Medical and Veterinary Entomology

Medical and Veterinary Entomology (2011) **25**, 104–108 doi: 10.1111/j.1365-2915.2010.00875.x

Spatial feeding preferences of ornithophilic mosquitoes, blackflies and biting midges

O. ČERNÝ[1], J. VOTÝPKA[1,2] and M. SVOBODOVÁ[1]

[1]Department of Parasitology, Faculty of Sciences, Charles University, Prague, Czech Republic and [2]Biology Centre, Institute of Parasitology, Czech Academy of Sciences, České Budějovice, Czech Republic

Abstract. The section of habitat used by particular bloodsucking insects when seeking bloodmeals may influence the spectrum of hosts to which they have access and consequently the diseases they transmit. The vertical distribution of ornithophilic bloodsucking Diptera (Culicidae, Simuliidae and Ceratopogonidae) was studied using bird-baited traps set at both ground and tree canopy levels. In total, 1240 mosquito females of eight species, 1201 biting midge females of 11 species, and 218 blackfly females of two species were captured during 2003–2005. *Culex pipiens* (L.) (Diptera: Culicidae) was found to prefer ground-level habitats, whereas *Anopheles plumbeus* (Stephens) (Diptera: Culicidae), biting midges [*Culicoides* spp. (Diptera: Ceratopogonidae)] and *Eusimulium angustipes* (Edwards) (Diptera: Simuliidae) preferred the canopy. The results of this study with regard to *Cx. pipiens* behaviour differ from those of most previous studies and may indicate different spatial feeding preferences in geographically separate populations. The occurrence of *E. angustipes* in the canopy is concordant with its role in the transmission of avian trypanosomes. These findings may be important for surveillance programmes focusing on ornithophilic Diptera which transmit various pathogenic agents.

Key words. *Culex, Culicoides, Eusimulium, Trypanosoma,* height preferences, ornithophilic Diptera.

Introduction

Bloodsucking Diptera, including ornithophilic species, are widely distributed vectors of numerous diseases caused by viruses, bacteria, protozoa and helminths. *Culex pipiens* and other ornithophilic mosquitoes facilitate transmission of West Nile virus (WNV) from birds to mammals; biting midges and blackflies transmit *Leucocytozoon*, *Haemoproteus* and *Plasmodium* (Haemosporida) to poultry and pets, and some species of ornithophilic biting midges may be involved in the transmission of a broad spectrum of arboviruses (Hubálek & Halouzka, 1996). The spatial preferences of ornithophilic haematophagous insects when seeking hosts can influence their host spectra and thus their roles as vectors of pathogens to wild and domestic birds. Nevertheless, reliable studies are scarce and the majority have focused on mosquitoes only; thus the spatial preferences of most species remain unknown.

In studies using bird-baited traps, mosquitoes were found to prefer the canopy level (Anderson *et al.*, 2004) or ground level (Flemings, 1959). Some studies show seasonally different preferences or no preference at all for some species (Service, 1971a; Lundström *et al.*, 1996; Darbro & Harrington, 2006). Therefore, the site of mosquito occurrence may differ according to the species' physiological status and geographic population differences. Ornithophilic biting midges probably prefer canopy level (Henry & Adkins, 1975; Braverman & Lindley, 1993; Garvin & Greiner, 2003); however, none of these studies used bird-baited traps. Blackflies seem to prefer bird-baited traps in the canopy over those at ground level, but the studies indicating this lacked statistical evaluation (Bennett, 1960; Anderson & DeFoliart, 1961; Kiszewski & Cupp, 1986).

Correspondence: O. Černý, MSc, Department of Zoology, Faculty of Sciences, Charles University in Prague, Viničná 7, 128 44 Prague, Czech Republic. Tel.: +420 77 674 1832; E-mail: ondras.cerny@gmail.com

