

# Mosquito Landing Rates on Nesting American Robins (*Turdus migratorius*)

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## ABSTRACT

We measured mosquito landing rates on adult and nestling American robins at nests with infrared cameras in Washington, D.C., and Maryland, United States. Mosquitoes landed on nesting robins almost exclusively between dusk and dawn. The mean number of mosquito landings per night was higher for adults ( $123.3 \pm \text{SE } 32.8$ ) than nestlings ( $37.26 \pm 14.8$ ). The fraction of mosquitoes landing at a nest on nestlings increased with decreases in adult brooding. Oral swabs from nestlings at these and 13 other robin, Gray catbird, and house finch nests were negative for West Nile virus (WNV). These results show that landing rates were higher on adults and that parental brooding reduces the landing rates of mosquitoes on nestlings. Key Words: Cutex—Modeling—Vector-borne—Arbovirus(es)—West Nile. Vector-Borne Zoonotic Dis. 7, 437–443.

## INTRODUCTION

**B**IRDS ARE THE PRIMARY HOSTS for many important mosquito-borne viruses, including Sindbis, St. Louis Encephalitis, Japanese Encephalitis, Eastern Equine Encephalitis, and West Nile viruses (WNV) (Stamm 1966). As of 1991, 79 of the 504 recognized arboviruses had been detected in birds (Calisher and Karabatsos 1988, Karabatsos 1985, Scott and Edman 1991). Though critical to the study of these arboviruses, the temporal and spatial patterns of mosquito feeding on birds remain poorly defined. This information, along with factors including mosquito abundance, lifespan, and vector competence, is key to understanding and predicting vector borne avian epizootics

and reducing their impact on human and animal health (Anderson and May 1991).

The biting rate of vectors on birds is an influential parameter in determining  $R_0$ , the basic reproductive ratio of a vector-borne pathogen, which in turn determines the epidemic potential of a virus (Anderson and May 1991). Several studies have measured hourly mosquito abundances (Curtis 1953, Haddow and Ssenkubuge 1965, Happold 1965, Haufe 1952, Nasci and Edman 1981, Service 1971, Trueman and McIver 1986), and others have quantified *in situ* avian host seeking behavior of mosquitoes using caged birds (Blackmore and Dow 1958, Dow et al. 1957, Edman et al. 1972, Hodgson et al. 2001, Nelson et al. 1976). However, none of these studies measured the

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biting rate of mosquitoes on birds under natural conditions.

Nesting adult birds and their nestlings spend large portions of their time in a fixed location and may be particularly attractive to mosquitoes because, as an aggregate, they give off large quantities of heat and carbon dioxide. Nestlings may be particularly accessible to biting mosquitoes because of an inability to avoid mosquitoes through flight (Blackmore and Dow 1958, Day and Curtis 1993), weak defensive behavior, incomplete feather coverage, or other factors (Scott and Edman 1991, Scott et al. 1990). Quantifying mosquito visitation of nestling birds may offer insight into their potential arbovirus exposure and contribution to epizootics.

We studied American robins (*Turdus migratorius*), a widely distributed short distance migrant and moderately competent WNV host (Komar et al. 2003), that has been hypothesized to play a role in the continental dispersal pattern of WNV (Rappole et al. 2000). Robins are an important host for *Culex pipiens* and *Culex restuans* mosquitoes in the eastern United States and an important species in the epidemiology of WNV (Apperson et al. 2004, Kilpatrick et al. 2006a,b, Molaei et al. 2006). In order to gain insight into avian arbovirus transmission, we quantified host and vector behavior during the nesting season by observing nesting robins for 24-h periods with infrared cameras.

## METHODS

### Study area

We studied American robin nests located near houses and apartment buildings throughout suburban Maryland (Annapolis, Baltimore, Manchester, Silver Spring) and Washington, D.C., United States. Nests were found by participants in Neighborhood Nestwatch, a Smithsonian citizen science program (Evans et al. 2005). All nests were in residential or suburban areas where robins fed on nearby lawns and from fruiting shrubs. Locations (latitude, longitude) for nests are given in Table 1.

### Observation protocol

During this study, we filmed nests during two periods of the nesting cycle: early (days 1–5) and late (days 6–14), with day 0 defined as the day when all the eggs had hatched. Nestlings open their eyes on day five (Howell 1942), and this provided an easy indicator of nestling development. The period prior to day five generally corresponds to a high degree of parental attendance and brooding (Clark 1985, Dunn 1975), and by implication may represent a lower level of exposure of nestlings to mosquitoes.

