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INTRASPECIFIC VARIATION IN KEY MORPHOLOGICAL CHARACTERS OF *CULISETA MELANURA* (DIPTERA: CULICIDAE)

THEODORE G. ANDREADIS[1] AND LEONARD E. MUNSTERMANN[2]

ABSTRACT. *Culiseta melanura* (Coq.), the enzootic vector of eastern equine encephalitis in North America, is polymorphic for a trait used as a key diagnostic character. The absence of white abdominal bands distinguishes this species in several prominent keys to North American mosquitoes. However, this is an environmentally induced, nongenetic trait that cannot be used as a key character for diagnosing *Cs. melanura.* In light trap collections, banded specimens occur in early spring and summer, and nonbanded adults appear in late summer–autumn. Larvae reared in laboratory conditions produce nonbanded adults. Progeny reared from banded mothers are uniformly nonbanded. Biochemical genetic results indicate that banding is not correlated with a distinctive genotype or presence of cryptic species. In 18 enzyme loci screened, neither diagnostic alleles nor large differences in allele frequencies were detected between field-collected representatives of the two forms. Genetic variability was relatively low in the 28-year-old laboratory colony (average heterozygosity = 7%; average number of alleles per locus = 1.4), whereas in field samples, the variability was typical of field populations (average heterozygosity = 12–19%; average number of alleles per locus = 1.6–1.8), with the presence of both polymorphic and private alleles. The population genetic profile and comparisons among geographically distinct populations represent the first such presentation for any species in the genus *Culiseta.*

INTRODUCTION

Traditionally, adult *Culiseta melanura* (Coq.) have been described as the only member of the genus *Culiseta* without basal pale bands on the dorsum of the abdomen. The taxonomic significance of this morphological trait is currently recognized by Darsie and Ward (1981:96–100), who use it as a principal diagnostic character to distinguish *Cs. melanura* from other species of *Culiseta* in their key to adult females.

During studies on the ecology of *Cs. melanura* at several freshwater swamp sites in Connecticut, the senior author (T.G.A.) collected large numbers of adult females that did not conform to the conventional description. Although these mosquitoes possessed the characteristic dark-scaled wings and hind tarsomeres, they bore, in addition, distinct bands of light-colored scales on the dorsum of the abdomen. This observation prompted us to review prior taxonomic descriptions of *Cs. melanura.* Several descriptions indicated that narrow basal bands of pale scales are variable on some abdominal segments (Dyar 1928, Matheson 1929, Carpenter and LaCasse 1955, Means 1987).

We subsequently undertook to document the widespread occurrence of this variable trait and to quantify its natural prevalence and distribution in field populations of *Cs. melanura.* Biochemical genetic profiles were compared to ascertain that the genotype does not parallel the morphological phenotype.

[1] The Connecticut Agricultural Experiment Station, 123 Huntington Street, P. O. Box 1106, New Haven, CT 06504.

[2] Department of Epidemiology and Public Health, Yale University School of Medicine, 60 College Street, P. O. Box 208034, New Haven, CT 06520-8034.

MATERIALS AND METHODS

Field sites: Adult female *Cs. melanura* populations were sampled at 10 freshwater swamp sites located in 5 counties throughout Connecticut (Fig. 1). All collection sites were mature hardwood swamps dominated by red maple, *Acer rubrum*; Atlantic white cedar, *Champaecyparis thyoides*; and eastern hemlock, *Tsuga canadensis.* The ground cover was predominantly *Sphagnum* spp. With the exception of two coastal sites (Fairfield and Waterford), all sites were inland. Elevations ranged from sea level to 365 m (Cornwall).

Field collections: Adults were collected weekly at each site with dry-ice-baited CDC miniature light traps (one trap per site) from June 27 to October 11, 1991, and from June 2 to September 30, 1992. Traps were set at the swamp perimeter during the late afternoon and were retrieved the following morning. Mosquitoes were transported to the laboratory, where they were cold anesthetized on a chill table and identified using the keys of Carpenter and LaCasse (1955), Darsie and Ward (1981), and Means (1987). Female *Cs. melanura* with complete bands of light-colored scales at the bases of the abdominal segments and females whose abdomens were entirely dark-scaled were recorded from each site. Females with small patches of light-colored scales on the sides of the abdomen were recorded as unbanded.

For comparison of biochemical variability, collections were taken from three of the same sites described above (Fig. 1) in September 1994 (Killingworth—light, banded form; Chester—dark, unbanded form) and in June 1995 (Chester—light, banded form; Cornwall—dark, unbanded form).

