (1990) Dose response of tsetse flies (*Glossina*) to carbon dioxide, acetone and octenol in the field. Physiol Entomol 15: 93-103.

Vale GA and Hall DR (1985) The role of 1-octen-3-ol, acetone and carbon dioxide in the attraction of tsetse flies, *Glossina* spp. Bull Entomol Res 75: 209-217.

Wenk P (1962) Anatomie des Kopfes von *Wilhelmia equina* L. Females (Simuliidae syn. Melusinidae, Diptera). Zool Jahrb Abt Anat Ontog Tiere 80: 81-134.

Wenk P and Schlorer G (1963) Wirstorientierung und Kopulation bei blutsaugenden Simuliiden (Diptera). Z. Tropenmed Parasitol 14: 177-191.

Yee WL and Anderson JR (1995) Trapping black flies (Diptera-Simuliidae) in northern California. 2. Testing visual cues used in attraction to CO_2-baited animal head models. J Vector Ecol 20: 26-39.