Electrophoretic Study of Eastern North American *Eurylophella* (Ephemeroptera: Ephemerellidae) with the Discovery of Morphologically Cryptic Species

DAVID H. FUNK, BERNARD W. SWEENEY, AND ROBIN L. VANNOTE

Stroud Water Research Center, Academy of Natural Sciences of Philadelphia, Avondale, Pennsylvania 19311

Ann. Entomol. Soc. Am. 81(2): 174–186 (1988)

ABSTRACT The genus *Eurylophella* includes nine species from eastern North America, one from western North America, and three from Europe. We examined 18 polymorphic enzyme loci in 82 populations (>2,000 individuals) of *Eurylophella* from 40 localities in eastern North America, representing eight of the nine currently recognized eastern species. Our data suggest that *Eurylophella* is actually represented by at least 15 species in eastern North America, some of which cannot be resolved morphologically. Although intraspecific comparisons revealed significant geographic variation in allele frequencies, there were no fixed allelic differences between populations. In contrast, most interspecific comparisons were characterized by the lack of shared alleles at several loci. Thus, >99% of the individuals studied could be sorted to species by electromorph characteristics. The average Nei's genetic distance (D) was 0.02 (range = 0–0.12) between conspecific populations and 0.74 (range = 0.11–1.87) between species. A phenogram generated from D values shows five groups of species branching at $D < 0.60$:

- *E. verisimilis, E. verisimilis-A, E. verisimilis-B, E. verisimilis-C, E. bicolor, E. minimella, E. prudentalis, E. aestiva,* and *E. aestiva-A;*
- *E. temporalis-A, E. temporalis-B,* and *E. temporalis-C;*
- *E.* sp. 1;
- *E. funeralis;* and
- *E. lutulenta.*

Interspecific D values for *Eurylophella* were in the upper range of reported literature values for congeneric insect species, a fact that supports the recent elevation of *Eurylophella* from subgeneric to generic status. Expected heterozygosities ranged from 0.03 to 0.21; those of most species averaged 0.10–0.12.

KEY WORDS Insecta, biochemical genetics, taxonomy, heterozygosity

THE GENUS *Eurylophella* comprises a small group of ephemerellid mayflies whose larvae are often abundant members of the macroinvertebrate fauna of rivers, streams, and some lakes of eastern North America. Although *Eurylophella* is easily recognized, its species are generally difficult to identify as larvae and often impossible to identify as adults (McDunnough 1931, Allen & Edmunds 1963). In recent years enzyme electrophoresis has been used to resolve species and clarify relationships among congeners in many animal groups. However, except in two studies (Saura et al. 1979, Zurwerra et al. 1986), multilocus enzyme electrophoresis has not been applied to ephemeropteran taxonomy. We initially undertook an electrophoretic study of two species (*E. funeralis* and *E. verisimilis*) to assess geographic variation in population structure and to verify our morphologically based concepts of these species (Sweeney et al. 1987). The discovery that *E. verisimilis* is in fact a complex of several species led us to broaden our original scope to include the other eastern species.

Eurylophella was considered a subgenus of *Ephemerella* until Allen (1979) elevated it to generic rank. He recognized 15 species, 11 of which are known from eastern North America, 1 from western North America, and 3 from Europe. Two of the eastern North American species, *E. doris* (Traver) and *E. triltneata* (Berner), subsequently were synonymized with *E. temporalis* (McDunnough) by Berner (1984), leaving a total of nine eastern species. McCafferty (1978) included *Dannella bartoni* (Allen) in *Eurylophella,* but Allen (1979) disagreed and erected a new subgenus within *Dannella* to accommodate *D. bartoni.* This species was not included in the present study for lack of material. We report here the results of an electrophoretic survey of over 2,000 adult *Eurylophella,* including all presently recognized eastern North American species except *E. coxalis* (McDunnough). Our data show clear biochemical differences among all species. We conclude that there are at least 15 species of *Eurylophella* in eastern North America including 7 undescribed species. A thorough morphological treatment including descriptions and a key is in preparation.