Laboratory rearing, morphological characterization: Further confirmation on the identity and associated variability of each adult form (banded and unbanded) was obtained through examination of 1) adults reared from field-collected larvae and 2) F_1

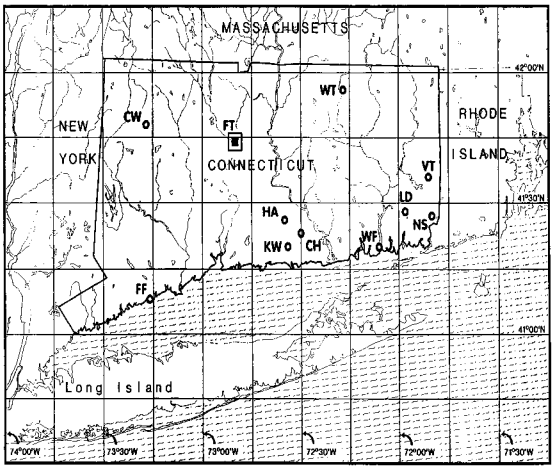

Fig. 1. Collection sites of *Culista melanura* in Connecticut overlaid on the major state watershed systems. CH = Chester, CW = Cornwall, FF = Fairfield, FT = Farmington, HA = Haddam, KW = Killingworth, LD = Ledyard, NS = North Stonington, VT = Voluntown, WF = Waterford, WT = Willington. Electrophoretic samples were taken from CH, CW, FT, and KW.

larval and adult progeny reared from field-collected females that oviposited in the laboratory.

Larval specimens (1st–4th instars) for the first study were collected from July through October from cool-water pools found at Chester, Cornwall, and Voluntown. Larvae were individually isolated in 30-ml plastic cups containing water from the habitat, maintained at 24 ± 2°C under a 14-h (plus 2 h dawn, dusk) photophase, and reared to adulthood. Larvae were fed a small quantity of an aqueous suspension of dried liver powder (1st–2nd instars) and finely ground cat chow (3rd–4th instars). After emergence, they were classified according to the presence or absence of abdominal bands.

Gravid female *Cs. melanura* for the second study were collected using CDC miniature light traps as described above. These females were initially examined for abdominal banding patterns at the time of collection and were then individually transferred into 20-cm^3 screened cases for oviposition. Each cage was supplied with a cotton pad soaked with a 3% (w/v) sucrose solution and a 30-ml paper-lined cup filled with distilled water. Females were maintained at 70% RH under the same temperature and photoperiod regime as in the larval studies. Egg rafts were individually transferred into 100 × 80-mm culture dishes containing 250 ml of distilled water. Sibling larvae were reared to pupation and were examined for key diagnostic characters. Pupae were individually transferred into 30-ml plastic cups for adult emergence, following which each adult (male and female) was examined and categorized according to the abdominal banding pattern.

Electrophoretic protocols—sampling: Samples for electrophoretic analysis were selected from light trap samples taken from three locales (Chester, Cornwall, and Killingworth). Two kinds of comparisons were performed. The first was to ascertain the genetic profile of adult mosquitoes with the distinctive morphological phenotypes. The second was to compare genetic variation of field populations with that of a laboratory colony established 28 years earlier (Wallis and Whitman 1969).

Electrophoretic protocols—biochemical methods: Standard polyacrylamide gel electrophoresis separated the mosquito proteins on a thin, 5% polyacrylamide, slab gel matrix. Buffer systems (Tris-borate-EDTA, Tris-citrate), histochemical stains, and specimen preparation have previously been described (Munstermann 1980, Matthews and Munstermann 1983, Eldridge et al. 1986). Fourteen enzyme assays revealed phenotypes of 18 genetic loci and 45 putative alleles in individuals representing the 2 experimental categories. The enzymes, Enzyme Commission numbers, and alleles used were as follows: aconitate hydratase, EC 4.2.1.3, *Aco*; adenylate kinase, EC 2.7.4.3, *Ak*; arginine kinase, EC 2.7.3.3, *Ark*; aspartate transaminase, EC 2.6.1.1, *Aat-1, Aat-2*; fumarate hydratase, EC 4.2.1.2, *Fum*; glucose-6-phosphate isomerase, EC 5.3.1.9, *Gpi*; glycerol-3-phosphate dehydrogenase, EC 1.1.1.8, *Gpd*; hexokinase, EC 2.7.1.1, *Hk-1, Hk-2, Hk-3, Hk-C*; isocitrate dehydrogenase, EC 1.1.1.42, *Idh-1, Idh-2*; malate dehydrogenase, EC 1.1.1.37, *Mdh-2*; malate dehydrogenase, decarboxylating, EC 1.1.1.40, *Me*; mannose-6-phosphate isomerase, EC 5.3.1.8, *Mpi*; octanol dehydrogenase, EC 1.1.1.73, *Odh*; phosphogluconate 2-dehydrogenase, EC 1.1.1.43, *Pgd*; phosphoglucomutase, EC 5.4.2.2, *Pgm*; α,α-trehalase, EC 3.2.1.28, *Tre-3*.

