Influence of elevation and avian or mammalian hosts on attraction of *Culex pipiens* (Diptera: Culicidae) in southern Ontario

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Abstract—We studied *Culex pipiens* L. in the Niagara Region of Ontario, Canada, to establish whether or not these mosquitoes are attracted to hosts other than birds at different elevations or as the season progresses. Guinea-pigs and chickens were used as representative mammalian and avian hosts, respectively. Bait animals were placed next to modified CDC miniature light traps (no light and no CO_2) hung 1.5 or 5 m above ground in a Niagara woodlot. The season was divided into three 6-week periods (early, middle, and late). Significantly more *C. pipiens* were captured at the 5 m than at the 1.5 m elevation. In general, chicken-baited traps were preferred over control and guinea-pig-baited traps, with one important exception: there was no significant difference among traps during the late period at 1.5 m elevation. The potential role of *C. pipiens* as a bridging vector of West Nile virus to humans is discussed.

Résumé—Nous avons étudié des *Culex pipiens* L. dans la région de Niagara en Ontario, Canada, afin de déterminer si ces moustiques sont attirés par des hôtes autres que des oiseaux en fonction de la hauteur et au cours de la saison. Des cobayes et des poulets ont servi d'hôtes représentatifs, respectivement des mammifères et des oiseaux. Les animaux appâts ont été placés près de pièges lumineux CDC miniatures modifiés (sans lumière, ni CO_2) pendus à 1,5 et à 5 m au-dessus du sol dans une région boisée de Niagara. La saison a été divisée en trois périodes de 6 semaines (précoce, médiane et tardive). Il y a eu significativement plus de captures de *C. pipiens* à la hauteur de 5 m qu'à 1,5 m. En général, les pièges munis d'un poulet sont choisis de préférence aux pièges témoins et à ceux qui contiennent un cobaye, sauf qu'exceptionnellement il n'y a pas eu de différence significative entre les pièges durant la période tardive à la hauteur de 1,5 m. Le rôle de *C. pipiens* comme vecteur-pont potentiel du virus du Nil occidental chez les humains fait l'objet d'une discussion.

[Traduit par la Rédaction]

Introduction

Since the discovery of West Nile virus (WNV) in New York in 1999, *Culex pipiens* L. (Diptera: Culicidae) has been regarded as the primary enzootic vector in eastern North America (Kulasekera *et al.* 2001; Petersen and Roehrig 2001). This mosquito purportedly feeds primarily on birds, although there is some evidence that it feeds on other hosts also (Wood *et al.* 1979; Apperson *et al.* 2002, 2004). Apperson *et al.* (2004) used serological and polymerase chain reaction analyses to identify blood meals of *C. pipiens* in New York, New Jersey, and Tennessee. Those from New York usually fed on birds (84.6%) and those from New Jersey on birds (34.7%) and mammals (38.0%, of which 10.8% had fed on humans); the remainder fed on amphibians and reptiles (Apperson *et al.* 2004). Furthermore, in Iowa, *C. pipiens*, *C. restuans* Theobald, and *C. salinarius* Coquillett fed mainly on birds but exhibited a midsummer increase in feeding on mammals (Ritchie and Rowley 1981). Most human WNV cases occur near the end of the mosquito season (Andreadis *et al.* 2004).

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Elevation and time during the season could affect the attraction of *C. pipiens* to birds *versus* mammals. In most areas of the Nearctic Region, the majority of female *C. pipiens* are captured in forest canopies (Main *et al.* 1966; Mitchell and Rockett 1979; Nasci and Edman 1981; Novak *et al.* 1981; Mitchell 1982; Lundstrom *et al.* 1996; Russell and Hunter 2005; Drummond *et al.* 2006; Andreadis and Armstrong 2007). In those studies, low numbers of *C. pipiens* were also found at ground level. Darbro and Harrington (2006) conducted studies using bird-baited traps at two different levels, ground and canopy, and found equal numbers of *C. pipiens*. If human WNV infection is caused by *C. pipiens* in Ontario, we would expect to find occasional attraction of *C. pipiens* to mammalian hosts at ground level. There may also be seasonal cues (*e.g.*, day length or temperature) or differences in host availability or accessibility (*e.g.*, nestlings have already fledged) that cause late-season mosquitoes to be more attracted to human hosts than are their early-season conspecifics. *Culex pipiens* may exhibit a late-season shift in feeding preference towards humans because of a decline in late-season bird abundance when birds migrate south (Kilpatrick *et al.* 2006).