In a previous study of avian trypanosomes, potential vectors of avian blood parasites were noted attacking young birds in raptor nests; the blackfly *Eusimulium angustipes* (Edwards, syn. *Eusimulium securiforme* Rubcov) was identified as a vector of *Trypanosoma avium* (Danilewsky) (Kinetoplastida: Trypanosomatidae) (Votýpka *et al*., 2002; Votýpka & Svobodová, 2004). In addition, several other trypanosomatid species were isolated from captured insects (Van Dyken *et al.*, 2006), including an as yet undescribed avian trypanosome from the gut of *Cx. pipiens* (L.) (Votýpka *et al.*, 2002). Although the prevalence of trypanosomes in mosquitoes was as high as 10%, the trypanosome occurring in *Cx. pipiens* gut has never been isolated from the blood of raptors bitten by infected mosquitoes. However, raptors were readily infected by *T. avium*, transmitted by blackflies (Votýpka *et al*., unpublished data, 2005). The current study was undertaken to determine the spatial feeding preferences of ornithophilic insects in order to elucidate their host-seeking habits and to identify the potential hosts and habitat of the culicine trypanosome.

Materials and methods

Field sites

Insect trapping was conducted during June and July 2003–2005, in Milovicky forest game preserve (Breclav district, southern Moravia, Czech Republic, 48°48′39″ N, 16°43′26″ E).

Sentinel hosts

Animal use was approved by the Ethical Committee of the Faculty of Science, Charles University, Prague. Chickens (*Gallus domesticus*, 63–80 days old, *c*. 800 g, bought at a local poultry farm) and Japanese quail (*Coturnix japonica*, adults, *c*. 280 g, Research Institute of Animal Production, Prague) were used as sentinel birds. Birds were placed in cages just before they were transported to field sites and returned within an hour of removing the insect traps the following morning. Birds had access to water during insect trapping. Their legs were marked with coloured tape and the birds were rotated daily between the different trap locations (canopy and ground).

Quail were used to compare their attractiveness with chickens. In 2005, cages with chickens or quail were placed on neighbouring trees at both canopy and ground level ($n = 4$).

Insect traps

Bird cages consisted of a double cage (inner cage: 50 × 40 × 30 cm; outer cage: 60 × 50 × 35 cm; mesh size 1.3 cm) with a roof made of Plexiglas to allow the visual orientation of vectors and to protect the birds against rain. Centers for Disease Control (CDC) light traps (John W. Hock Co., Gainesville, FL, U.S.A.) without light bulbs were placed near the bird cages. Captured insects were collected in 20 × 20 × 20-cm nylon nets connected to the traps. Two traps were used at each trapping site; one was set at the tree canopy level (close to the top of a tree, *c*. 13 m above ground) and the second was set at ground level (1.5 m above the ground) on a neighbouring tree (distance *c*. 25–30 m). Traps were set before dusk and were picked up the next morning. Collections were made for a total of 39 trap-nights (four in 2003, 16 in 2004 and 19 in 2005).

Insect processing

Insects were aspirated from traps, knocked out in a freezer, sorted by family and stored at −20°C prior to identification. Only females were considered. Mosquitoes were stored dry in parafilm-sealed Petri dishes; blackflies and midges were stored in 70% ethanol. Insects were determined using standard keys (Kramář, 1958; Chvála *et al*., 1980).

Data analysis

For each insect taxon, we compared the numbers of individuals recorded for the same trapping date and site, according to trap height. Taxa found in traps more than five times at each level were included in the statistical analysis. The normality of data distribution was tested using the Kolmogorov–Smirnov test. Data were tested using the Wilcoxon rank test (rank sum method). All tests were run using S PLUS 6.2 (Insightful Corp., New York, NY, U.S.A.).

In 2005, in addition to chickens, quail were used as bait to compare the influences of bird species used for attraction. The numbers of insects captured at each site, date and level were compared as described to establish any differences in results according to bird species.

Results and Discussion

This study examined the spatial feeding preferences of ornithophilic insects to elucidate the behaviour of these vectors in an area of high trypanosome prevalence in raptors and in insects that attack nestlings (Votýpka *et al.*, 2002; Votýpka & Svobodová, 2004).

For all vector taxa examined, numbers of individuals did not significantly differ between traps baited with chickens or with quail. Despite the limited number of replications, the current results are consistent with those of Darbro & Harrington (2006) for mosquitoes and indicate that chickens and quail are similarly attractive to the insect species under consideration. Because the possibility that quail and chicken might differ in attractiveness was excluded, data for both bird sentinel species were pooled for further analysis.