Reference standards on each gel (Munstermann 1988) consisted of individual specimens from a laboratory colony of *Aedes aegypti* (L.) (ROCK strain) with low genetic variability. The ratio of migration of the gel bands of the 2 species provided the allele designation (Table 2). A second standard for comparison was represented by the 28-year-old colony of *Cs. melanura* established by Wallis and Whitman (1969). Biochemical genetic data were summarized with the BIOSYS-1 population genetics program (Swofford and Selander 1981).

RESULTS

Description: Two distinct morphological variants of adult female *Cs. melanura* were observed in the light trap collections: a light form and a dark form. The light form had conspicuous basal bands of light-colored scales on each abdominal tergite (Fig. 2A) that in some individuals widened slightly on the sides to small lateral patches. The integument of the scutum was golden brown to amber with dark brown setae, and the prespiracular bristles were very light and difficult to see. The wing

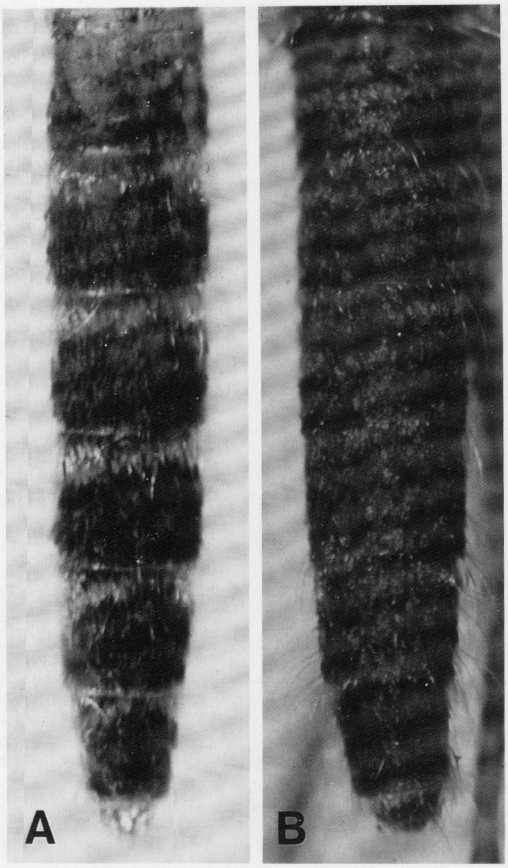

Fig. 2. Dorsal view of abdominal tergites of a banded light female (A) and an unbanded dark (B) female *Culiseta melanura.*

scales were entirely dark and the hind tarsomeres were unbanded.

The dark form possessed all of the characters noted in most descriptions. The abdominal tergites were entirely dark-scaled and without pale bands (Fig. 2B). The integument of the scutum was dark brown, as were the setae and prespiracular bristles. The wing scales were all dark and the hind tarsomeres were unbanded.

Field collections: The comparative abundance and distribution of each of the two morphological forms of adult female *Cs. melanura* collected in the light trap surveys are shown in Table 1 and Fig. 3. In 1991, a distinct separation in the abundance of each morphological variant was observed. The banded light form predominated during the early and mid-summer months (June through mid-August) while the unbanded dark form predominated during the late summer and early fall (mid-August through September) (Fig. 3). An examination of the comparative abundance and distribution of the two morphological variants at each of the field sites during 1991 (Table 1) revealed no clear pattern of occurrence: the banded form was more abundant at 3 sites, while the unbanded form was more abundant at 5 sites, many of which were located in the same county.

A different distribution and abundance pattern was observed in 1992. As in 1991, no unbanded dark forms were collected in June or early July, and all first generation adults that emerged from overwintered larvae were light in color with abdominal bands (Fig. 3). However, unlike in 1991, these banded *Cs. melanura* females were continuously found throughout the entire sampling period, and,

Table 1. Prevalence of banded and unbanded forms of adult female *Culiseta melanura* collected at various field sites in Connecticut, 1991–92.