Materials and Methods

Mayfly larvae were collected from 40 locations in eastern North America (Table 1; Fig. 1). Larvae were returned alive to our laboratory and reared

0013-8746/88/0174-0186$02.00/0

Table 1. Site codes, latitudes, longitudes, and locality names for sampling sites where species of *Eurylophella* were collected, including total number of individuals run electrophoretically

Site code	Latitude	Longitude	Locality	Species[a]														
				EV	EVA	EVB	EVC	EM	EB	EF	ETA	ETB	ETC	EP	EA	EAA	EL	ES1
QU1	50°19′35″N	65°57′33″W	Trapper Cabin Creek	—	—	—	—	—	—	30	—	—	—	—	—	—	—	—
QU2	50°18′41″N	65°57′10″W	Beaver Creek	1	—	—	—	—	—	30	—	—	—	—	—	—	—	—
QU3	50°16′15″N	65°37′33″W	Pigou River	57	—	—	—	—	—	—	—	—	—	1	—	—	—	—
ME1	46°03′13″N	68°26′36″W	Crystal Brook	29	1	—	—	—	—	—	—	—	—	—	—	—	—	—
ME2	45°54′13″N	69°02′16″W	Nesowadnehunk Stream, trib	—	—	—	—	—	—	30	—	—	—	—	—	—	—	—
ME4	45°51′50″N	68°31′23″W	Swift Brook	2	—	23	—	—	—	—	—	—	—	—	—	—	—	—
NH1	43°56′43″N	71°42′08″W	Norris Brook	—	—	—	—	—	—	26	—	—	—	—	—	—	—	—
VT5	43°16′35″N	73°00′16″W	Emerald Lake	—	—	—	—	—	—	—	—	—	—	—	—	—	37	—
VT2	43°13′47″N	73°07′11″W	Goodman Brook	—	—	—	—	—	—	30	—	—	—	—	—	—	—	—
VT3	43°06′04″N	73°14′31″W	Battenkill River	30	1	24	—	—	3	—	—	—	—	—	—	—	—	—
VT6	43°05′52″N	73°08′31″W	Battenkill River	—	—	—	—	—	—	—	—	—	—	35	—	—	—	—
NY1	42°10′31″N	75°01′02″W	West Branch Delaware River	36	1	1	—	—	—	—	—	—	—	—	—	—	—	—
NY2	42°09′18″N	74°37′30″W	East Branch Delaware River	30	—	—	—	—	—	—	—	—	—	—	—	—	—	—
NY4	42°04′38″N	75°24′21″W	West Branch Delaware River	30	—	—	—	—	—	—	—	—	—	—	—	—	—	—
NY5	42°04′19″N	75°00′25″W	East Branch Delaware River	59	21	4	—	—	—	1	—	—	—	—	—	—	—	—
NY6	42°01′30″N	75°07′14″W	East Branch Delaware River	18	—	30	—	—	1	—	—	—	—	—	4	—	—	—
NY7	42°00′10″N	75°23′03″W	West Branch Delaware River	30	—	—	—	—	—	—	—	—	—	—	—	—	—	—
NY3	41°58′18″N	75°02′23″W	Beaverkill River	29	—	36	—	2	1	—	—	—	—	—	—	—	—	—
PA5	41°54′23″N	75°20′00″W	Starlight Lake	—	—	—	—	—	—	—	30	—	5	—	—	—	—	—
PA6	41°52′02″N	75°15′50″W	Delware River	6	—	28	—	2	40	—	—	—	—	—	30	—	—	—
PA8	41°49′11″N	75°56′00″W	Wyalusing Creek, trib	8	26	—	—	—	—	—	—	—	—	—	—	—	—	—
PA1	41°45′27″N	75°44′46″W	Nine Partners Creek	—	—	—	—	—	—	55	—	—	—	—	—	—	—	—
PA2	41°36′45″N	76°00′58″W	Meshoppen Creek	40	—	25	—	10	30	—	—	—	—	—	30	—	—	—
PA7	41°22′46″N	75°17′43″W	Lake Lacawac	—	—	—	—	—	—	—	48	—	2	—	—	—	—	—
PA4	39°51′47″N	75°47′07″W	White Clay Creek	146	—	—	—	—	—	31	—	—	—	—	—	54	—	—
DE1	39°42′58″N	75°45′53″W	White Clay Creek, trib	—	—	—	—	—	—	59	—	—	—	—	—	—	—	—
DE2	39°21′18″N	75°40′55″W	Blackbird Creek	30	—	—	—	—	—	—	—	36	—	—	29	—	—	—
DE3	39°00′37″N	75°31′47″W	Pratt's Branch	38	—	—	—	—	—	—	—	—	—	—	—	—	—	—
VA2	38°45′51″N	78°02′04″W	Jordan River	35	—	—	—	—	—	—	—	—	—	—	5	—	—	—
DE4	38°33′06″N	75°19′18″W	Sheep Pen Ditch	—	—	—	—	—	—	—	—	—	—	33	—	—	—	—
VA3	37°28′28″N	78°39′27″W	Slate River	30	—	—	—	—	—	30	—	—	—	30	—	—	—	—
VA4	37°23′22″N	79°33′05″W	Big Otter River	29	—	—	—	—	—	1	—	—	—	—	—	—	—	—
VA5	37°18′41″N	80°30′59″W	Sinking Creek	—	—	—	34	—	—	—	—	—	—	—	—	—	—	—
NC3	36°08′21″N	79°10′13″W	West Fork Eno River	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
NC1	36°07′49″N	79°10′33″W	West Fork Eno River	30	—	—	—	—	—	—	—	—	—	—	—	—	—	—
NC2	35°38′30″N	79°58′00″W	Uwharrie River	18	—	—	—	—	4	—	—	35	—	—	3	—	—	—
SC2	34°54′53″N	83°04′20″W	Cranes Creek	—	—	—	—	—	—	34	—	—	—	—	—	—	—	—
GA1	34°40′24″N	83°21′17″W	Panther Creek	30	—	—	—	—	—	—	—	6	—	—	—	—	—	—
SC4	34°25′29″N	81°36′18″W	Indian Creek	—	—	—	—	—	—	—	—	36	—	8	—	—	—	—
SC3	33°59′55″N	82°23′01″W	Horton Creek	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—
				801	50	171	34	14	79	357	78	113	7	107	101	54	37	1

