Habitat preference of host-seeking *Coquillettidia perturbans* (Walker) in relation to birds and eastern equine encephalomyelitis virus in New Jersey

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ABSTRACT: *Coquillettidia perturbans* (Walker) has been implicated as a bridge vector of eastern equine encephalomyelitis virus in North America. Eastern equine encephalomyelitis virus epizootics occur regularly in wild birds in New Jersey with little or no involvement of susceptible dead end hosts even though high populations of *Cq. perturbans* are present. Several factors may limit eastern equine encephalomyelitis (EEE) virus transfer from birds to mosquito bridge vectors like *Cq. perturbans* (Walker), including bird/mosquito density, differences in bird/mosquito habitat preference, mosquito host preference, mosquito host-seeking behavior and mosquito avoidance behavior by birds. Our investigations focused on the host-seeking activities of *Cq. perturbans* at Colliers Mills Wildlife Management Area in Ocean County, New Jersey. We first examined the abundance of host-seeking *Cq. perturbans* at a height of 1.0 m in each of three habitats: forest, open field and marsh. We collected significantly more mosquitoes in the forest as compared to the marsh. Forest and field collections did not differ significantly from one another nor did field and marsh. Second, we examined the temporal and vertical distribution of host-seeking *Cq. perturbans* in a sylvan habitat at three heights (1.0, 2.5, and 6.0 m) from dusk to dawn. We found that *Cq. perturbans* did significantly more host-seeking during the night period than either the evening or morning. Host-seeking mosquitoes also tended to stay near the ground and at no time did they ascend into the forest canopy. Host-seeking *Cq. perturbans* also tended to stay near the ground and at no time did they ascend into the forest canopy. Host-seeking *Cq. perturbans* and their apparent spatial disparity to roosting/nesting birds are discussed as a possible barrier to the transmission of EEE virus in New Jersey. *Journal of Vector Ecology* 26(1): 103-109. 2001.

Keyword Index: *Coquillettidia perturbans*, host-seeking, EEE, birds.

INTRODUCTION

*C. perturbans* (Walker), a competent bridge vector of eastern equine encephalomyelitis (EEE) virus, is found throughout North America where suitable habitat exists (Darsie and Ward 1981, Boromisa et al. 1987 and Morris 1988). In New Jersey EEE virus is believed to recur in permanent resident returning summer resident birds in early spring before it is detected in the enzootic mosquito vector *Culicoida melanura* (Coquillet) (Crans et al. 1994). Frequently, EEE virus is amplified and reaches epizootic levels in bird and *C. melanura* populations in late summer and early fall. Bridge vector mosquitoes, like *Cq. perturbans*, must be present for the transfer of virus from the bird/*C. melanura* cycle to susceptible dead end hosts. Although human involvement in the transmission of EEE virus is relatively rare, epizootics regularly occur among horses and exotic birds such as emu and pheasant (Kissling 1958, Sofield et al. 1983, Crans and Schulze 1986 and Tully et al. 1992). In a number of North American equine epizootic foci, *Cq. perturbans* was the most prevalent mosquito collected and considered the probable vector species (Carter et al. 1981, Francy 1982, Sofield et al. 1983, Crans and Schulze 1986 and Nasci et al. 1993).

In general, three criteria must be met for the transfer of virus from bird to mosquito. First, a viremic bird with sufficiently high titer must be present; second, the mosquito must locate and accept the avian host, and third it must acquire a blood meal.