Collection site (county and town)	1991			1992		
	No. collected	% banded	% unbanded	No. collected	% banded	% unbanded
Fairfield						
Fairfield	74	20.2	79.8	104	82.7	17.3
Litchfield						
Cornwall	—	—	—	66	92.4	7.6
Middlesex						
Chester	258	42.6	57.4	895	81.5	18.5
Haddam	221	57.0	43.0	931	86.7	13.3
Killingworth	220	35.5	64.5	1,053	88.1	11.9
New London						
Ledyard	211	68.7	31.3	238	94.5	5.5
North Stonington	167	40.7	59.3	239	81.2	18.9
Voluntown	558	70.6	29.4	395	81.8	18.2
Waterford	262	41.6	58.4	260	89.2	10.8
Tolland						
Willington	—	—	—	411	83.7	16.3
Overall total	1,971	53.0	47.0	4,592	85.6	14.4

Table 2. Sample sizes and allele frequencies for 14 isoenzyme loci in 4 Connecticut populations of *Culiseta melanura*, representing the 2 morphotypes, "light" (banded) and "dark" (unbanded) forms. Alleles are named by relative migration to *Aedes aegypti* ROCK[1] strain reference standard. NT = not tested. Four additional loci showed no variation—*Fum, Gpd, Hk-1,* and *Pgm.*

Enzyme locus/ alleles	Colony (Farmington) light form	Field populations: Chester light form	Chester dark form	Cornwall dark form	Killingworth light form
Aat-1					
2.00	0	0		0.091	
0.68	**1.000**	**1.000**		**0.909**	
n	8	21	NT	22	NT
Aat-2					
0.96	0.063	0.071	0.222	0.159	0.250
0.88	0	0.024	0	0	0
0.73	**0.938**	**0.881**	**0.778**	**0.818**	**0.750**
0.56	0	0	0	0.023	0
0.53	0	0.024	0	0	0
n	16	21	9	22	8
Aco[1]					
1.17	0	0	0.083	0.024	0
1.09	**1.000**	**0.850**	**0.806**	**0.905**	**1.000**
1.03	0	0.150	0.111	0.071	0
n	24	20	18	21	15
Ak					
1.09	0	0.048	0	0	0.029
0.91	**1.000**	**0.952**	**1.000**	**1.000**	**0.971**
n	24	21	18	22	17
Ark					
0.88	**1.000**	**1.000**	**0.944**	**1.000**	**1.000**
0.79	0	0	0.056	0	0
n	12	21	18	19	18
Gpi					
1.21	0.063	0.048	0	0	
1.18	0.167	0.381	0.750	0.571	
1.13	**0.771**	**0.571**	**0.250**	**0.381**	
1.09	0	0	0	0.048	
n	24	21	18	21	NT
Hk-3					
1.08	0.053	0	0.385	0	
1.04	**0.947**	**1.000**	**0.615**	**1.000**	
n	19	12	13	9	NT
Hk-C					
1.11	0.105	0	0.167	0.056	
1.06	**0.895**	**1.000**	**0.833**	**0.944**	
n	19	12	9	9	NT
Idh-1					
1.02	0	0	0.029	0.023	0
0.75	**1.000**	**1.000**	**0.971**	**0.977**	**1.000**
n	23	21	17	22	16
Idh-2					
1.04	0	0	0	0.023	0
0.90	**1.000**	**0.976**	**0.889**	**0.955**	**1.000**
0.80	0	0	0.028	0	0
0.74	0	0.024	0.083	0.023	0
n	24	21	18	22	8
Mdh-2					
1.16	0.188	0.405	0.222	0.214	0.667
1.00	**0.813**	**0.595**	**0.778**	**0.786**	**0.333**
n	24	21	18	21	6
Me					
1.00	0	0.024	0	0	
0.90	**1.000**	**0.952**	**0.971**	**0.977**	
0.82	0	0.024	0.029	0.023	
n	24	21	17	22	NT
Odh					
1.00	0	0.111	0	0	0
0.83	**1.000**	**0.889**	**1.000**	**1.000**	**1.000**
n	18	9	15	11	6
Tre-3[1]					
1.00	0.125	0.238	0.294	0.318	0.059
0.96	**0.875**	**0.571**	**0.706**	**0.659**	**0.941**
0.93	0	0.190	0	0.023	0
n	24	21	17	22	17

[1] Note that though the *Aedes aegypti* strain is a laboratory colony of more than 50 years, it is polymorphic for 2 alleles in at each of 2 loci—*Aco* and *Tre-3*. In these cases, the slowest *Aedes aegypti* allele was chosen as the reference band to establish the relative migration of the *Cs. melanura* alleles.