[a] EV, *E. verisimilis;* EVA, *E. verisimilis-A;* EVB, *E. verisimilis-B;* EVC, *E. verisimilis-C;* EM, *E. minimella;* EB, *E. bicolor;* EF, *E. funeralis;* ETA, *E. temporalis-A;* ETB, *E. temporalis-B;* ETC, *E. temporalis-C;* EP, *E. prudentalis;* EA, *E. aestiva;* EAA, *E. aestiva-A;* EL, *E. lutulenta;* ES1, *E.* sp. 1.

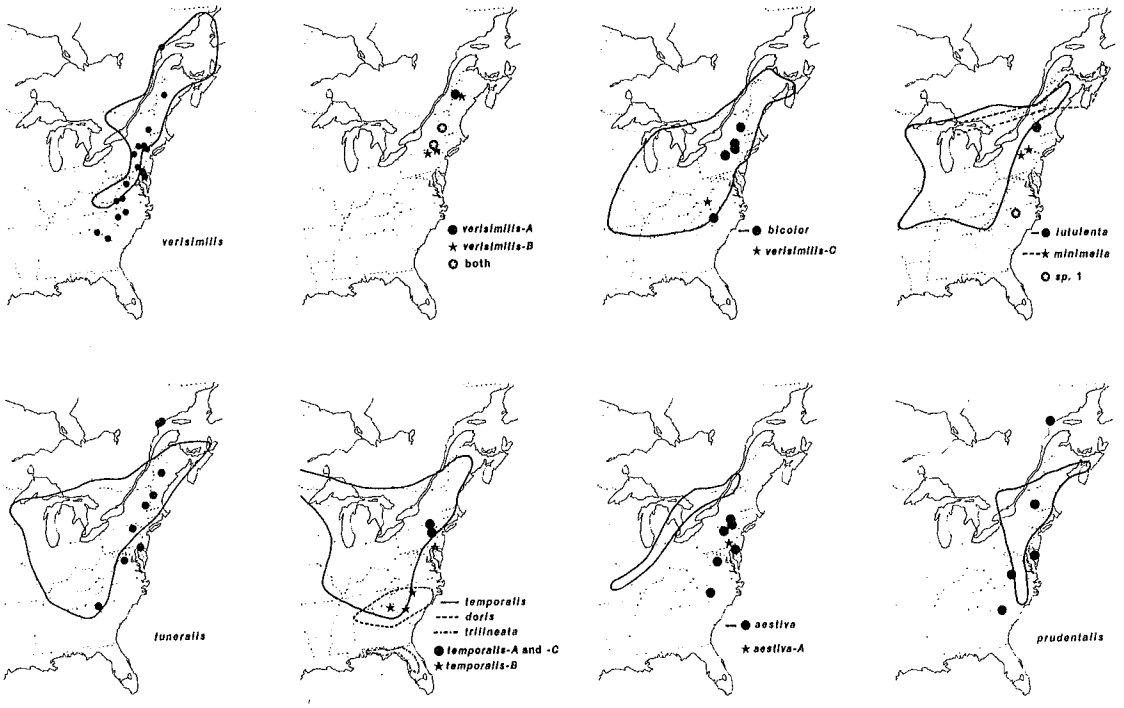

Fig. 1. Geographic distribution of several eastern North American species of *Eurylophella* as delimited in Allen & Edmunds (1963) and the location of populations (symbols) tested electrophoretically in this study.

