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# AVIAN HOST PREFERENCE BY VECTORS OF EASTERN EQUINE ENCEPHALOMYELITIS VIRUS

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*Abstract.* An important variable in the amplification and escape from the enzootic cycle of the arboviral encephalitides is the degree of contact between avian hosts and mosquito vectors. To analyze this interaction in detail, blood-fed mosquitoes that were confirmed vectors of eastern equine encephalomyelitis (EEE) virus were collected in 2002 from an enzootic site in central Alabama during the time this virus was actively transmitted. Avian-derived blood meals were identified to the species level of the host, and the proportion derived from each species was compared with the overall composition of the avifauna at the study site. The EEE vector mosquito species fed significantly more on some bird species and less on other species than expected given the overall abundance, biomass, or surface area of the local avifauna. When viewed collectively, these data suggest that these mosquitoes are selectively targeting particular avian species.

## INTRODUCTION

The ecology of several arboviruses of public health importance in North America involves collateral interaction with avian populations. Viruses within the family Flaviviridae such as St. Louis encephalitis (SLE) virus and West Nile (WN) virus are examples of viruses for which this is true, as is eastern equine encephalomyelitis (EEE) virus, a member of the family Togaviridae. While different mosquito vectors may be involved, a crucial step in the maintenance and dissemination of these viruses is their amplification within certain avian hosts. Infected native species usually do not show undue morbidity or mortality, but there are occasional exceptions to this pattern. For example, some introduced species are susceptible to EEE virus and the strain of WN virus currently circulating in the United States causes significant mortality in several native bird species, especially corvids such as the blue jay (*Cyanocitta cristata*) and the American crow (*Corvus brachyrhynchos*).[1]

Tracing the movement of EEE virus through both mosquito and avian populations is essential to understanding the ecology of this virus and to developing effective means to control outbreaks. Because EEE follows a pattern typical of other viral encephalitides endemic to the United States, it also serves as a general model of seasonal virus amplification. Passerine birds are the major enzootic reservoirs and early transmission among the local avifauna is believed to be initiated by ornithophilic species such as *Culiseta melanura.*[2] Once enzootic transmission has begun, the virus becomes available to other mosquitoes such as *Aedes vexans* and *Coquillettidia perturbans* that have more catholic host feeding preferences.[3,4] These mosquitoes then serve as "bridge vectors" by transmitting the virus to mammalian species, such as horses and humans, which are dead-end hosts for EEE virus because they do not develop viremias high enough to infect mosquitoes.

Although the over-arching features of the ecology of EEE virus transmission are understood, several specific aspects of the biology of the enzootic cycle remain unresolved. For example, it is not clear what factors are responsible for triggering an outbreak of active infection and transmission. Furthermore, the peak of transmission of EEE virus in enzootic foci generally occurs in the late summer and early fall. This is long after migrating birds have arrived in a given area and the timing does not correlate with the peak population densities of *Cs. melanura,* suggesting that the development of active transmission cannot be ascribed solely to an increase in the vector population.[5]

The dynamics of transmission of any arthropod-borne infection is a complex function of many factors, including the intensity of infection in the vertebrate reservoir, the competence of the vector, and the degree of contact of the vector with the infected vertebrate host reservoir. Data regarding the degree of contact of the vector and host populations can be assayed by identifying the sources of blood meals in recently blood-fed vector mosquitoes. Until recently however, it was impossible to identify avian-derived blood meals to the species level. This prevented measurement of the level of vector contact with different bird species in a given area, a variable that is essential in identifying those avian species that are most likely to serve as important amplifiers for arboviral enzootics. Furthermore, because it has not been possible to identify the source of avian blood meals to the species level, temporal changes in host choice by ornithophilic mosquitoes could not be accurately determined. These changes may also directly influence the development and maintenance of avian enzootics as well as spill-over into human and other animal populations.[6]

Recently, a method based upon a vertebrate-specific polymerase chain reaction amplification of a portion of the cytochrome B gene followed by heteroduplex analysis has been shown to be capable of identifying avian blood meals to the species level.[7] Here, we describe the use of this method to identify the sources of avian-derived blood meals in four species found to carry EEE virus collected at a study site in Macon County, Alabama in 2002. The frequency of blood meals taken from each bird species has been compared with the overall abundance of that species at the site. The data demonstrate that certain avian species are significantly over- or under-represented in the mosquito blood meals than what is predicted based upon their abundance at the site.