For the first criteria, Crans et al. (1994) found that permanent resident and summer resident birds, as compared to migrant and winter resident birds, were more likely to be involved in the EEE virus cycle. In one year of their study (1982), they collected five after hatch year (AHY) birds that were viremic 37-51 days prior to the first detection of virus in mosquitoes,
indicating that the annual EEE virus cycle begins in birds, not mosquitoes. Amplification of virus and a
subsequent epidemic is thought to be due to an influx of susceptible fledgling birds that coincides with a
second generation of newly emerged Cs. melanura (Crans 1990 and Crans et al. 1994). An epidemic then
sets the stage for the increased chance of EEE virus transmission to nulliparous host-seeking Cq. perturbans.
For the second criteria, mosquitoes utilize a number of cues to first locate, and second to accept or reject, a
potential host. A general cue like carbon dioxide may elicit host-seeking behavior in a wide-range of host
specific mosquito species regardless of the source. However, when a mosquito locates a potential host
through long-range broad-based cues another set of highly specific, short-range cues ultimately lead to host
acceptance or rejection. Host range may be remarkably broad for species like Aedes vexans (Edman and Downe
1964) or highly specific as reported for Culex territans (Crans 1970). Although the ornithophilic nature of Cs.
melanura is documented and widely accepted (Jobbins et al. 1961 and Crans 1963), the extent to which Cq.
perturbans seeks out birds is less clear. Downe (1962) and Murphy et al. (1967) reported that Cq. perturbans
showed a preference for avian rather than mammalian hosts; however, Crans (1963) found that only 1 out of
41 blooded wild-caught mosquitoes contained avian blood. Similarly, Edman (1971) found that of 673 blood
meals identified from Cq. perturbans, 91% contained mammalian blood and the remaining 9% avian. As
shown in the previous studies Cq. perturbans will feed on mammals and birds making it an ideal bridge vector
for the transfer of EEE virus from viremic birds to susceptible dead end hosts.

For the third criteria, mosquitoes encounter a
number of obstacles that prevent them from acquiring
the blood needed for egg production. Each host taxa,
mammal, bird or otherwise, has both physical (long hair,
scales etc) and behavioral adaptations (physical dexterity
to remove mosquitoes or shielding exposed areas) that
limit or completely prevent mosquitoes from obtaining
a blood meal. Adult birds in particular, are covered
with dense feathers and assume sleeping postures that
challenge species like Cs. melanura and Cq. perturbans.
Birds also utilize mosquito avoidance behavior, but this
can vary dramatically within and between species.
Edman and Kale (1971) found that within the order
Ciconiiformes (herons) the degree of mosquito
avoidance behavior could be correlated directly to the
success of a mosquito acquiring a complete blood meal.
Juvenile birds of many species are more vulnerable to
blood feeding, because they are inexperienced at
mosquito avoidance and have fewer feathers for
protection; consequently they are more important in the
amplification and transmission of EEE virus (Blackmore
and Dow 1958).

Serological studies of wild birds and sentinel
chickens and virus isolations from mosquitoes indicate
that EEE virus is present nearly every year in New
Jersey, at times reaching epizootic levels, yet during most
of these episodes, few or no cases of EEE virus are
reported in susceptible dead end hosts. A number of
possibilities exist that may explain the low transmission
of EEE virus to dead end hosts in the presence of viremic
birds and bridge vector mosquitoes. These include bird/
mosquito density, differences in bird/mosquito habitat
preference, mosquito host preference, mosquito host-
seeking behavior and mosquito avoidance behavior by
birds. Our investigation focused on the temporal and
spatial parameters of host-seeking that are involved in
the acquisition of virus from resident birds to the bridge
vector Cq. perturbans.

We examined two hypotheses relating to bird/
mosquito interaction: 1) Cq. perturbans females host-
seek preferentially in sylvan habitats where birds most
typically roost and nest versus open field and marsh
habitats and 2) Cq. perturbans females exhibit changes
in both temporal and vertical distribution when host
seeking in a sylvan habitat.

MATERIALS AND METHODS

Field studies were conducted at Colliers Mills
Wildlife Management Area in Ocean County, New
Jersey. For the first part of the study three ABC
(American Biophysics Corp. Jamestown RI) traps
supplemented with carbon dioxide [CO₂] and 1-octen-
3-ol [octenol] were placed at a height of 1.0 m in each
of three habitats: forest, open field and marsh. This part
of the study was replicated five times (14, 30 July and
4, 6, 19 August 1998). Traps were operated from late
afternoon until late morning the following day. Trap
catches were returned to the laboratory, where they were
sorted, counted and pooled within habitat. Mean Cq.
perturbans per three traps was calculated for each habitat
and each collection date. To test the first hypothesis of
habitat preference, we compared the mean number of
Cq. perturbans caught in the three habitats throughout
the study period (five dates) with a two way ANOVA
(Zar, 1984, SPSS GLM version 7.5.1, and Norusis,
1995).