to subimagines in flow-through polypropylene trays (23 by 45 by 22 cm deep), which were checked several times per day during the emergence period. Subimagines were then reared to imagines (hereafter called adults) in small (1 liter) freezer containers with screening on top. All associated larval exuviae were preserved in 80% EtOH and referenced individually (whenever possible) to frozen adults. Genitalia were removed from many of the males and preserved with exuviae. When two or more individuals emerged together, exuviae were referenced by groups. When mixtures of species were present, only adults individually associated with exuviae were electrophoresed. All of the preserved material is deposited at the Stroud Water Research Center of the Academy of Natural Sciences of Philadelphia.

Adults were stored individually at −60°C until electrophoresed. Allozymes were separated by horizontal starch gel electrophoresis using methods described in Sweeney et al. (1986, 1987). A total of 34 enzyme systems was screened. Of these, 24 were scorable in at least one species and yielded data on 32 presumptive gene loci (see Table 5 in Sweeney et al. [1987] for full enzyme names and enzyme commission numbers and buffer systems used). The following loci could not be reliably scored in one or more *Eurylophella* species because of insufficient activity on gels (i.e., too faint to read) or uninterpretable banding patterns, and so were eliminated from the present analysis: *Aat-1, Aat-2, Ao, Aph, Hbd, Est-1, Est-2, Est-3, Gdh, Isdh-2, Lap-1, Ldh, Me,* and *Xdh. Adk* was easily scored but was not "discovered" until late in the study. The remaining 18 loci (*Mdh-1, Mdh-2, a-Gpdh, Hex, Sod-1, Sod-2, Gpi, Pgm, 6pgd, G6pdh, Isdh-1, Mpi, Acp, Ald, G3pdh, Lap-2, Est-5, Est-4*) could be scored unambiguously in all 15 species, and all were polymorphic in at least 1 species.

Expected heterozygosity, or average gene diversity (*Hexp*), was defined and calculated as described by Nei (1978). Unbiased estimates of genetic distance (*D*) and identity (*I*) were calculated according to Nei (1978) and used to construct phenograms by the unweighted pair-group method of cluster analysis (UPGMA) (Sneath & Sokal 1973).

Results

Discovery of New (or Presently Unrecognized) Species

Eurylophella verisimilis. Our sampling of *E. verisimilis* at eight locations on the upper Delaware River in New York revealed strikingly different electromorphs in one population and a severe deficiency of heterozygotes at certain loci. About 25% (21 out of 84) of individuals from site NY5 formed a distinct group (hereafter referred to as *E. verisimilis-A) characterized by fixed allelic substitutions at two loci (Sod-1* and *6pgd)* and nearly fixed differences at four other loci (*Mdh-1, Pgm, G6pdh,* and *Acp;* Table 2).

Larvae that McDunnough (1930) described as *E. verisimilis* were not reared and were not des-

Table 2. Allele frequencies for 15 species of *Eurylophella* averaged by species, including 1,426 individuals from 52 populations