## MATERIALS AND METHODS

**Study site.** The study site, located in the Tuskegee National Forest in Macon County, Alabama, has been described previously in detail.[8] Within the 10–15-acre site, there has been

extensive re-forestation over depleted farmland that was abandoned in the early 1900s. Five beaver ponds that are interconnected and fluctuate in size and depth provide standing water for much of the year.

**Estimation of bird densities.** Point counts were used to estimate bird densities at the study site. Point count censuses generally provide a more accurate estimate of true bird densities than do mist net censuses.[9,10] Point counts made in early summer when birds are maximally detectable can act as a measure of relative bird abundance for the entire summer period in the southern United States, because woodland birds in the southeastern United States move little during this period.[11] We conducted 12 point counts on May 30, 2002, using previously described methods.[10,12] Briefly, points were established every 200 meters along a trail through the center of the study area. Counts began at first light and ended at 9:00 PM. Point counts lasted three minutes and all birds seen or heard within 100 meters of the observer during the three-minute counts were recorded. The abundance of birds estimated from these point counts was essentially the same as abundance estimates made by mapping the territories of singing males throughout the spring. We used point count data because this estimate includes non-territorial species that could not be accurately censused by territory mapping (Hill GE, unpublished data).

**Collection of blood-fed mosquitoes.** Vacuum collections were made twice a week from resting boxes[13] and natural resting sites beginning the first week of May and continuing through the end of September 2002. Collections from each box/site were made twice a week on the same day of each week and at approximately the same time (8:30–10:30 AM) each day. Collections were carried out in the wooded interior of the site where birds had been collected or counted. Live material was returned to the laboratory, sorted, and identified using a chill table and binocular microscope, and blood-fed individuals were frozen at –70°C.

**Identification of blood meals.** Blood meals were identified using polymerase chain reaction–heteroduplex analysis (PCR-HDA) assays as previously described.[14] Briefly, total genomic DNA prepared from blood fed mosquitoes was used as a template in a nested PCR amplification with primers that were designed to specifically amplify vertebrate cytochrome B sequences. First-stage PCR amplifications were conducted in a solution containing 60 mM Tris-HCl (pH 8.5), 15 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 200 μM each of dATP, dCTP, dGTP, and dTTP, 0.2 μM of each primer, 1.25 units of *Taq* DNA polymerase (Roche Biochemicals, Indianapolis, IN), and 2.5 μL of DNA template. The sequence of the primers used in the PCR were as follows: 5′- CCCCTCAGAATGATATTTGTCCTCA-3′ and 5′-CCATCCAACATCTCAGCATGATGAAA-3′.

Reactions began with an incubation at 95°C for 3.5 minutes, followed by 40 cycles at 95°C for 30 seconds, 60°C for 50 seconds, and at 72°C for 40 seconds. The reaction was completed by incubation at 72°C for five minutes. Nested amplifications were carried out in a 50-μL volume containing 60 mM Tris-HCl (pH 9.5), 15 mM $(NH_4)_2SO_4$, 2.0 mM $MgCl_2$, 200 μM each of dATP, dCTP, dGTP, and dTTP, 0.2 μM of each primer, 1.25 units of *Taq* DNA polymerase (Roche Biochemicals), and 0.5 μL of the first step amplification product. The sequence of the primers used in the nested PCR were 5′-TCWRCHTGATGAAACTTCGG-3′ and 5′- GTTGCYATKAGGGYYAGGAG-3′ where W = A or T, R = A or G, H = A, C or T, Y = C or T, and K = G or T. Amplification conditions were 95°C for three minutes, followed by 40 cycles at 95°C for 30 seconds, 55°C for one minute, and 72°C for one minute. The reaction was completed with a seven-minute extension at 72°C.