For the second part of the study, ABC traps were
equipped as described above. Three traps were placed
in a sylvan habitat at each of three heights (1.0, 2.5, and
6.0 m) for a total of nine traps per collection date. Traps
were placed randomly throughout the forest and those
at 2.5 and 6.0 m were operated on a pulley system. Trap heights were chosen to reflect the shrub layer, mid-canopy and upper-canopy of the forest. Mosquito collections were taken hourly from one hour before sunset to one hour after sunrise and were sorted and counted on site. To simplify the analysis, we pooled the hourly data for each height into three time periods (evening, night, and morning), each containing approximately four hours. Six replicates of this study were conducted (July 26-27, July 31-Aug.1, Aug. 7-8, 1997; July 14-15, July 22-23 and Aug. 12-13, 1998). To test the second hypothesis of temporal and vertical distribution, we compared the mean number of *Cq. perturbans* caught during the three pooled time periods (evening, night and morning) at the different trap heights using a two way ANOVA, with Bonferroni as the comparison of the means test.

**RESULTS**

For the first hypothesis of habitat preference, we found no significant interaction between the mean number of host-seeking *Cq. perturbans* for date and habitat type. Mosquitoes tended to behave similarly on each date, (F=1.62, p=0.160) and therefore we examined simple main effects. The mean number of mosquitoes significantly differed among the three habitat types of forest, marsh and field (F=3.79, p=0.034). We found more host-seeking mosquitoes in the forest than in the marsh (Bonferroni = 23.9, p=0.038). Field and marsh did not significantly differ in the number of mosquitoes (Bonferroni = 17.8, p=0.174) as neither did forest and field (Bonferroni = 6.13, p=1.0). The second hypothesis had two parts: vertical and temporal distribution within a sylvan habitat. For vertical distribution we examined data from 1997 and 1998. For the 1997 data, we found a significant three-way interaction among date, height, and period (F=2.17, p=0.044) and therefore we examined the relationship between height and period for each date. This interaction reflects the difference in the total number of mosquitoes caught per night, with July 22-23 trap catches as much as six times the number of mosquitoes caught as on the other dates. As with the 1997 data, we found significant interactions for all three 1998 dates and thus we examined the differences among the means for height while controlling period (July 14-15: F=5.81, p=0.003; July 22-23: F=3.213, p=0.037; August 12-13: F=6.646, p=0.002). Again, we found that significantly more mosquitoes visited the 1.0 m trap compared to the traps set at higher heights (Table 1a). There were never any significant differences between the 2.5m and 6.0m traps.

In general for both years, a trap placed at 1.0 m attracted significantly more mosquitoes than either a 2.5 m or a 6.0 m trap. The latter two trap counts did not differ significantly from each other in the number of mosquitoes caught within any period or date. Additionally, we did not observe any changes vertically in mosquito host-seeking during the three time periods on any date; host-seeking *Cq. perturbans* remained near the forest floor and did not move into the forest canopy at any time during the night. Temporally, *Cq. perturbans* appears to increase in numbers to highest peaks during the middle of the night, however, only three increases were significant during 1997 and 1998 (Table 1b). Two of the significant comparisons were from the evening period into the middle of the night period in the 1.0 m trap during the early part of the season.

These significant changes, in addition to the changes noted above for vertical distributions, contribute to the
Figure 1. Mean number of *Cq. perturbans* collected from three habitat types on five dates in 1998 at Colliers Mills Wildlife Management Area, Ocean County, NJ. N=3 for each mean.