Species:[a]	EV	EVA	EVB	EVC	EM	EB	EF	ETB	ETA	ETC	EP	EA	EAA	EL	ES1
n:	563	47	143	34	14	77	55	113	78	7	106	97	54	37	1
No. pops:	16	2	5	1	3	4	1	4	2	2	4	5	1	1	1
Locus															
Mdh-1															
1.30	0.02	—	0.01	—	—	—	—	—	—	—	—	—	—	—	—
1.00	0.96	0.01	0.99	0.94	1.0	0.71	0.98	—	1.0	1.0	1.0	0.01	—	—	—
0.93	—	0.01	—	—	—	—	—	—	—	—	—	*[b]	—	—	—
0.73	0.02	—	—	—	—	—	—	—	—	—	*	—	—	—	1.0
0.66	*	0.98	*	0.06	—	0.27	0.02	0.99	—	—	—	0.99	1.0	1.0	—
0.51	—	—	—	—	—	0.02	—	—	—	—	—	*	—	—	—
0.35	—	—	—	—	—	—	—	0.01	—	—	—	—	—	—	—
Mdh-2															
1.00	1.0	1.0	0.91	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.81	1.0	1.0
0.50	—	—	0.09	—	—	—	—	—	—	—	—	—	0.19	—	—
a-Gpdh															
1.39	*	—	—	—	—	—	—	—	—	—	0.05	*	—	—	—
1.24	—	—	—	—	—	—	—	0.01	—	—	—	—	—	—	—
1.20	*	—	—	—	—	0.17	—	—	—	—	*	—	—	—	—
1.14	—	—	0.01	—	—	—	—	—	—	—	—	—	—	1.0	—
1.08	*	—	—	—	—	—	—	—	—	—	—	0.02	—	—	—
1.00	0.97	1.0	0.98	1.0	1.0	0.83	1.0	0.98	1.0	1.0	0.95	0.98	1.0	—	1.0
0.78	0.03	—	0.01	—	—	—	—	—	—	—	—	—	—	—	—
0.77	—	—	—	—	—	—	—	0.01	—	—	—	—	—	—	—
Hex															
1.06	0.02	—	0.02	—	—	—	—	—	—	—	—	—	—	—	—
1.04	—	—	—	—	—	—	—	—	—	—	—	—	—	1.0	—
1.03	0.06	0.01	—	—	—	—	—	—	—	—	—	—	—	—	—
1.00	0.92	0.98	0.95	1.0	1.0	1.0	1.0	*	—	—	1.0	1.0	1.0	—	1.0
0.97	—	—	—	—	—	—	—	—	0.01	—	—	—	—	—	—
0.96	—	—	—	—	—	—	—	0.99	0.99	1.0	*	—	—	—	—
0.94	—	0.01	0.03	—	—	—	—	—	—	—	—	—	—	—	—
Sod-1															
1.33	0.02	—	0.01	0.46	—	0.01	—	—	—	—	0.01	0.01	0.02	—	—
1.30	—	0.01	—	—	—	—	—	—	—	—	—	—	—	1.0	—
1.25	—	—	—	—	—	—	0.01	—	0.14	—	—	—	—	—	—
1.00	0.98	—	0.98	—	—	—	—	—	—	—	—	0.02	0.02	—	—
0.96	—	0.99	—	0.54	1.0	0.99	0.97	0.97	0.86	1.0	0.99	0.95	0.96	—	1.0
0.72	—	—	0.01	—	—	—	—	0.03	—	—	—	—	—	—	—
0.48	—	—	—	—	—	—	0.02	—	—	—	—	0.02	—	—	—
Sod-2															
2.00	—	—	—	—	—	—	—	—	—	—	—	—	—	1.0	—
1.82	—	—	—	0.19	—	—	—	—	—	—	—	—	—	—	—
1.00	1.0	1.0	1.0	0.81	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	—	1.0
Gpi															
1.42	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.0
1.28	0.05	—	—	—	—	0.01	0.01	0.01	—	1.0	*	—	—	—	—
1.24	*	—	—	—	—	—	—	0.01	—	—	—	—	—	—	—
1.18	—	0.01	—	—	—	0.03	—	—	—	—	—	—	—	—	—
1.08	—	—	—	—	—	—	—	0.01	—	—	—	—	—	0.01	—
1.03	—	—	—	—	—	—	0.99	—	—	—	—	—	—	—	—
1.00	0.95	0.95	0.97	1.0	—	0.89	—	0.96	1.0	—	0.68	0.01	—	0.99	—
0.77	—	—	—	—	—	—	—	0.01	—	—	0.32	—	—	—	—
0.74	*	—	0.01	—	—	—	—	—	—	—	—	—	—	—	—
0.72	—	0.04	—	—	0.55	0.07	—	—	—	—	—	0.99	1.0	—	—
0.52	*	—	0.02	—	—	—	—	—	—	—	—	—	—	—	—
0.47	—	—	—	—	0.45	—	—	—	—	—	—	—	—	—	—
Pgm															
1.09	0.01	0.06	—	—	—	—	—	—	—	—	—	—	—	—	—
1.08	0.08	—	0.01	—	—	—	—	—	—	—	—	—	—	—	—
1.04	0.01	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1.03	—	—	—	—	—	—	—	0.11	—	—	0.03	—	—	1.0	—
1.02	—	0.90	—	0.01	—	0.09	—	—	—	—	—	—	—	—	—
1.00	0.85	0.03	0.95	—	—	—	—	0.01	0.33	—	—	—	—	—	—
0.96	—	—	—	—	—	0.88	—	—	—	—	—	—	—	—	—
0.95	0.03	—	0.03	0.19	—	—	—	0.88	—	1.0	0.94	—	—	—	—
0.94	—	—	—	—	0.10	—	0.05	—	—	—	—	0.02	—	—	—