Two aliquots of 6 μL of the PCR product were then separately mixed with 6 μL of PCR product driver derived from northern cardinal (*Cardinalis cardinalis*) and Carolina chickadee (*Poecile carolinensis*). The combined sample and driver PCR products were mixed with 8 μL of 10 mM Tris-HCI (pH 8.0), 1 mM EDTA and overlaid with 10 μL of mineral oil. The mixture was denatured at 99°C for 2.5 minutes and allowed to form heteroduplex products by slow cooling to room temperature. An aliquot (14 μL) of each heteroduplex solution was mixed with 6 μL of loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol, 108 mM Tris-boric acid, pH 8.0, and 2.7 mM EDTA). Ten microliters of this mixture was loaded onto a 5% polyacrylamide/urea gel (29:1 acrylamide:bisacrylamide, 1 M urea) prepared in 108 mM Tris-boric acid (pH 8.0), 2.7 mM EDTA. Electrophoresis was conducted on 20 cm × 20 cm Protean II Xii system (Bio-Rad, Hercules, CA) at 12 mA per gel for 18 hours in 89 mM Tris-boric acid (pH 8.3), 2.5 mM EDTA. Gels were stained in Sybr green (Molecular Probes, Eugene, OR) and homoduplex and heteroduplex patterns were visualized under ultraviolet light. Samples were grouped based upon their HDA product mobilities in the northern cardinal and Carolina chickadee HDA assays. The DNA sequence of one or more representatives of each group were then determined to obtain an unambiguous identification of the source of the blood meal to the species level. In those cases where a group was found to be overrepresented in the blood meals, multiple representatives were sequenced to confirm the HDA identification. In all cases, the DNA sequence data confirmed the identity as predicted by HDA. Representative samples producing a positive result in the PCR amplification, but no HDA patterns on the avian HDAs were further analyzed by DNA sequencing to confirm that they were not derived from avian hosts.

**Feeding index and statistical analysis.** Feeding indicies for each species were calculated using the method described by Kay and others.[15] The feeding index of each species was calculated by comparing the number of blood meals taken from a given bird species to the overall number of avian blood meals identified. Feeding indicies were calculated from the abundance data and from the abundance data corrected for both overall biomass and surface area, as proposed by Kay and others.[15] The total biomass of each species was estimated by multiplying the published average weight of adults of each bird species[16] by the total estimated abundance. Overall estimated surface areas were roughly estimated by taking the two-thirds power of the total biomass. In calculating feeding indices for species for which no blood meals were detected, a value of one was used for the number of blood meals found and the feeding index value was expressed as less than the calculated value. Similarly, species whose blood was found in the mosquitoes tested but which were not identified in the point counts were assigned a point count value of 1. The feeding index was calculated and reported as greater than this calculated value.

Models based on the multinomial distribution were used to estimate the level of significance of the overall feeding indi-

ces. In these analyses, the raw point count data and corrected data for biomass and surface area were all used as described earlier in this report. In each case, bird species for which no blood meals were detected and that represented less than 5% of the total avifauna were grouped. Likelihood ratio tests were used to perform the tests. Because of the relatively small sample sizes, the usual determination of *P* values for the tests based on the asymptotic (large sample) behavior of the natural logarithm of the likelihood ratio statistic was not used. Instead, *P* values based on simulations from the Null Hypothesis distribution were used. In each case, the reported *P* value is based on 1,000 samples drawn for the distribution under the null hypothesis. *P* values less than 0.05 were considered to be significant. In cases where zero cells appeared in the profiles being compared, a count of one was placed in the cell.

## RESULTS

A total of 447 blooded mosquitoes representing 15 species (Figure 1) were collected during the 2002 season at the Tuskegee National Forest site. The most commonly collected blood-fed species was *Culex erraticus,* which represented 63% of the total (Figure 1). This was not surprising, given that surveys of the overall mosquito fauna at the Tuskegee National Forest study site showed that 43% of the individual mosquitoes collected at this site during 2002 were *Cx. erraticus*.