We filmed nests using high resolution, infrared cameras and illuminators and recorded in time lapse (1:3) to 8-h VHS tapes (Super Cir-

TABLE 1. MEAN ( $\pm 1$  SE) MOSQUITO LANDING RATES (PER 24-H PERIOD) ON ADULTS AND NESTLINGS

Latitude (N)	Longitude (W)	Day 0	B.S.	Landing rate on nestlings <sup>a</sup>	Landing rate on adults <sup>b</sup>
38.96522	76.56542	5/13	4	0.5	12.5
38.98647	76.58270	5/19	4	8.5	90.5
38.96992	76.47463	5/24	3	2	84.5
39.71095	76.78627	5/28	3	105.5	268
38.99693	77.05048	6/5	4	113	445
39.02292	77.04110	6/12	4	1	22
38.91233	76.51563	6/19	4	61.5	143.5
38.98225	77.02033	7/5	3	10	67
38.89097	77.02297	7/4	3	33 <sup>c</sup>	21 <sup>c</sup>
38.90852	77.06622	7/9	2	47 <sup>c</sup>	54 <sup>c</sup>
39.38878	76.67285	7/11	1	24 <sup>c</sup>	1 <sup>c</sup>

<sup>a</sup>SE (nestling landing rate) = 4.3.

<sup>b</sup>SE (adult landing rate) = 1.5.

<sup>c</sup>Based on one day of observation.

Date of hatching (day 0) and number of nestlings (brood size, B.S.) are given for each nest.

cuits, TX). This allowed for 24-h observations at each nest under all illumination conditions. We attached cameras by Velcro tape to nearby objects about 45 cm from the center of the nest cup and at about a 45-degree angle above the nest. Adjustments to nest illumination were achieved by placing electrical tape over the majority of the camera's infrared diodes.

#### Film analysis

We counted the number of mosquito landings on adults and nestlings in 15-min intervals throughout each 24-h observational period. The resolution of our cameras was insufficient to identify mosquitoes. However, we have trapped mosquitoes using CDC light traps over the past 4 years (2003–2006) at seven nearby sites in residential and suburban areas in Maryland and Washington (Kilpatrick et al. 2006a,b). At each of these sites, >90% of the mosquitoes trapped were *Culex pipiens*, *Cx. Restuans*, or *Aedes albopictus*, and 93% of the *Culex* mosquitoes were molecularly identified (Crabtree et al. 1995) as *Cx. pipiens*. *Aedes albopictus* is thought to be a highly anthropophilic and mammalophilic species, whereas *Cx. pipiens* and *Cx. restuans* feed more frequently on birds (Gingrich and Williams 2005, Kilpatrick et al. 2005). As a result, it is likely that a large fraction of the mosquitoes observed landing on robins were *Cx. pipiens* and a smaller number were likely *Cx. restuans*. However, we cannot exclude the possibility that the mosquitoes landing on birds were other, difficult to trap, species. We were also unable to differentiate between a successful feeding event and a mosquito that merely landed on the host but did not probe or take a blood meal. As a result, the observed landing rates are an upper bound on the biting rate of mosquitoes.

While measuring mosquito visitation rates, we also quantified adult nesting behavior. When neither parent was seen at the nest with our camera, we recorded the nest as unattended. When an adult was brooding, we divided it into two behaviors, full and partial brooding. Partial brooding occurred when the breast of the bird was in contact with nestlings and roughly 20–70% of the nest cup was covered. Full brooding occurred when the breast of the bird con-

tacted the nestlings and more than 70% of the nest cup was covered. We determined the effect of parental brooding on late stage nestling exposure to mosquito landings by correlating the fraction of the night that the nestlings were fully brooded with the fraction of mosquito landings on nestlings. Both variables were arcsin square root transformed before analysis.

#### Sample collection, RNA extraction, and RT-PCR analysis

We swabbed each nestling orally after each of the two 24-h observation periods (Komar and Spielman 1994) for WNV detection. We placed individual swabs in vials containing 1.25 mL of BA-1 (M199 nutrient medium, antibiotics, 1% bovine albumin, and 20% heat-inactivated fetal bovine serum) and maintained them on ice for delivery to the laboratory. Samples were then stored at  $-80^{\circ}\text{C}$  until RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR) analysis. Adults generally resumed normal behavior within a half hour of our leaving a site.

We isolated RNA from 140  $\mu\text{L}$  of the sample medium using the QIAamp<sup>®</sup> Viral RNA Mini Kit (QIAGEN, Valencia, CA). We performed RT-PCR using the Taqman<sup>®</sup> One-step RT-PCR system (Applied Biosystems). Primers and probes were based on the published sequence of the NY99 strain (GenBank Accession No. AF196835) (Lanciotti et al. 2000).

#### Statistical analysis

Data were analyzed by analysis of variance (ANOVA).