In total, 1240 female mosquitoes (eight species), 1201 biting midges (11 species), and 218 blackflies (two species) were captured (Table 1). All insect taxa [with the exception of *Culicoides kibunensis* (Tokunaga) (Diptera: Ceratopogonidae)] captured during the study showed a significant preference for either canopy or ground-level habitats (Fig. 1). Individual

Table 1. Bloodsucking insects captured in bird-baited traps during 2003–2005.

Species	*n**	%†
Mosquitoes (Diptera: Culicidae)		
Culex pipiens pipiens	1144	92.2
Anopheles plumbeus	63	5.1
Aedes vexans	24	1.9
Mansonia richiardii	5	0.4
Culiseta morsitans	1	0.1
Aedes cataphylla	1	0.1
Aedes communis	1	0.1
Aedes geniculatus	1	0.1
Mosquitoes, total	1240	
Biting midges (Diptera: Ceratopogonidae)		
Culicoides festivipennis	499	41.6
Culicoides kibunensis	249	20.7
Culicoides minutissimus	174	14.5
Culicoides circumscriptus	135	11.2
Culicoides pictipennis	40	3.3
Culicoides duddingstoni	34	2.8
Culicoides simulator	31	2.6
Culicoides truncorum	28	2.3
Unidentified *Culicoides* spp.	7	2.6
Culicoides obsoletus	2	0.1
Culisoides reconditus	1	0.1
Culicoides segnis	1	0.1
Biting midges, total	1201	
Blackflies (Diptera: Simuliidae)		
Eusimulium angustipes	217	99.5
Simulium morsitans	1	0.5
Blackflies, total	218	

*Total numbers of caught insects.
†Percentage of given species of group abundance.

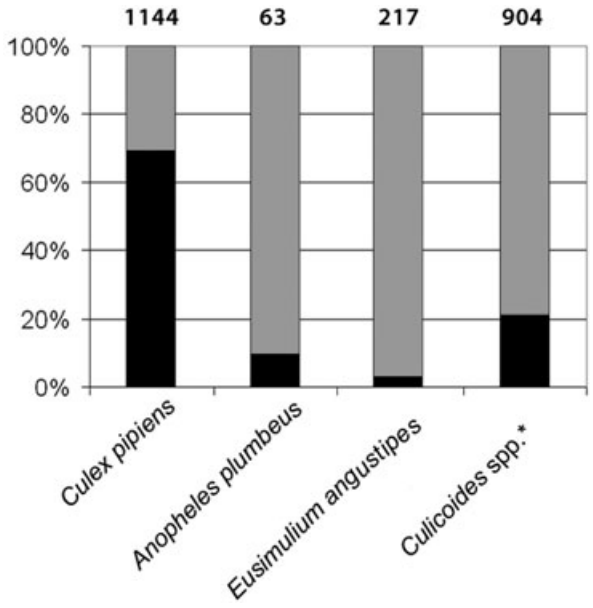

Fig. 1. Relative proportion of bloodsucking insect taxa caught at ground level (black) and canopy level (grey). Only taxa caught more than five times at each level were included. *Four species of biting midge (*Culicoides festivipennis*, *Culicoides kibunensis*, *Culicoides minutissimus* and *Culicoides circumscriptus*) are combined as *Culicoides* spp. Numbers indicate the total number of specimens.

taxa of bloodsucking insects exhibited different feeding level preferences: whereas *Cx. pipiens* preferred ground-level feeding, *Anopheles plumbeus* (Stephens), all biting midges species (*Culicoides* spp.) caught, and the blackfly *E. angustipes* preferred the canopy level.

Culex pipiens significantly preferred ground level (68% caught at ground level; $Z = -3.41$, $P < 0.001$), whereas *An. plumbeus* preferred the canopy level (88%; $Z = 3.22$, $P < 0.01$). The trapping methods used in previous studies of the spatial distribution of mosquitoes mostly varied from that used in the present study (e.g. Novak *et al*., 1981). Service (1971a) considered *Cx. pipiens* to prefer the canopy. However, by using unbaited traps these authors focused on non-specific flight levels of host-seeking mosquitoes; the current study evaluated mosquito preferences for avian hosts placed at different levels above the ground. Another study using CDC light traps baited with CO_2 found no height preference for *Cx. pipiens* or *Culex torrentium* (Diptera: Culicidae), but species determination (*Cx. pipiens* vs. *Cx. torrentium*) was not performed (Lundström *et al*., 1996). Exact determination of *Cx. pipiens* and *Cx. torrentium* (Martini) females using morphological features is laborious and almost impossible (Vinogradova, 2000). Nevertheless, males of *Cx. torrentium* have never been reported from the lowlands of south Moravia (Vaňhara & Rettich, 1998); therefore these mosquitoes are considered to be *Cx. pipiens sensu stricto*.