Studies conducted in 2001 had demonstrated that EEE was endemic at the Tuskegee National Forest study site and that EEE virus was detected in five mosquito species (*Cx. erraticus, Cs. melanura, Ae. vexans, Cq. perturbans*, and *Ur. sapphirina*).[8] A total of 13,259 mosquitoes representing these five potential EEE vector species collected from the Tuskegee National Forest study site in 2002 were therefore examined for the presence of EEE virus using a reverse transcriptase–PCR, as previously described.[8] Eastern equine encephalomyelitis virus was detected in all five species (Table 1). Previous studies had demonstrated that these species, with the exception of *Ur. sapphirina* select avian hosts,[2,17–19] which are believed to be the major enzootic hosts for EEE in the United States. Host-feeding studies of *Ur. sapphirina* have not been published. However, host selection studies of *Ur. iowii,* a species that is a member of the same subgenus as *Ur. sapphirina*, indicated a host-feeding preference for frogs, toads, and salamanders.[20] For this reason, *Ur. sapphirina* was excluded from further analysis. Blooded mosquitoes were collected representing three of the four ornithophilic mosquito species found to be infected with EEE virus. DNA isolated from these blooded mosquitoes and the isolated DNAs were used as a template in the avian-specific PCR-HDA described in the Materials and Methods. The source of the blood meal was determined by HDA and confirmed by DNA sequencing, as described in Materials and Methods. All of the *Ae. vexans* and *Cq. perturbans* blood-fed mosquitoes and 80% of the *Cx. erraticus* collected were analyzed in this manner. Of the 264 samples examined, 198 (75%) produced detectable blood meal derived PCR products. As expected, HDA analysis of the PCR products revealed that all three species fed upon avian hosts. A total of 25% of the blood meals were avian in origin in the *Ae. vexans* analyzed, while this proportion was 48% in *Cx. erraticus,* and 33% in *Cq. perturbans.*

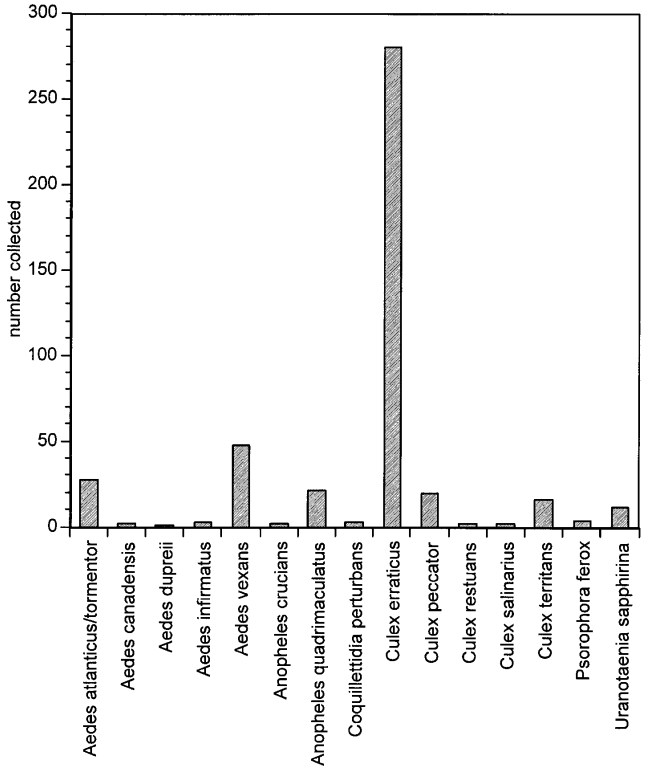


FIGURE 1. Blooded mosquitoes collected at the Tuskegee National Forest study site during the period May–September 2002.

Of the 90 mosquitoes found to be harboring avian blood, seven had mixed meals derived from two or more avian species. These were not characterized further. HDA analysis of the remaining 83 samples revealed 18 different patterns, suggesting that the mosquitoes had fed upon 18 different avian species. Comparison of the DNA sequences derived from representatives of each group confirmed that all were derived from avian hosts. A positive identification could be made for 14 of these groups. The remaining four groups, representing five individual blood meals, were also clearly avian in origin as BLAST searches of the Genbank DNA sequence databank revealed highly significant matches to multiple avian cytochrome B genes. However, exact or nearly exact matches (> 95% identity) were not obtained for these groups, suggesting that these meals were derived from bird species for which cytochrome B sequences are not yet available.