Table 2. Continued

Species:[a]	EV	EVA	EVB	EVC	EM	EB	EF	ETB	ETA	ETC	EP	EA	EAA	EL	ES1
n:	563	47	143	34	14	77	55	113	78	7	106	97	54	37	1
No. pops:	16	2	5	1	3	4	1	4	2	2	4	5	1	1	1
Locus															
0.93	0.02	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.91	—	—	0.01	—	—	—	—	—	—	—	—	—	—	—	—
0.90	—	—	—	—	—	—	—	—	0.65	—	—	—	—	—	—
0.89	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.0
0.88	—	0.01	—	0.80	—	—	—	—	—	—	—	—	—	—	—
0.87	—	—	—	—	0.80	0.03	0.95	*	0.02	—	0.03	0.01	0.09	—	—
0.83	—	—	—	—	—	—	—	—	—	—	—	0.01	0.04	—	—
0.78	—	—	—	—	0.08	—	—	—	—	—	—	0.74	0.72	—	—
0.75	—	—	—	—	—	—	—	—	—	—	—	0.02	—	—	—
0.71	—	—	—	—	—	—	—	—	—	—	—	0.02	—	—	—
0.68	—	—	—	—	0.02	—	—	—	—	—	—	0.16	0.15	—	—
0.65	—	—	—	—	—	—	—	—	—	—	—	0.02	—	—	—
6pgd															
1.30	—	—	—	—	—	—	0.99	0.95	—	—	—	—	—	—	1.0
1.00	0.99	—	1.0	1.0	0.08	0.98	—	—	—	—	1.0	0.99	0.99	—	—
0.55	—	—	—	—	—	—	0.01	—	—	—	—	—	—	—	—
0.54	*	—	*	—	—	—	—	0.01	—	—	—	—	—	—	—
0.52	—	—	—	—	—	—	—	—	—	—	—	—	—	1.0	—
0.30	—	—	—	—	—	—	—	0.04	1.0	1.0	—	—	—	—	—
0.25	*	—	—	—	—	—	—	—	—	—	—	0.01	0.01	—	—
0.08	*	1.0	—	—	0.92	0.02	—	—	—	—	—	—	—	—	—
G6pdh															
1.54	—	0.01	—	—	—	—	—	—	—	—	—	—	—	—	—
1.17	*	0.99	—	—	0.25	*	—	—	—	—	*	—	—	—	—
1.00	0.88	—	0.02	—	0.75	0.99	0.99	*	—	0.13	0.61	0.97	—	1.0	—
0.94	—	—	—	—	—	—	—	—	—	—	—	—	0.01	—	—
0.88	—	—	—	—	—	—	—	—	—	—	—	—	0.99	—	—
0.81	0.12	—	0.98	0.99	—	*	0.01	0.99	1.0	0.87	0.34	0.03	—	—	1.0
0.67	—	—	*	0.01	—	—	—	—	—	—	0.05	—	—	—	—
0.62	—	—	—	—	—	—	—	0.01	—	—	—	—	—	—	—
Isdh-1															
1.00	0.95	1.0	1.0	1.0	—	1.0	—	1.0	1.0	0.95	0.02	—	1.0	—	—
0.97	—	—	—	—	—	—	—	—	—	—	—	—	—	0.95	1.0
0.84	0.05	—	—	—	—	—	1.0	—	—	0.05	0.98	0.98	—	—	—
0.75	—	—	—	—	1.0	—	—	—	—	—	—	—	—	—	—
0.68	—	—	—	—	—	—	—	—	—	—	—	—	—	0.05	—
0.51	—	—	—	—	—	—	—	—	—	—	—	0.02	—	—	—
Mpi															
1.29	—	—	—	—	—	—	—	—	—	—	0.02	—	—	—	—
1.19	—	0.07	*	0.01	—	—	—	—	—	0.13	0.88	—	—	—	—
1.16	—	—	—	—	—	—	—	0.01	—	—	—	—	—	—	—
1.12	0.01	—	—	—	—	—	—	—	—	—	0.09	—	—	—	—
1.10	0.02	0.02	0.14	0.22	—	0.03	—	—	—	—	0.01	—	—	—	—
1.06	—	—	—	—	—	—	—	0.06	—	0.87	—	—	—	—	—
1.02	—	—	—	—	—	—	1.0	—	—	—	—	—	—	0.03	—
1.00	0.95	0.91	0.74	0.75	0.02	0.97	—	—	0.89	—	—	0.03	—	—	1.0
0.95	—	—	—	—	—	—	—	0.91	—	—	—	—	—	—	—
0.94	—	—	—	—	0.98	—	—	—	—	—	—	—	—	—	—
0.92	—	—	—	—	—	—	—	—	0.11	—	—	—	—	0.96	—
0.89	0.01	—	0.12	—	—	*	—	—	—	—	—	0.93	0.90	—	—
0.85	—	—	—	—	—	—	—	0.01	—	—	—	—	—	—	—
0.81	0.01	—	*	—	—	—	—	—	—	—	—	—	—	0.01	—
0.78	—	—	—	—	—	—	—	—	—	—	—	0.04	0.05	—	—
0.74	—	—	—	—	—	—	—	0.01	—	—	—	—	—	—	—
0.72	—	—	—	—	—	—	—	—	—	—	—	—	0.05	—	—
Acp															
2.33	—	—	—	—	—	0.01	—	—	—	—	—	—	—	—	—
1.75	*	—	—	—	0.08	—	0.98	—	—	—	—	0.05	—	—	—
1.38	*	0.68	0.19	0.09	—	—	—	—	—	—	*	0.89	0.94	—	—
1.00	0.96	0.29	0.64	0.91	—	0.98	0.02	0.98	1.0	1.0	*	—	—	—	—
0.81	—	—	—	—	0.92	—	—	—	—	—	0.94	0.06	—	—	—
0.65	—	—	—	—	—	—	—	—	—	—	—	—	—	1.0	—
0.57	—	—	—	—	—	—	—	—	—	—	—	—	0.06	—	—
0.36	0.04	0.03	0.17	—	—	0.01	—	0.02	—	—	0.06	—	—	—	1.0
Ald															
1.05	—	—	—	—	—	—	—	—	—	—	—	—	—	1.0	1.0