Figure 2. Mean number of *Cq. perturbans* caught in light traps at three heights at Collier Mills Wildlife Management Area, NJ. Each bar represents the average of three trap totals per hour over a four to five hour period. Periods covered early evening, the middle of the night, and early morning hours. Note the peak in total mosquito numbers in late July for both years representing a seasonal peak emergence.
significance of the period by height interaction. In short, *Cq. perturbans* became more active during the middle of the night but did not change heights.

**DISCUSSION**

Our findings indicate that *Cq. perturbans* preferentially host-seek in the forest and to a lesser extent the field. Their host-seeking behavior places them in the roosting and nesting habitat of birds like Blue Jay (*Cyanocitta cristata*), Wood Thrush (*Hylocichla mustelina*) and Eastern Tufted Titmouse (*Parus bicolor*), species known to have a high prevalence of EEE viremic individuals (McLean et al. 1985 and Crans et al. 1994). Although we found birds and mosquitoes in the same general habitat, the location of each within the habitat is more important as it relates to the cycling of EEE virus. The success of a mosquito acquiring a blood meal hinges on host compliance in time and space, and birds are most vulnerable to mosquitoes when quiescent (i.e. roosting or nesting). Information regarding both the roosting habits of birds and the host-seeking behavior of *Cq. perturbans* is either incomplete or contradictory. McNeel (1932) in an observational study stated that *Cq. perturbans* showed its greatest host-seeking activity at dusk. Snow and Pickard (1957) present data indicating *Cq. perturbans* host-seeking activity peaks between 1930-2000 hrs. Many diurnally active birds remain active at dusk and into late evening (pers. obs.), a time the two previously mentioned studies indicate as the peak host-seeking period of *Cq. perturbans*. On the three dates of the second part of our study, *Cq. perturbans* exhibited its greatest host-seeking activity during the night period, between 2200-0100 hrs. This is the period when most diurnally active birds have completely settled for the night and would seem a more opportune time for acquiring a blood meal.

Although many studies indicate preferred roosting habitats of a number of avian species, evidence of specific heights at which actual roosting occurs is
lacking. Skutch (1989) generalized avian roosting behavior in light of nesting preferences. Birds that nest on the ground will roost on the ground or higher up, whereas species that nest in the canopy are less likely to roost lower down. For nesting preferences, Crans et al. (1994) recognized six classes of nest habitat: ground, low (1-3 m), cavity, medium (3-7 m), high (>7 m) and variable height. They found that birds nesting in the 1-3 m category had the highest percentage of individuals positive for EEE virus antibody. This is important because we found that Cq. perturbans do significantly more host-seeking near the ground effectively reducing bird/mosquito interaction and subsequently the transmission of EEE virus.

Snow and Pickard (1957) and Downe (1962) reported that Cq. perturbans exhibit a significant upward movement at dusk and a downward movement from the forest canopy at dawn. It would appear that this vertical movement increases its chances of acquiring a blood meal from a roosting bird, yet, at no time during the six dates of our study did we observe a vertical change in host-seeking by Cq. perturbans. Our findings are similar to those of Bast and Rehn (1963) who collected significantly more Cq. perturbans at 5 ft. as compared to 25 ft. in a sylvan habitat in New Jersey, but contradict Burgess and Haufe (1960) who collected significantly more mosquitoes at 25 ft. as compared to 5 or 50 ft in a sylvan habitat in Marmora, Ontario. Because Cq. perturbans is an opportunistic species utilizing a wide range of hosts, we recognize that the density of one kind of host may determine mosquito choice, and therefore host-seeking behavior; however, at our study site birds were by far the most prevalent vertebrate hosts. Furthermore, apparent differences observed in the vertical distribution of host-seeking Cq. perturbans at different sites may, as suggested by Bates (1949) and Nasci (1985), be attributed to vertical gradients in temperature, humidity and illumination, behavior associated with mating and oviposition, and location of larval habitats.

Our findings indicate that Cq. perturbans and their potentially viremic avian hosts share the same habitat in New Jersey, however, the temporal and spatial differences of each within the habitat limits their contact and likely reduces the chance of EEE virus transmission.

REFERENCES CITED