The two most common avian hosts were the Carolina chickadee and the yellow-crowned night-heron (*Nyctanassa violacea*). Yellow-crowned night-herons made up 27% of the avian-derived blood meals in *Cx. erraticus,* but were not seen

TABLE 1

Eastern equine encephalomyelitis in the Tuskegee National Forest in Alabama

| Species | Positive pools/total pools tested | Minimum infection rate (infected/1,000) |
|---|---|---|
| *Aedes vexans* | 3/60 | 1.13 |
| *Culiseta melanura* | 1/7 | 40.0 |
| *Coquillettidia perturbans* | 1/11 | 4.46 |
| *Culex erraticus* | 8/215 | 0.80 |
| *Uranotaenia sapphirina* | 1/10 | 2.65 |

in the five avian meals in *Ae. vexans or* the single avian derived meal in *Cq. perturbans* (Table 2). Carolina chickadees made up 19% of the avian derived meals in *Cx. erraticus.* The Carolina chickadee was also the single avian host seen in *Cq. perturbans,* but was not seen in the five avian derived meals in *Ae. vexans* (Table 2). When the data for all the vector species were combined, 37 (44%) of 83 avian meals were derived from the Carolina chickadee or the yellow-crowned night-heron (Table 2).

The simplest hypothesis for host selection by avian generalist feeders is that it is dependent upon relative host abundance. By this model, the abundance of the different avian species at a given site should be proportional to their abundance in blood meals. To test this hypothesis, the composition of the avifauna at the Tuskegee National Forest study site was assessed as described in the Materials and Methods. These data are summarized in Table 3. The abundance of each species was then compared with the number of mosquito-derived blood meals to identify species that were either over- or under-represented in the blood meals when compared with the number of birds observed at the site, as described in the Materials and Methods (Table 4). Five species (yellow-crowned night-heron, Carolina chickadee, great blue heron [*Ardea herodias*], northern mockingbird [*Mimus polyglottos*], and wild turkey [*Meleagris gallopavo*]) were found to be significantly over-represented in the blood meals when compared with their abundance as estimated by point count, while four species (Acadian Flycatcher [*Empidonax virescens*], northern cardinal, red-bellied woodpecker [*Melanerpes carolinus*], and white-eyed vireo [*Vireo griseus*]) were significantly under-represented in the blood meals when compared with their estimated abundance.

Mosquito attraction to hosts involves a variety of factors, including $CO_2$ output, heat, and moisture that enable the mosquito to distinguish the potential host from the background.[21] These factors are all related to the overall size of the potential host. Similarly, once a mosquito has identified a host, successful feeding will be affected by the exposed surface area of the host available to the mosquito. For these reasons, point count data were normalized for the mass and surface area of each bird species, and the adjusted abundance data were compared with the blood meal prevalence data. Four species were found to be significantly over-represented in the blood meals when compared with the biomass adjusted abundance data, while four species were significantly under-represented (Table 4). Similarly, four species were over-represented in the blood meals when compared with the data adjusted for surface area, while one species was under-represented. Three species (Carolina chickadee, northern mockingbird, and yellow-crowned night-heron) were over-represented in the blood meals in all three analyses (Table 4).

*Culex erraticus,* the predominant mosquito species at the Tuskegee National forest study site, was present throughout the spring and summer of 2002. It was therefore of interest to determine if the host-feeding preference of this vector changed over time. The temporal pattern of the host choice for *Cx. erraticus* is shown in Figure 2. The proportion of avian-derived blood meals in *Cx. erraticus* was greatest in the spring and early part of the summer, and decreased thereafter. Interestingly, the pattern of feeding on the yellow-crowned night-heron was highly restricted, with all meals derived from this most commonly targeted host being taken in a six-week period from the middle of May through the end of June. In contrast, meals taken from the Carolina chickadee and northern mockingbird were distributed more evenly throughout the season (Figure 2).

TABLE 2

Avian blood meals in the four vector species for eastern equine encephalomyelitis at the Tuskegee National Forest study site