Table 2. Continued

Species:[a]	EV	EVA	EVB	EVC	EM	EB	EF	ETB	ETA	ETC	EP	EA	EAA	EL	ES1
n:	563	47	143	34	14	77	55	113	78	7	106	97	54	37	1
No. pops:	16	2	5	1	3	4	1	4	2	2	4	5	1	1	1
Locus															
1.00	0.84	0.99	0.98	—	1.0	1.0	—	—	0.01	0.13	1.0	1.0	1.0	—	—
0.75	—	—	—	—	—	—	—	1.0	0.99	0.87	—	—	—	—	—
0.70	0.16	0.01	0.02	1.0	—	—	1.0	—	—	—	—	—	—	—	—
G3pdh															
1.00	1.0	1.0	1.0	0.22	1.0	1.0	1.0	1.0	1.0	1.0	0.93	1.0	1.0	1.0	1.0
0.71	—	—	—	0.77	—	—	—	—	—	—	0.07	—	—	—	—
0.55	—	—	—	0.01	—	—	—	—	—	—	—	—	—	—	—
Lap-2															
1.07	—	—	—	—	—	—	—	—	—	—	0.05	—	—	—	—
1.06	0.02	0.05	0.03	—	—	—	—	—	—	0.43	—	0.02	—	—	—
1.04	—	—	—	—	—	0.04	—	—	—	—	—	—	—	—	—
1.00	0.88	0.88	0.97	0.56	0.98	0.87	—	0.96	1.0	0.47	0.88	0.87	0.61	1.0	1.0
0.96	—	—	—	0.44	0.02	0.01	—	0.04	—	0.10	0.07	—	—	—	—
0.93	0.09	0.07	—	—	—	0.08	0.04	—	—	—	—	0.11	0.19	—	—
0.89	0.01	—	—	—	—	—	0.96	—	—	—	*	—	0.19	—	—
0.79	—	—	—	—	—	—	—	—	—	—	—	—	0.01	—	—
Est-5															
0.51	—	—	—	—	—	—	—	—	—	—	—	—	—	0.27	—
0.47	—	—	—	—	—	—	—	0.07	—	—	0.04	—	—	—	—
0.44	—	—	—	—	—	—	—	—	—	—	—	—	—	0.73	—
0.43	0.03	—	—	—	0.48	0.02	0.12	—	—	—	—	—	—	—	0.50
0.40	*	—	—	—	—	—	—	0.52	0.97	—	—	—	—	—	—
0.38	0.01	—	—	—	0.20	—	—	—	—	—	—	—	—	—	—
0.36	0.12	0.11	0.01	—	0.30	0.16	0.58	—	—	—	0.06	0.07	0.06	—	0.50
0.34	—	—	—	—	—	—	—	0.41	0.03	—	*	—	—	—	—
0.32	0.07	—	0.70	0.01	—	—	—	—	—	—	—	—	—	—	—
0.30	0.42	0.79	—	—	0.02	0.73	0.08	—	—	0.95	0.43	—	—	—	—
0.29	—	—	—	—	—	—	—	—	—	—	—	0.23	0.05	—	—
0.25	0.02	—	0.28	0.40	—	0.04	0.14	—	—	—	—	0.03	—	—	—
0.23	0.33	0.10	—	—	—	0.04	0.08	—	—	0.05	0.42	0.20	0.24	—	—
0.22	—	—	—	—	—	—	—	—	—	—	—	0.09	—	—	—
0.21	*	—	—	—	—	—	—	—	—	—	—	0.02	—	—	—
0.20	—	—	0.01	0.52	—	—	—	—	—	—	—	—	—	—	—
0.17	*	—	—	—	—	0.01	—	—	—	—	0.05	0.26	0.52	—	—
0.13	—	—	—	0.07	—	—	—	—	—	—	—	—	—	—	—
0.10	—	—	—	—	—	—	—	—	—	—	—	0.10	0.13	—	—