## RESULTS

#### Mosquito visitation

We obtained video on eight nests for both early and late nestling stages, and three nests that were filmed only once. Over the full 24-h period, there were a mean of  $170.3 \pm \text{SE } 58.6$  mosquito landings on adults and  $24.8 \pm 22.1$  on nestlings at eight early stage nests, and  $89.1 \pm 36.3$  landings on adults and  $46.4 \pm 20.4$  on nestlings at 11 late stage nests (Fig. 1). Landing

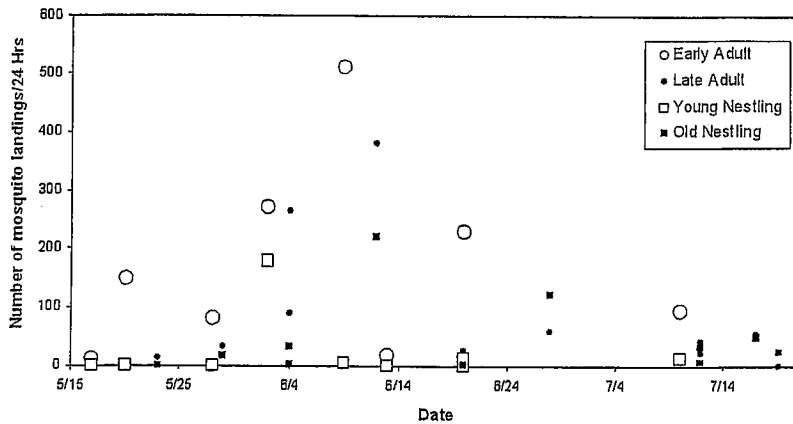


FIG. 1. Mosquito landing rates per 24-h period at 11 American robin nests during early (days 1–5 post-hatching) and late (days 5–11) stages of the nestling period in 2003 in Maryland and Washington, D.C.

rates were significantly higher on adults than nestlings (ANOVA on log-transformed data with 0.5 added to zero counts:  $F_{1,10} = 13.77$ ,  $p = 0.002$ ). Landing rates on adults or nestlings were not significantly different between early and late stage nests (ANOVA, as before:  $F_{1,13} = 1.81$ ,  $p = 0.128$ ). Over 99% of all mosquito observations at robin nests occurred from 2000 to 0600 h (sunrise to sunset; Fig. 2).

The fraction of mosquito landings on nestlings in late stage nests was significantly negatively correlated with the fraction of the night that parents fully brooded the nestlings (Fig. 3). Brooding fraction decreased significantly as the summer progressed (correlation with Julian date:  $r = -0.62$ ;  $n = 11$ ;  $p = 0.042$ ).

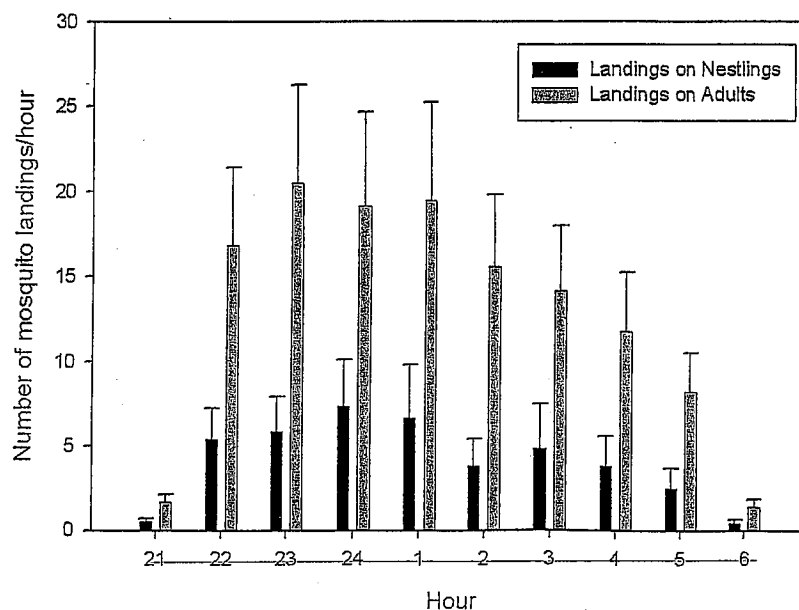
We swabbed nestlings at nine robin nests in

May, six in June (one overlapping with May), and six in July. We also swabbed nestlings at three Gray catbird nests in June and seven in July. Finally, we swabbed nestlings at one House Finch nest during June. All of these swabs tested negative for WNV (165 robin, 53 Gray catbird, and six House finch swabs).