| Source of blood* | *Aedes vexans* (n = 5) | *Coquillettidia perturbans* (n = 1) | *Culex erraticus* (n = 84) |
|---|---|---|---|
| Blue jay | 0 | 0 | 1 |
| Brown-headed cowbird | 0 | 0 | 2 |
| Carolina chickadee | 0 | 1 | 15 |
| Great blue heron | 1 | 0 | 9 |
| Green heron | 0 | 0 | 2 |
| House finch | 0 | 0 | 2 |
| Mixed meal | 1 | 0 | 6 |
| Northern cardinal | 1 | 0 | 2 |
| Northern mockingbird | 0 | 0 | 8 |
| Orchard oriole | 0 | 0 | 1 |
| Tufted titmouse | 0 | 0 | 3 |
| Wild turkey | 2 | 0 | 3 |
| Wood duck | 0 | 0 | 4 |
| Yellow-crowned night-heron | 0 | 0 | 21 |
| 90% Black-capped chickadee | 0 | 0 | 1 |
| 91% Lark sparrow | 0 | 0 | 1 |
| 91% *Toxostoma curvirostre* | 0 | 0 | 1 |
| 93% Orchard oriole | 0 | 0 | 2 |

* Some species, although clearly avian in origin, did not match any sequences in the Genbank database with a sufficient level of identity to allow an unambiguous identification. These species are indicated by the percentage match to the species whose sequence most closely matched that of the sample in question.

TABLE 3

Density of bird species at the Tuskegee National Forest Site

| Species | % point count (n = 284) | % avaian meals* (n = 90) |
|---|---|---|
| Northern cardinal | 10.6 | 3.3 |
| Acadian flycatcher | 6.3 | 0.0 |
| White-eyed vireo | 6.3 | 0.0 |
| Red-bellied woodpecker | 6.0 | 0.0 |
| American crow | 5.6 | 0.0 |
| Northern parula | 5.6 | 0.0 |
| Tufted titmouse | 5.6 | 3.3 |
| Carolina chickadee | 5.3 | 17.8 |
| Brown-headed cowbird | 1.4 | 2.2 |
| Wood duck | 1.1 | 4.4 |
| Great blue heron | 1.1 | 11.1 |
| Great egret | 0.7 | 0.0 |
| Yellow-crowned night-heron | 0.4 | 23.3 |
| Blue jay | 0.0 | 1.1 |
| House finch† | 0.0 | 1.1 |
| Orchard oriole | 0.0 | 1.1 |
| Green heron | 0.0 | 2.2 |
| Wild turkey | 0.0 | 5.6 |
| Northern mockingbird | 0.0 | 8.9 |
| Others‡ | 44.0 | 0.0 |

* Multiple host meals (7.8% of total) and unidentified avian species (5.5% of total) are not included in the table, but were included in the percentage calculations.

† Although not an observed natural species, house finches ere maintained as sentinels for arboviral transmission at the site.

‡Species (n = 28) that represented less that 5% of the birds in the point count survey and from which blood meals were not detected.

## DISCUSSION

Eastern equine encephalomyelitis virus is maintained primarily in an enzootic cycle involving the local avifauna. The importance that each particular bird species plays in this pro-

TABLE 4

Bird species over-represented or under-represented in blood meals when compared to their observed abundance at the Tuskegee National Forest study site*

| Species | Count feeding index | Mass feeding index | Area feeding index |
|---|---|---|---|
| Carolina chickadee | 4.70 | 51.56 | 17.48 |
| | $P = 0.00001$ | $P = 0.00001$ | $P = 0.00001$ |
| Northern mockingbird | > 32.81 | > 71.44 | > 40.99 |
| | $P = 0.00001$ | $P = 0.00001$ | $P = 0.00001$ |
| Yellow-crowned night-heron | 255.94 | 16.97 | 22.23 |
| | $P = 0.00001$ | $P = 0.00001$ | $P = 0.00001$ |
| Wild turkey | > 19.65 | > 0.29 | > 0.93 |
| | $P = 0.00001$ | $P = 0.00001$ | NS |
| Orchard oriole | 3.72 | 16.4 | 7.46 |
| | NS | $P = 0.00001$ | $P = 0.009$ |
| Great blue heron | 13.98 | 0.47 | 1.14 |
| | $P = 0.00001$ | $P = 0.00001$ | NS |
| Acadian flycatcher | < 0.19 | < 1.66 | < 0.61 |
| | $P = 0.026$ | NS | NS |
| Northern cardinal | 0.34 | 0.87 | 0.47 |
| | $P = 0.031$ | NS | NS |
| White-eyed vireo | < 0.19 | < 1.88 | < 0.66 |
| | $P = 0.036$ | NS | NS |
| Red-bellied woodpecker | < 0.20 | < 0.36 | < 0.22 |
| | $P = 0.039$ | NS | NS |
| American crow | < 0.22 | < 0.04 | < 0.05 |
| | NS | $P = 0.00001$ | $P = 0.00001$ |
| Great egret | < 1.83 | < 0.21 | < 0.32 |
| | NS | $P = 0.02$ | NS |
| Pileated woodpecker | < 0.60 | < 0.22 | < 0.22 |
| | NS | $P = 0.024$ | NS |