Contrary to the current results, American populations of *Cx. pipiens* were reported to prefer the canopy level in studies using bird-baited or CO_2-based traps(Anderson *et al*., 2004; Andreadis & Armstrong, 2007). However, the species in the study by Anderson *et al*. (2004) may in fact have been *Culex restuans* (Theobald) (Diptera: Culicidae), which the authors did not distinguish. Darbro & Harrington (2006) used polymerase chain reaction techniques to distinguish *Cx. pipiens* and *Cx. restuans* and concluded that *Cx. restuans* preferred the canopy level, whereas *Cx. pipiens* showed no preference when chickens or sparrows were used as bait.

Habitat structure might also influence mosquito behaviour: in the study by Anderson *et al*. (2004), scrub vegetation was high and closely knit, whereas at the current locality scrub was about 1 m high and was rather scarce as a result of extensive grazing by game (red and fallow deer). Scrub may provide mosquitoes with resting sites, but it may also affect the occurrence of potential hosts. Anderson *et al.* (2004) hypothesized that the *Cx. pipiens* preference for the canopy can be associated with the presence of sufficient numbers of birds nesting or roosting. In the current study, the lower scrub storey probably resulted in higher numbers of birds closer to the ground. Mosquito trypanosome is probably transmitted to insectivorous birds at ground levels because its transmission requires that the mosquito be ingested (Szabová & Svobodová, unpublished data, 2008). This may explain why this trypanosome has not been noted in raptor blood although birds of prey do not display any defensive

anti-mosquito behaviour (Edman *et al.*, 1974; Svobodová, unpublished data, 2003).

Consistently with previous studies (Service, 1971a; Novak *et al*., 1981), *Aedes vexans* (Meigen) (Diptera: Culicidae) was found to show a high preference for ground-level feeding ($n = 24$, 70% caught at ground level; $Z = -2.11$, $P < 0.05$). However, the numbers of specimens caught were considerably lower than those for *Cx. pipiens*, although *Aedes* mosquitoes attacked us during trap setting. The catches may thus represent mosquitoes attracted by the operator, rather than the bird. In any case, *Aedes* occur near the ground as they did not follow the investigators to the canopy during trap setting.

Biting midge s species (Diptera: Ceratopogonidae) tested together preferred the canopy level (79% of captures were made at canopy level; $Z = 3.80$, $P < 0.001$), as did the various species when tested individually: *Culicoides minutissimus* (Zetterstedt), $n = 174$, 85% ($Z = 3.15$, $P < 0.01$); *Culicoides festivipennis* (Kieffer), $n = 452$, 73% ($Z = 3.33$, $P < 0.001$), and *Culicoides circumscriptus* (Kieffer), $n = 133$, 83% ($Z = 3.67$, $P < 0.001$). Results for *C. kibunensis* ($n = 241$, 58%; $Z = 1.94$, $P = 0.052$) were very close to the 0.05 significance border.

Biting midges were shown to differ in their height preferences (Henry & Adkins, 1975). In our study, most of the biting midge species preferred or strongly tended to prefer [*C. kibunensis*, syn. *Culicoides cubitalis* (Edwards, 1939)] the canopy level, which is consistent with results obtained using CDC light traps in the U.S.A. (Garvin & Greiner, 2003), and blacklight suction traps in Israel, where the ornithophilic species [*C. circumscriptus* and *Culicoides cataneii* (Clastrier, 1957)] were caught in traps placed 20–26 m above the ground (Braverman & Lindley, 1993). By contrast, Service (1971b) caught higher numbers of several biting midge species, including *C. kibunensis* and *Culicoides pictipennis* (Staeger, 1939) near the ground, when using unbaited suction traps. However, as in the study by Service (1971a), the results of Service (1971b) are probably more focused on non-specific flying levels of biting midges when seeking hosts, whereas we studied the preferences of biting midges for hosts placed at different levels above the ground.