We used chickens and guinea-pigs as representative host animals to test whether the attraction of *C. pipiens* to bird hosts is affected by elevation and (or) time in the season. We hypothesized that if *C. pipiens* is both the primary enzootic vector and a contributing bridge vector of WNV in southern Ontario, then this species will be less ornithophilic at lower elevations (where humans are most likely to be bitten by mosquitoes) and later in the season (when humans are usually infected).

Materials and methods

Location

The study was conducted in a 17.23 ha Carolinian woodlot (approximately 43°04′N, 79°10′W) in the Niagara Region of Ontario, Canada (as described in Russell and Hunter 2005), from the first week of June to the last week of September in 2004.

Trap placement

Miniature light traps (Centers for Disease Control (CDC), Model 512, John W. Hock Company, Gainesville, Florida) were placed in nine trees at elevations of approximately 1.5 and 5 m (for a total of 18 traps per night). Selected trees were separated from each other by at least 200 m.

Trap modification

CO_2 and light sources were removed from each animal-baited trap. Light sources were removed from control traps. Each test animal (guinea-pig or chicken) was contained in a wire-mesh cage attached to the CDC trap (Fig. 1) (Brock University Animal Care Approval No. 04-04-07).

Test animals

Adult (6–12 months old) male Hartley guinea-pigs weighing between 700 and 1000 g were supplied by Charles River Laboratories (Saint-Constant, Quebec). Male dual-purpose-breed chickens aged 2 weeks to 1 month and equivalent in size to the guinea-pigs were acquired from Bonnie's Chick Hatchery (Elmira, Ontario).

Animal placement, setup, and collection

On each trapping night, guinea-pigs and chickens were caged individually in the CDC traps. In total, six guinea-pig-baited traps, six chicken-baited traps, and six CO_2-baited (2 kg of dry ice) control traps were used each night. Three guinea-pig-baited, three chicken-baited, and three control traps were randomly assigned to individual trees and placed at one of the two elevations. After each trapping night, captured mosquitoes were removed from the traps and transported to Brock University for sorting and identification. Test animals were placed in the traps at 1600 and removed from the traps at 0800 the following morning. New test animals were used for the next night of trapping.

The trapping weeks were divided into three 6-week periods: early (weeks 23–28; 30 May to 10 July), middle (weeks 30–35; 18 July to 28 August), and late (weeks 36–41; 29 August to 9 October). Week 29 was excluded from the analysis because it included only one trapping night (*versus* the standard two nights per week).

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Fig. 1. Photographs of a modified CDC miniature light trap (with CO_2 and light sources removed) with a small wire cage used to hold either a guinea-pig or chicken as a mosquito attractant.

Sorting, identification, and analysis

Captured insects were placed in a −20°C freezer for approximately 30 min to kill them, and all non-mosquitoes were removed and discarded. The mosquitoes were then identified to species.

Statistical analyses

The average number of mosquitoes captured per 6-week period was calculated using the number of females collected over 12 trap-nights (2 trap-nights per week).

To determine whether the average numbers of mosquitoes caught differed between host traps and elevations, data were analyzed using a two-way ANOVA. Owing to non-normality of the data, the two-way ANOVA was conducted using a randomization program with repeated measures. Code lines for the randomization were developed using Microsoft C++ software (Microsoft Corporation, 2005). Because the randomization program and a standard two-way ANOVA produced the same level of significance, the standard test was used with its *post hoc* Tukey test using SigmaStat (Systat Software Inc., 2005) to discern where significant differences occurred among the different trap types.