* Feeding indices and statistical significance were calculated from the raw point count data and the point count data adjusted for biomass and surface area as described in the Materials and Methods. A feeding index of greater than 1 indicates that a particular species was over-represented in the blood meals when compared to the abundance data, while an index of less than 1 indicated that a particular species was under-represented. Only species found to be significantly over-represented or under-represented in one or more of the analyses are shown. NS = not significant.

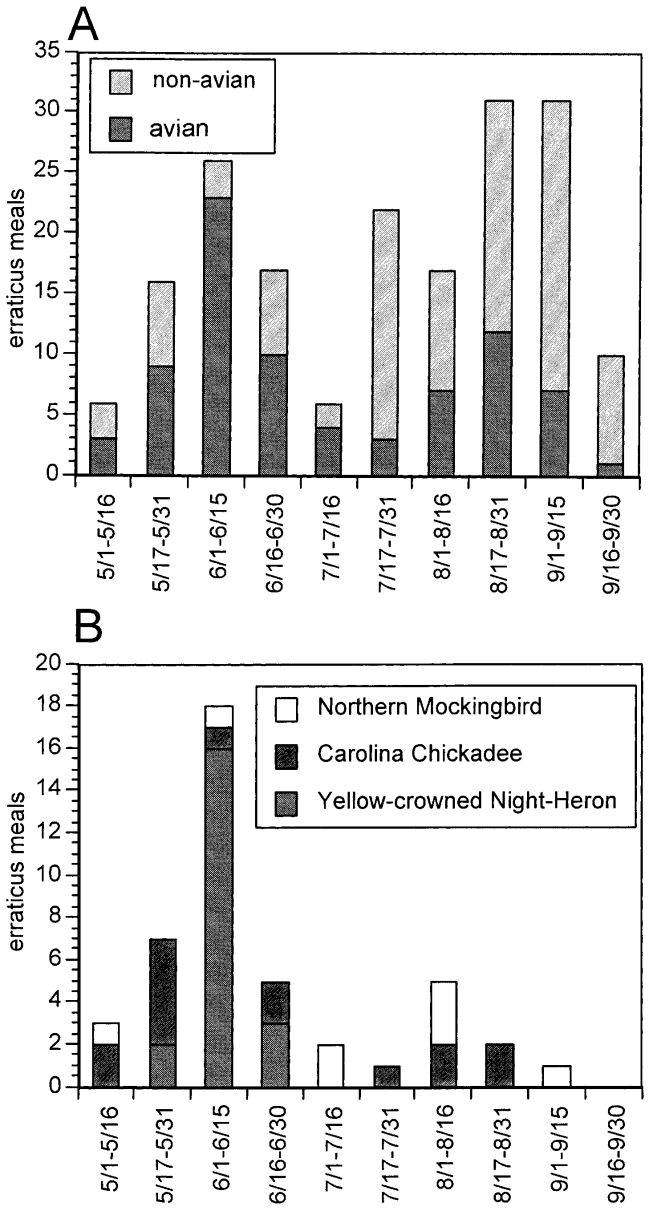


FIGURE 2. Temporal distribution of blood meal hosts in *Culex erraticus* collected in the period May–September 2002. **A**, Distribution of avian and non-avian derived blood meals identified from *Cx. erraticus* from May to September 2002. **B**, Distribution of the blood meals derived from the three most common avian host species from May to September 2002.