In the current study, *E. angustipes* blackflies showed a significant preference for host seeking in the canopy, with 93% of individuals caught at canopy level ($Z = 5.77$, $P < 0.001$). The ornithophily of *E. angustipes* has been previously noted (Chvála *et al*., 1980). The spatial preferences of ornithophilic blackflies have been studied in Canada, where higher numbers of woodland species, including *Eusimulium latipes* (Diptera: Simuliidae), were trapped in the canopy at up to 8 m above the ground than at ground level (Bennett, 1960). *Simulium meridionale* (Diptera: Simuliidae) and *Eusimulium aureum* (Diptera: Simuliidae) were more attracted to turkey and pheasant placed in the canopy than at ground level (Anderson & DeFoliart, 1961; Kiszewski & Cupp, 1986). Importantly, the canopy is also the site of *Leucocytozoon smithi* (Sporozoa: Leucocytozoidae) transmission to turkey hosts (Kiszewski & Cupp, 1986).

Raptors in the area of study were readily found to be infected with several species of haemosporida and *T. avium*, transmitted by *Eusimulium* spp. (Votýpka *et al.*, 2002; Votýpka & Svobodová, 2004). The findings of the current study are in agreement with this observation as both the vector and the host occur in the same part of the habitat: the nest height of buzzards in this locality is around 12 m (Voříšek, unpublished data, 1998). However, this does not exclude the possibility that blackflies act as vectors of different blood parasites to other bird taxa because blackflies may behave differently in forests and open habitats, as suggested by Bennett (1960). Blackflies were shown to feed on passerines in Sweden (Malmqvist *et al.*, 2004) which is consistent with the finding that *T. avium* transmitted to raptors by blackflies is infectious to passerines (Votýpka *et al.*, 2002; Votýpka & Svobodová, 2004), and *T. avium* has been isolated from wild passerines in the same locality (Zídková *et al.*, unpublished data, 2005).

Although many Diptera species are opportunistic, the blood-feeding preferences of other species are host-specific or are still unknown. The preference for canopy feeding observed in most insect species in the current study may be the result of avian host availability in the forest canopy, and suggests that these insects are ornithophilic. Spatial distribution may thus be related to the importance of these vectors in disease transmission.

Acknowledgements

We thank Petr Voříšek, Lenka Zídková and Kateřina Rezková for technical assistance in the field, and Petr Volf for discussion on trap design. This work was supported by the Czech Republic Ministry of Education (project MSMT0021620828) and the Grant Agency of Charles University, Prague (216/2005/B-bio/PrF).

References

Anderson, J.R. & DeFoliart, G.R. (1961) Feeding behaviour and host preferences of some blackflies (Diptera: Simuliidae) in Wisconsin. *Annals of the Entomological Society of America*, **54**, 716–729.

Anderson, J.F., Andreadis, T.G., Main, A.G. & Kline, D.L. (2004) Prevalence of West Nile virus in tree canopy-inhabiting *Culex pipiens* and associated mosquitoes. *American Journal of Tropical Medicine and Hygiene*, **71**, 112–119.

Andreadis, T.G. & Armstrong, P.M. (2007) A two-year evaluation of elevated canopy trapping for *Culex* mosquitoes and West Nile virus in an operational surveillance programme in the northeastern United States. *Journal of the American Mosquito Control Association*, **23**, 137–148.

Bennett, G.F. (1960) On some ornithophilic bloodsucking Diptera in Algonquin Park, Ontario, Canada. *Canadian Journal of Zoology*, **38**, 377–389.

Braverman, Y. & Lindley, J.R. (1993) Effect of light trap height on catch of *Culicoides* (Diptera: Ceratopogonidae) in Israel. *Journal of Medical Entomology*, **30**, 1060–1063.

Chvála, M., Hůrka, K., Chalupský, J., Knoz, J., Minář, J. & Országh, I. (eds) (1980) *Bloodsucking Flies and Botflies (Diptera)*, *Fauna ČSSR*, Vol. 22. Academia, Prague [in Czech].

Darbro, J.M. & Harrington, L.C. (2006) Bird-baited traps for surveillance of West Nile mosquito vectors: effect of bird species, trap height, and mosquito escape rates. *Journal of Medical Entomology*, **43**, 83–92.

