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DETECTION OF MULTIPLE BLOODFEEDING IN *CULISETA MELANURA* USING A HISTOLOGIC TECHNIQUE

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ABSTRACT. We used a histologic technique to detect multiple bloodfeeding in a single gonotrophic cycle by *Culiseta melanura.* In a laboratory study with colonized mosquitoes, 77% (10/13) of known multiple meals were detected when the interval between meals was 24–30 h. Outside that range, known multiple meals were undetectable with this method. No multiple blood meals were detected in 653 wild engorged mosquitoes collected during 3 consecutive years from the Pocomoke Swamp in Maryland. Although previous studies have shown that *Cs. melanura* will feed twice in the same evening, it rarely, if ever, takes multiple blood meals at 24-h intervals. Our study also indicates that a thorough laboratory standardization is required prior to application of the histologic technique to species for which it has not been studied. This would include a time-series analysis to define species-specific limits for detecting known multiple meals.

This study attempted to use a histological technique to detect multiple bloodfeeding by *Culiseta melanura* (Coquillett), the enzootic mosquito vector of eastern equine encephalomyelitis (EEE) virus in North America (Scott and Weaver 1989). The technique we used was originally developed for detection of multiple feeding by *Culex nigripalpus* (Theobald) (Romoser et al. 1989) and *Aedes aegypti* (Linn.) (Scott et al. 1993).

Multiple feeding by mosquitoes is important because an increase in the frequency of host contact by a vector correspondingly increases the probability of pathogen transmission and, therefore, the reproductive rate of an arthropod-borne parasite (Scott et al. 1993). One way that a mosquito can increase host contacts is to imbibe more than one blood meal during each gonotrophic cycle. This kind of mosquito feeding scenario is potentially important to EEE virus epidemiology. In laboratory studies, we found that infected *Cs. melanura* that probed, but imbibed no visible amounts of blood from, 2 different chicks in succession transmitted EEE virus to both birds (L. H. Lorenz and T. W. Scott, unpublished data). Results from a field study that used chickens inoculated with alkali metals as experimentally marked hosts document that *Cs. melanura* will engage in multiple feeding behavior (Anderson et al. 1990). Those authors reported that wild *Cs. melanuara* fed on more than one chicken in a single evening. In combination, these studies suggest that an infected *Cs. melanura* could transmit EEE virus to more than one avian host in an evening.

We used the histologic technique to examine the occurrence of multiple feeding in order to determine whether 1) wild *Cs. melanura* imbibe multiple meals from natural as well as from experimental avian hosts and 2) multiple meals by this species are imbibed during the same evening or are separated by one or more days. The procedures for histologic preparation and the criteria for determining whether a mosquito took more than one blood meal are described in detail by Scott et al. (1993). In brief, specimens were fixed in alcoholic Bouin's solution, embedded in paraplast, sectioned, stained with a modified Azan stain, and viewed with bright-field microscopy.

Prior to examination of wild mosquitoes, a time-series analysis was conducted to standardize the technique for *Cs. melanura.* Individual mosquitoes from the Farmington colony (Wallis and Whitman

Table 1. A laboratory time-series analysis on detection of multiple blood feeding by *Culiseta melanura.*

Time between meals (hours)	Time from 2nd meal until fixation (hours)	
	0	18–36
2–18	0/6[1]	0/7
24	5/8	—[2]
30	5/5	—
50	0/1	—
Total	10/20	0/7

[1] Mosquitoes containing detectable multiple meals/number of mosquitoes examined.

[2] Dashes indicate that no mosquitoes were examined for that time interval.

Table 2. Engorged *Culiseta melanura* collected from the Pocomoke Swamp and examined histologically for multiple bloodfeeding.

Year	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Total
1985	0	7	16	22	16	65	135	32	1	294
1986	5	0	2	3	10	33	109	10	0	172
1987	0	0	21	15	10	42	74	26	0	188
Total	5	7	39	40	36	140	318	68	1	654

1969) were induced to imbibe multiple blood meals at time intervals varying from 2 to 50 h. For the first feeding, the mosquitoes were interrupted after being allowed to imbibe what appeared from external examination to be only ⅓–⅔ of a replete blood meal. During the 2nd meal, mosquitoes were allowed to feed to repletion. Sources of blood for these laboratory studies were house sparrows (*Passer domesticus*) or chickens. Enticing colonized *Cs. melanura* to feed twice in a single gonotrophic cycle was difficult. After exposing more than 2,000 mosquitoes to chicks in 12 different feeding experiments, we were able to obtain approximately 75 that took partial meals and, of those, only 27 took a 2nd meal.

None of the mosquitoes that imbibed 2 meals separated by <24 h or >30 h exhibited histologic characteristics of multiple feeding. However, we detected multiple feeding in 77% (10/13) of the mosquitoes that took 2 meals separated by 24–30 h (Table 1).

Field specimens were collected from the Pocomoke Cypress Swamp, which is located near Pocomoke City, Maryland (Lorenz et al. 1990). Mosquitoes were captured in 3 areas: resting boxes (Edman et al. 1968) set up along the edge of the swamp, resting boxes set up about 100 m from the swamp in an upland wooded setting, and a buried plywood box halfway between the swamp and the upland resting boxes (Lorenz et al. 1990). Mosquitoes were collected as early as March and as late as November over a 3-year period (Table 2). None of the 654 field-collected mosquitoes contained histologic evidence indicating that they had imbibed a multiple blood meal.

Culiseta melanura generally seek blood meals in early evening; a feeding peak occurs 1–2 h after sunset (Nasci and Edman 1981; L. H. Lorenz and T. W. Scott, unpublished data). Results from our laboratory time-series study indicate that the histologic technique would detect multiple feeding by *Cs. melanura* if the meals were taken during the feeding peak on successive evenings, but not if they were taken within a single evening or if they were separated by more than one evening. From our analysis, we are able to conclude that this mosquito rarely, if ever, feeds on successive nights.

Differences in the results obtained from laboratory studies with *Cs. melanura* reported herein vs. those previously published for *Cx. nigripalpus* (Romoser et al. 1989) and *Ae. aegypti* (Scott et al. 1993) highlight the need for thorough standardization of this procedure before it is applied across species. For example, with *Ae. aegypti,* multiple meals separated by as brief an interval as 1 h were detectable. For *Cs. melanura,* the minimum detectable separation increased to 24 h.

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