Bold numbers denote most common allele frequencies.

with a few exceptions, they were consistently more abundant than the unbanded form (Fig. 3). The relative abundance of the banded form was universally evident at all 10 sites, where the overall percentage of banded females ranged from 81 to 94% (Table 1).

Laboratory rearings: Totals of 74 male and 86 female *Cs. melanura* were reared to adulthood from field-collected larvae. In every instance, the emerging adults were dark brown and were without distinct bands of light-colored scales on the dorsum of the abdomen. The 4th-instar larvae were unremarkable and possessed all of the key characters for the species (i.e., antenna, comb scales, pecten teeth, siphonal index, ventral row of siphonal setae) as described in Carpenter and LaCasse (1955) and Means (1987).

We were able to induce 7 field-collected female *Cs. melanura* to oviposit in the laboratory and produce eggs that hatched. Each female was of the characteristic light form with distinct abdominal bands. A total of 92 males and 83 females (average F_1 progeny/female = 13.1 male and 10.3 female) were successfully reared to adulthood. As in the previous study with field-collected larvae, all emerging adults were dark brown and the abdominal tergites were entirely dark-scaled. Fourth-instar larvae were essentially identical to those collected

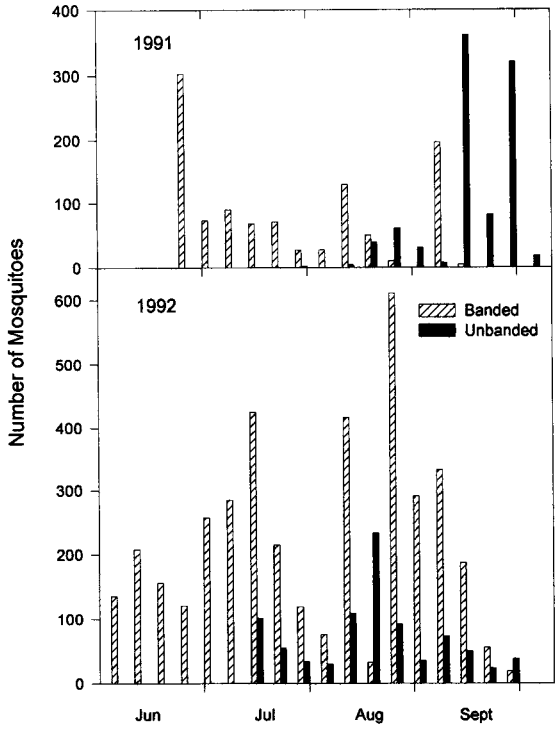

Fig. 3. Total weekly collections of banded and unbanded forms of *Culiseta melanura*, 1991–92.

from the field, and no unusual morphological features were detected.

Population genetic structure: The data matrix consisted of allele frequencies for each form, as shown in Table 2. A total of 2,720 genes were sampled—868 from banded light individuals, 1,136 from nonbanded dark forms, and 716 from banded light specimens from the laboratory colony. The genetic profile of each sample, in terms of average heterozygosity and average numbers of alleles per locus, is presented in Table 3. Genetic distances (Nei's) between each of the sample pairs from the field ranged from 0.005 (Chester light form and Cornwall dark form) to 0.036 (Chester dark form and Killingworth light form). The larger genetic distance indices were equally distributed among light form–light form pairs and light form–dark form pairs. The low magnitude of these values is well within ranges expected between populations of the same species. The laboratory colony showed a substantially reduced level of genetic variability for both average heterozygosity and number of alleles (Table 3); this did not affect the genetic distances, which ranged from 0.010 to 0.027 in pairwise comparisons with each of the field samples.

Voucher specimens: A selection of 4th-instar larval and adult specimens, representing the banded light and unbanded dark morphological variants of *Cs. melanura,* are in the collection of the senior author and will be placed in the entomological collection of the Peabody Museum of Natural History at Yale University, New Haven, CT.