Edman, J.D., Webber, L.A. & Schmidt, A.A. (1974) Effect of host defences on the feeding pattern of *Culex nigripalpus* when offered a choice of blood sources. *Journal of Parasitology*, **60**, 874–883.

Flemings, M. (1959) An altitude biting study of *Culex tritaeniorhynchus* (Giles) and other associated mosquitoes in Japan. *Journal of Economic Entomology*, **52**, 490–492.

Garvin, M.C. & Greiner, E.C. (2003) Ecology of *Culicoides* (Diptera: Ceratopogonidae) in southcentral Florida and experimental *Culicoides* vectors of the avian haematozoan *Haemoproteus danilewskyi* Kruse. *Journal of Wildlife Disease*, **39**, 170–178.

Henry, L.G. & Adkins, T.R. Jr (1975) Vertical distribution of biting midges in coastal South Carolina. *Annals of Entomological Society of America* **68**, 321–324.

Hubálek, Z. & Halouzka, J. (1996) Arthropod-borne viruses of vertebrates in Europe. *Acta Scientiarum Naturalium Brno*, **10**, 1–95.

Kiszewski, A.E. & Cupp, E.W. (1986) Transmission of *Leucocytozoon smithi* (Sporozoa: Leucocytozoidae) by blackflies (Diptera: Simuliidae) in New York, U.S.A. *Journal of Medical Entomology*, **23**, 256–262.

Kramář, J. (1958) *Biting Mosquitoes—Culicinae (Diptera)*. Nakladatelství Československé Akademie Věd, Prague [in Czech].

Lundström, J.O., Chirico, J., Folke, A., & Dahl, C. (1996) Vertical distribution of adult mosquitoes (Diptera: Culicidae) in southern and central Sweden. *Journal of Vector Ecology*, **21**, 159–166.

Malmqvist, B., Strasevicius, D., Hellgren, O., Adler, P.H. & Bensch, S. (2004) Vertebrate host specificity of wild-caught blackflies revealed by mitochondrial DNA in blood. *Proceedings of the Royal Society London Series B* **271** (Suppl.), 152–155.

Novak, R.J., Peloquin, J. & Rohrer, W. (1981) Vertical distribution of adult mosquitoes (Diptera: Culicidae) in a northern deciduous forest in Indiana. *Journal of Medical Entomology*, **18**, 116–122.

Service, M.W. (1971a) Flight periodicities and vertical distribution of *Aedes cantans* (Mg.), *Ae. geniculatus* (Ol.), *Anopheles plumbeus* Steph. and *Culex pipiens* L. (Dipt., Culicidae) in southern England. *Bulletin of Entomological Research*, **60**, 639–651.

Service, M.W. (1971b) Adult flight activities of some British *Culicoides* species. *Journal of Medical Entomology*, **8**, 605–609.

Van Dyken, M., Bolling, B.G., Moore, C.G., Blair, C.D., Beaty, B.J., Black, W.C. IV & Foy, B.D. (2006) Molecular evidence for trypanosomatids in *Culex* mosquitoes collected during a West Nile virus survey. *International Journal for Parasitology*, **36**, 1015–1023.

Vaňhara J.F. & Rettich F. (1998) Culicidae. *Diptera of the Pálava Biosphere Reserve of UNESCO, I. Folia Facultatis Scientiarum Naturae Universitatis Masarykiana Brunensis Biol*, Vol. 99 (ed. by R. Rozkošný & J. Vaňhara), pp. 97–105, Universitas Masarykiana, Brno.

Vinogradova, E.B. (2000) *Culex pipiens pipiens Mosquitoes: Taxonomy, Distribution, Ecology, Physiology, Genetics, Applied Importance and Control*. Pensoft, Sofia.

Votýpka, J. & Svobodová, M. (2004) *Trypanosoma avium*: experimental transmission from blackflies to canaries. *Parasitology Research*, **92**, 147–151.

Votýpka, J., Oborník, M., Volf, P., Svobodová, M. & Lukeš, J. (2002) Trypanosoma avium of raptors (Falconiformes): phylogeny and identification of vectors. *Parasitology*, **125**, 253–263

Accepted 7 April 2010
First published online 17 May 2010