## DISCUSSION

The biting rate of mosquitoes on birds determines the probability of transmission of pathogens between mosquitoes and birds. Our results suggest that some female robins receive 2,000–6,000 mosquito landings during each nestling period and possibly a similar amount

FIG. 2. Hourly mosquito landing rates from all nests combined ( $\pm 1$  SE).



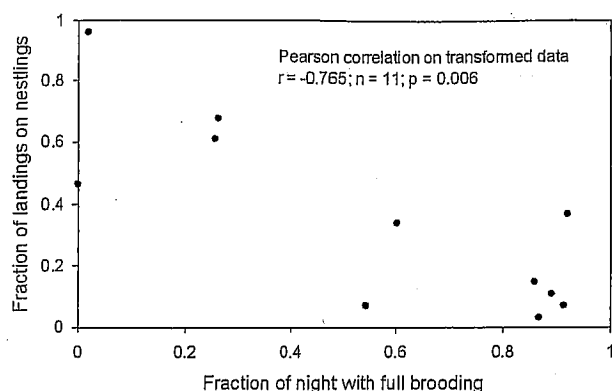


FIG. 3. The influence of brooding behavior of parental birds on the feeding of mosquitoes on nestlings.

during incubation. Although we are unable to determine the fraction of these landings that led to successful bites, their exposure to mosquito-borne pathogens, including WNV, may be substantial.

The landing rates we observed may be overestimates of the biting rates of mosquitoes on birds, because not all landings may have resulted in successful feeding or even probing (when viruses and other pathogens are transmitted). Adult robins sat on the nest with their heads tucked under their feathers and sealed their feet inside the nest. Thus, bare areas where mosquitoes would most easily feed, including the nares, eyes, and feet (Scott and Edman 1991), were protected from exposure.

If our landing rates are overestimates of contact rates, this would partly explain why none of the oral swabs from nestling robins, gray catbirds, or house finches were positive for WNV. However, we cannot rule out small sample sizes and chance, or that the nestlings were infected but not shedding virus from their oral cavities when we swabbed them. Laboratory infection studies suggest that while robins do shed WNV from the cloaca (they were not orally swabbed), they do so at low titers and only for 2 days (Komar et al. 2003).

Previous work has shown that adult birds and older nestlings respond to increasing mosquito visitation with an approximately linear increase in anti-mosquito behavior (Scott and Edman 1991). We did not note this behavioral shift, although adult birds did occasionally exhibit anti-mosquito behavior in the form of feather preening and the attempted ingestion

of mosquitoes. Attempted ingestion was seen definitively at three nests and more ambiguously at others. While the lack of increase in behavior may suggest that nesting birds are protected from mosquitoes, it is also possible that feeding by mosquitoes simply did not induce defensive behavior. One previous study suggested that robins have low levels of anti-mosquito behavior, at least in comparison to European Starlings (Hodgson et al. 2001).

Our data showed that a key determinant of mosquito landing rates on late-stage nestlings is the brooding behavior of the parent, which decreases as the season progresses. However, late-stage nestling exposure may have been offset by improved anti-mosquito behavior (Scott and Edman 1991) and increased feather coverage. Though unprotected by feathers, young nestlings are often completely covered by their parent during night brooding and thus have reduced exposure to mosquitoes.

Our research has provided preliminary insights into the exposure of nesting adult and nestling birds to mosquitoes. Birds appear to be exposed to large numbers of host-seeking mosquitoes each night, with substantial heterogeneity between individuals. In addition, due to the late season rise in prevalence of WNV and other arboviruses, our study suggests that early-breeding-season nestling birds may suffer less risk of exposure to mosquito-borne pathogens. Our study also highlights two challenges for estimating mosquito feeding rates on free-living birds: capturing the mosquitoes that landed on the birds, and determining whether they have probed or obtained a blood meal. Clearly the patterns of mosquito feeding on adult, nestling, and fledgling birds are important topics that may provide insight into the epidemiology of WNV and other arboviruses.

#### ACKNOWLEDGMENTS

Scott Shone (of Johns Hopkins University) and Jeannine Dorothy (of Maryland Mosquito Control) both provided information regarding potential mosquito species that we might find at our nests. We thank the Nestwatch volunteers for providing access and information on

American robins nesting in their yards. This work was supported by the U.S. Department of Agriculture, Grant Agreement Number U50/CCU320544-01 from the Centers for Disease Control and Prevention (CDC), NIAID contract NO1-AI-25490, and the National Science Foundation (NSF) grants DEB-0089565 and EF-0622391. This material is based upon work supported under a National Science Foundation Graduate Research Fellowship. Its contents are solely the responsibility of the authors and do not represent the official views of the CDC or the NSF.

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