cess involves several variables, including the susceptibility of the particular bird species to infection with the virus, the length of time an infected bird will remain infectious to a mosquito vector, and the degree of contact between the bird species and the vector mosquito. The latter parameter has been difficult to quantify because methods to identify avian blood meals to the species level have only recently become available. The data presented in this report indicate that documented EEE mosquito vectors at the Tuskegee National Forest study site fed much more often on some species than predicted by a null model based on the abundance of adult birds. The most striking example of this was the yellow-crowned night-heron. Overall, roughly 23% of the avian blood meals that were identified to the species level were derived from the yellow-crowned night-heron, although this species represented less than 1% of the adult birds detected at the site. Given the apparent high degree of contact between yellow-crowned night-herons and local vector populations, these data suggest that this species may play an important role in the ecology of EEE virus in central Alabama and probably elsewhere in the southeastern United States, where the yellow-crowned night-heron and EEE are co-endemic. Previous studies have shown that the yellow-crowned night-heron is susceptible to EEE virus.[22] Thus, this species meets the physiologic and/or ecologic criteria necessary for consideration as a putative virus amplifier.

All of the blood meals derived from the yellow-crowned night-herons were found in a period of six weeks from mid–May to the end of June. Although we did not locate any yellow-crowned night-heron nests on the study site in 2002, late May–early June coincides with the period of time when yellow-crowned night-herons are likely to be nesting. During nesting, parent yellow-crowned night-herons are likely to forage more and perhaps at different times of the day than when they are not nesting, which could make them more vulnerable to being fed upon by mosquitoes. In addition, nestling birds may be more vulnerable to being fed on by mosquitoes than adults, and nestling yellow-crowned night-herons may account for the brief period of intense feeding on yellow-crowned night-herons by mosquitoes.

Another possible explanation for the over-representation

of yellow-crowned night-herons and Carolina chickadees and the corresponding under-representation of other species in the blood meals is that mosquitoes prefer some hosts more than others, or that the resting behavior of certain avian species combines with the foraging behavior of the vectors to make certain species particularly vulnerable to attack. Mosquito species differ in their overall preference for different classes of host (e.g., mammals versus birds versus reptiles), in the times of day that they are most active in seeking blood meals, and the heights at which they forage.[23–27] Similarly, bird species differ in the height at which they forage, nest, and roost. They also differ in whether they nest in cavities, in vegetation, or on the ground. Finally, different species vary in how actively they kill mosquitoes that approach them. Any or all of these factors may play a role in shaping what species are most frequently fed upon by mosquitoes.[28]

The yellow-crowned night-heron and the Carolina chickadee were the resident species most clearly and consistently over-represented in the mosquito blood meals, but a number of other species were either over- or under-represented in blood meals compared with expectations of a null model based on adult abundance, and adult abundance corrected for total biomass and surface area. Most of the species that were found to be under-represented in one or more of these analyses are native to southeastern swamp forests. It is possible that birds that have had a long evolutionary history with the endemic mosquito species in these wet southern woods may have developed behavioral or physiologic adaptations that reduce their susceptibility to the native mosquito fauna. Yellow-crowned night-herons would be a conspicuous exception to this trend given that they are endemic to southern swamp forest and marsh, but they were the most over-represented in mosquito blood meals of all bird species.

Northern mockingbirds and wild turkeys were found to be common hosts for the mosquitoes collected at the site, although these bird species were not observed in the point count survey. Although turkeys were not observed during the point count survey, signs of this species were noted throughout the summer of 2002, suggesting that this species was present at the site. Turkeys in this area are actively hunted and are very wary of humans, which is probably why they were missed in the point count survey. In contrast, northern mockingbirds are birds of open habitats, not dense swamp forest. It is possible that northern mockingbirds were transiently present at the site, and not actual residents. Such transients might have been missed in the point count. Alternatively, the detection of blood derived from these open-country birds in mosquitoes that we sampled suggests that some blood-engorged mosquitoes collected at the Tuskegee National Forest site may have traveled from the nearest open habitat, a distance of roughly 1 km, after feeding. All three mosquito species examined here are known to forage in both woodland and forest habitats, and *Cx. erraticus* and *Ae. vexans* are capable of foraging flights of 2 km or more.[23,24,26,27] While northern mockingbirds may have been over-represented based on a null model constructed from the abundance of forest birds, they may not have been over-represented if compared with a null model constructed from the abundance of birds over a broader area that included pasture, cropland, and urban environments. Experiments to document the degree of movement of mosquitoes within and outside the Tuskegee National Forest study site are currently underway.

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