DISCUSSION

This study documents and reaffirms the existence of two distinct morphological variants of adult *Cs. melanura*; a light golden brown form that has distinct basal bands of light-colored scales on the dorsum of the abdomen, and a dark brown form that is entirely dark-scaled with no basal bands on the abdomen (Fig. 2). Both forms occur concurrently and are widely distributed in the freshwater swamp habitats of Connecticut (Fig. 1). However, assuming there is no bias in the atractiveness of the light traps, our collections indicate that the less conventional banded light variant is more common, especially during the early and mid-summer months. This would include most if not all 1st-generation adults that emerge from overwintered larvae. Adults of subsequent generations of this multivoltine mosquito appear to be more variable.

Table 3. Assessment of heterozygosities for 18 isoenzyme loci among 5 geographic/temporal samples of *Culiseta melanura.*

Criterion	Colony (Farmington) light form	Field populations			
		Chester light form	Chester dark form	Cornwall dark form	Killingworth light form
No. of loci examined	18	18	16	18	9
Mean sample size per locus	19.9	17.9	15.0	18.2	12.3
Average heterozygosity (unbiased estimate)	0.074	0.142	0.194	0.128	0.117
Average heterozygosity (direct count)	0.063	0.164	0.179	0.130	0.099
Average no. of alleles/locus	1.39	1.78	1.81	1.83	1.56
Percent loci polymorphic (5% level criterion)	33	33	50	39	33
Percent loci polymorphic (1% level criterion)	33	50	69	56	44

The difference in coloration and associated abdominal banding within *Cs. melanura* populations varies from one generation to the next and from 1 year to another within a given locality. This suggests that the expression of these morphological traits is environmentally induced. The laboratory rearing studies support this conclusion, but the critical environmental factors (e.g., temperature, light, food) have not been determined.

The population genetic profile and comparisons among geographcally distinct populations of *Cs. melanura* represent the first such presentation for any species in the genus *Culiseta.* Substantial population genetic substructuring was not discerned among the morphological phenotypes of this species, in terms of either differential gene frequencies or diagnostic biochemical loci. Rare alleles unique to each population ("private" alleles) were noted in each of the 3 field populations, although this may be sampling artifact due to small sample size. *Culiseta melanura* can be characterized as a species of intermediate vagility, with a documented dispersal of 4–10 km (Howard et al. 1989). Because of the dispersed and specific nature of the larval habitat, migration of individuals (gene flow) is probably restricted. Therefore, the relative homogeneity of the frequencies of polymorphic alleles along with the presence of low-frequency private alleles may be a reflection of intermittent or low levels of dispersal among these habitats.

The reduced genetic variability exhibited by the laboratory colony (Table 3) is not uncommon, particularly in species which are difficult to colonize (Munstermann 1994). In addition to loss of low-frequency alleles that were present in field samples, polymorphisms at certain loci may signal regions of the genome in a state of balanced polymorphism or even balanced lethal systems. However, polymorphisms in the laboratory colony were also polymorphic in the field—*Aat-2, Gpi, Hk-3, Hk-C, Mdh-2, Me,* and *Tre-3* (Table 2). Because studies of *Cs. melanura* biology and transmission dynamics of eastern equine encephalitis have been based on this colony (e.g., Scott et al. 1984), inferences must be tempered by recognizing that it represents a restricted subset of genetic variability extant in field populations.

Clearly, the diagnostic keys to adult females of the genus *Culiseta* currently employed by Darsie and Ward (1981:96–99) cannot reliably identify those female *Cs. melanura* that possess basal bands of light-colored scales on the dorsum of the abdomen. These individuals will be incorrectly identified as *Culiseta impatiens* (Walker), an unrelated but widespread species that similarly inhabits dense forests in the northeastern and north-central United States (Carpenter and LaCasse 1955, Darsie and Ward 1981, Means 1987).

This taxonomic problem is effectively addressed in the keys of Carpenter and LaCasse (1955:82) and Means (1987:46), in which no reference to abdominal banding is made and *Cs. melanura* and *Cs. impatiens* are distinguished by differences in wing venation. We strongly recommend that any future diagnostic key to the genus *Culiseta* be accordingly revised to accommodate this morphologically variable trait in *Cs. melanura,* and, in the interim, we propose the addition of the following couplet for insertion into the key of Darsie and Ward (1981: 99):

8. Points of origin of mediocubital and radiomedial cross veins separated by more than the length of either cross vein ..... *melanura*
– Points of origin of mediocubital and radiomedial cross veins separated by less than the length of either cross vein ..... *impatiens*

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