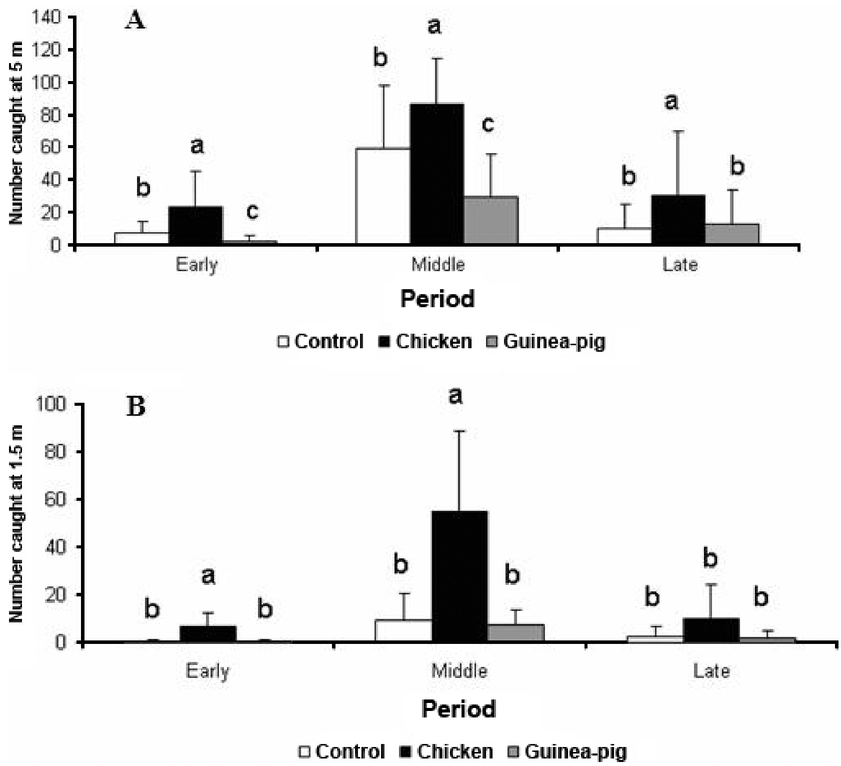
Results

Over the course of the trapping season 43 135 mosquitoes were identified in 666 trap catches. *Culex pipiens* was the most abundant species, accounting for 50.4% of the sample, followed by *Aedes vexans* (Meigen) at 18.4%, *Ochlerotatus stimulans* (Walker) at 7.6%, *O. cantator* (Coquillett) at 7.3%, *O. trivittatus* (Coquillett) at 5.8%, and *C. restuans* at 1.0%. The remaining combined species accounted for 9.5% of the samples.

Elevations

The numbers of *C. pipiens* captured at the 1.5 and 5 m elevations for the early period were 46.3 ± 26.1 (mean ± SD) and 226.7 ± 156.1, respectively, showing that more *C. pipiens* were captured at the higher elevation ($F_{1,213}$ = 40.86, $P < 0.001$). In the middle period, fewer *C. pipiens* were captured at 1.5 m (572.5 ± 400.5) ($F_{1,213}$ = 52.06, $P < 0.001$) than at 5 m (2192.3 ± 1145.3), and similarly in the late period, with 91.0 ± 104.7 at 1.5 m ($F_{1,213}$ = 17.86, $P < 0.001$) and 446.8 ± 564.7 at 5 m.

Fig. 2. Numbers of *Culex pipiens* (mean ± SD) caught in CDC light traps with three different bait types over each of three periods at 5 m (A) and 1.5 m (B) elevations in the Niagara Region of Ontario. The same letter above a bar (within a period) indicates that the values are not significantly different (early period, weeks 23–28; middle period, weeks 30–35; late period, weeks 36–41; there were two trap-nights per week).



