

## OBSERVATIONS ON MULTIPLE BLOODFEEDING IN FIELD-COLLECTED *CULISETA MELANURA*

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**ABSTRACT.** This study examined field populations of *Culiseta melanura* for evidence of partial blood meals and multiple feeding behavior. Engorged specimens that had  $\frac{1}{2}$  or less abdominal distention together with eggs in stage I-II of ovarian development were recorded as mosquitoes that had taken partial blood meals. Multiple blood meals were credited to engorged specimens with fresh blood as well as partially digested blood surrounded with separate peritrophic membranes and eggs in stage III or IV of ovarian development. Results indicated that only 17 of 532 *Cs. melanura* were captured with a partial meal, indicating that natural populations feed to repletion more than 95% of the time. Less than 1% of the specimens showed physical evidence of feeding on multiple hosts. *Culiseta melanura* appears to be highly efficient at obtaining a full blood meal and seems to be extremely reluctant to refeed once ovarian development has been initiated. As a result, multiple feeding does not appear to be important in the amplification of virus by this species.

### INTRODUCTION

We examined field populations of *Culiseta melanura* (Coq.) for evidence of partial blood meals to assess feeding efficiency in nature. Specimens were also examined for multiple meals to determine frequency of contact with more than one host within a gonotrophic cycle. *Culiseta melanura* is the enzootic vector of eastern equine encephalomyelitis (EEE) virus, a natural infection of birds along the Atlantic flyway of North America (Scott and Weaver 1989). The mosquito plays a primary role in the amplification of the virus in bird populations each year (Crans et al. 1994) and appears to be responsible for epizootics that precede equine and human involvement (Morris 1988).

Feeding by an infected mosquito within a short time on more than one host due to defensive behavior of the host will result in increased probability of disease transmission (Edman et al. 1974). Partial blood meals frequently indicate that a mosquito was unable to imbibe completely during the act of feeding and, as a result, may complete the meal on another host (Klowden and Lea 1979).

Lorenz and Scott (1996) reported that infected *Cs. melanura* could transmit EEE virus in the laboratory by merely probing on susceptible chicks. They were able to induce multiple feeding with this species in the laboratory but found no histologic evidence that *Cs. melanura* engaged in multiple feeding under field conditions. Anderson et al. (1990) found limited evidence for multiple feeding in the wild using rubidium and cesium blood markers in experimental chicken flocks.

### MATERIALS AND METHODS

From June 2 to October 25, 1993, we sampled *Cs. melanura* from 10 resting boxes each at 4 locations in southern New Jersey and examined all engorged specimens for evidence of partial meals and multiple bloodfeeding. The specimens were transported alive from the field sites in pint paper

cartons covered with moist sponges, placed in a refrigerator at 4°C to retard blood meal digestion, and anesthetized with chloroform for species identification and processing. Engorged specimens that visually exhibited  $\frac{1}{2}$  or less abdominal distention were separated and later dissected to determine Christophers' stage of ovarian development (Christophers 1911) as modified by Mer (1936). Mosquitoes from this group with follicles in stage I-II were counted as specimens that had taken a partial (and possibly interrupted) blood meal.

Fully engorged mosquitoes were examined for evidence of feeding on more than one host by opening the midgut and separating the contents. A fresh blood meal surrounded by a peritrophic membrane in combination with a partially digested blood meal in a separate membrane suggested that blood was probably taken from more than one host. We confirmed the result if ovarian development had progressed to Christopher's stage III or IV (as a result of the first meal) even though fresh blood was present in the abdomen (Detinova 1962).

### RESULTS AND DISCUSSION

Table 1 presents partial as well as multiple feedings in the 532 engorged *Cs. melanura* we collected during this investigation. Only 2.1-4.9% of the specimens were captured with partial meals ( $\chi^2 = 2.328$ ,  $df = 4$ ,  $P = 0.676$ ). This suggests that wild populations of *Cs. melanura* are able to feed to repletion more than 95% of the time. The experiment we conducted, however, does not confirm that feeding to repletion involved only one host. If *Cs. melanura* were interrupted by host defensive behavior during the act of taking the initial blood meal (Edman and Kale 1971) and refeed on a second host within a few hours, the specimen would have shown evidence for complete feeding with undetectable peritrophic membranes separating the contributions of the 2 hosts involved. Anderson et al. (1990) provide evidence for this type of interrupted

Table 1. Partial and multiple blood meals in field-collected *Culiseta melanura* from southern New Jersey.

Month	No. engorged	Partial meal <sup>1</sup>		Multiple meal <sup>2</sup>	
		No.	%	No.	%
June	97	2	2.1	0	0
July	147	4	2.7	1	0.7
August	163	8	4.9	2	1.2
September	116	3	2.6	2	1.7
October	9	0	0	0	0
Total	532	17	3.2	5	0.9

<sup>1</sup> Specimens where the blood meal distended the abdomen by  $\frac{1}{2}$  or less by external examination and had follicles in stage I-II of ovarian development.

<sup>2</sup> Specimens that contained fresh blood as well as partially digested blood when dissected and had follicles in stage III or IV of ovarian development.

feeding in their results with chickens injected with alkali blood markers. In their studies, however, only 1% of the *Cs. melanura* captured near marked chicken flocks took blood from more than one host on the same evening, suggesting that multiple host acceptance might be relatively uncommon with this species.

Less than 1% of the specimens we examined showed evidence of multiple blood meals separated by a period of one or more days ( $\chi^2 = 1.989$ ,  $df = 4$ ,  $P = 0.738$ ). This agrees with the observations of Lorenz and Scott (1996) who found that colonized *Cs. melanura* were extremely reluctant to feed twice in a single gonotrophic cycle and who believed that the mosquito rarely, if ever, feeds on successive nights.

*Culiseta melanura* appears to be highly efficient at obtaining a full blood meal and seems to be extremely reluctant to refeed once ovarian development has been initiated. As a result, multiple feeding does not appear to be important in the amplification of virus by this species. Further studies are required to determine how persistent this species is in response to host defensive behavior and the sig-

nificance of multiple probing in the dissemination of virus by infected specimens.

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