Trap types

The numbers of *C. pipiens* caught in the three trap types differed significantly during all periods (early: $F_{2,213} = 38.75$, $P < 0.001$; middle: $F_{2,213} = 35.82$, $P < 0.001$; late: $F_{2,213} = 9.32$, $P < 0.001$). During the early and middle periods, at the lower elevation the chicken-baited traps caught more *C. pipiens* than did the guinea-pig-baited or control traps ($P < 0.05$), with attraction to guinea-pig-baited and control traps being equal ($P > 0.05$) (Fig. 2). During the late period, there were no significant differences among the three trap types ($P > 0.05$) (Fig. 2). In the early and middle periods, at the higher elevation most *C. pipiens* were captured in the chicken-baited traps and fewest in the guinea-pig-baited traps ($P < 0.05$) (Fig. 2). In the late period, more *C. pipiens* were caught in the chicken-baited traps ($P < 0.05$), and attraction to guinea-pig-baited and control traps was equal ($P > 0.05$) (Fig. 2).

Discussion

Our finding of the greatest numbers of *C. pipiens* at the 5 m elevation was similar to the results of other studies (Main *et al.* 1966; Mitchell and Rockett 1979; Nasci and Edman 1981; Novak *et al.* 1981; Mitchell 1982; Lundstrom *et al.* 1996; Anderson *et al.* 2004; Andreadis *et al.* 2004; Russell and Hunter 2005; Drummond *et al.* 2006; Andreadis and Armstrong 2007), and was not surprising.

With the exception of the lower elevation during the late period, *C. pipiens* was attracted to chicken-baited traps more than to control and guinea-pig-baited traps. Ritchie and

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Rowley (1981) studied blood meals from *C. pipiens*, *C. restuans*, and *C. salinarius* from Iowa and found a midsummer increase in feeding on mammals. Although their grouping consisted of three different species of *Culex* L., they noted that the increase in feeding occurred after *C. pipiens* had replaced *C. salinarius* as the dominant *Culex* species feeding on mammals. Tempelis *et al.* (1967) examined the feeding patterns of mosquitoes from Colorado and noted that most *C. pipiens* with blood meals from mammals were encountered in late summer. We found that at the lower elevation during the late period there was no significant difference between the three trap types, demonstrating that *C. pipiens* likely feeds on mammals at a time when WNV infection rates are at their highest (Andreadis *et al.* 2004). In contrast, Hamer *et al.* (2009) detected no change in the attraction of *C. pipiens* to mammals over the season in the greater Chicago area and suggested that the increase in human WNV cases was mostly an amplification effect.

The feeding preference of *C. pipiens* has been shown to vary among locations in the United States of America. Populations from New Jersey were found to have fed on humans, and no significant difference in preference for birds or mammals was found in Delaware populations (Apperson *et al.* 2002; Apperson *et al.* 2004; Gingrich and Williams 2005). In contrast, populations in New York and Connecticut were found to feed primarily on birds (Apperson *et al.* 2004; Gingrich and Williams 2005; Molaei *et al.* 2006).

In summary, we found that *C. pipiens* were most strongly ornithophilic during the early period (weeks 23–28; 30 May to 10 July) and middle period (weeks 30–35; 18 July to 28 August) at the 5 m elevation, and showed weak ornithophily during the late period (weeks 36–41; 29 August to 9 October) at the 1.5 m elevation. This *late season*, *low elevation* decrease in ornithophily suggests that in southern Ontario, *C. pipiens* has the potential to be a contributing bridge vector of WNV. This corresponds to both *where* humans are likely to encounter *C. pipiens* (*i.e.*, at lower elevations) and *when* humans are likely to contract WNV (*i.e.*, late in the season) from infected *C. pipiens* (Andreadis *et al.* 2004; Campbell *et al.* 2002).

Clearly, *C. pipiens* is an ideal enzootic vector of WNV in southern Ontario, based on its behaviour throughout the season. It is found primarily in the forest canopy (where birds are likely to be encountered), and is attracted primarily to birds (the main host for WNV). In contrast, its role as a contributing bridge vector is limited to the 1.5 m elevation during the final weeks of the WNV season.

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