Est-4															
0.65	—	—	—	—	—	—	—	0.01	—	—	*	—	—	—	—
0.61	—	—	—	—	—	—	—	0.12	—	—	—	—	—	—	—
0.59	—	—	—	—	—	—	—	0.02	0.10	—	—	—	—	—	—
0.57	—	—	—	—	—	—	—	0.64	—	—	—	—	—	—	—
0.55	*	—	—	—	—	0.01	—	—	*	0.57	—	—	—	—	—
0.53	—	—	—	—	—	—	0.36	—	—	—	—	—	—	—	—
0.52	0.01	—	—	0.03	—	*	—	0.20	0.90	0.43	—	—	—	—	—
0.51	0.05	—	—	0.13	—	—	—	—	—	—	—	0.03	0.28	—	—
0.50	0.20	0.02	0.03	0.10	—	0.07	—	—	—	—	—	—	0.05	—	—
0.48	—	—	—	—	—	—	0.28	0.01	—	—	—	—	—	—	—
0.47	0.04	—	—	0.36	—	0.34	—	—	—	—	—	0.08	0.01	1.0	—
0.45	0.45	0.46	0.72	—	1.0	0.43	—	—	—	—	—	*	0.04	—	—
0.43	—	—	—	—	—	—	0.36	—	—	—	—	—	—	—	—
0.42	0.09	—	—	0.23	—	0.13	—	—	—	—	0.03	0.22	0.12	—	—
0.41	—	—	—	—	—	—	—	—	—	—	0.09	—	—	—	—
0.40	0.10	0.04	0.23	—	—	0.01	—	—	—	—	—	0.02	0.01	—	—
0.39	—	—	—	—	—	—	—	—	—	—	0.08	—	—	—	—
0.37	0.05	—	—	0.12	—	—	—	—	—	—	0.75	0.61	0.41	—	—
0.35	—	0.43	0.01	—	—	—	—	—	—	—	—	—	—	—	—
0.34	—	—	—	—	—	—	—	—	—	—	0.05	0.02	—	—	—
0.33	*	—	*	0.03	—	—	—	—	—	—	—	—	—	—	—
0.31	—	—	—	—	—	—	—	—	—	—	—	0.02	—	—	—
0.30	—	0.05	—	—	—	—	—	—	—	—	—	—	—	—	—
0.25	—	—	—	—	—	—	—	—	—	—	—	—	0.08	—	—
0.10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.0

[a] EV, *E. verisimilis*; EVA, *E. verisimilis-A*; EVB, *E. verisimilis-B*; EVC, *E. verisimilis-C*; EM, *E. minimella*; EB, *E. bicolor*; EF, *E. funeralis*; ETA, *E. temporalis-A*; ETB, *E. temporalis-B*; ETC, *E. temporalis-C*; EP, *E. prudentalis*; EA, *E. aestiva*; EAA, *E. aestiva-A*; EL, *E. lutulenta*; ES1, *E.* sp. 1.

[b] *, <0.01.

Table 3. Observed and expected *G6pdh* genotype frequencies for *Eurylophella* from DE3 and the Delaware River[a]

Genotype	DE3		Delaware River					
	verisimilis $n = 30$		Unsorted *"verisimilis"* $n = 354$		*verisimilis* $n = 260$		*verisimilis*-B $n = 94$	
	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected
1.0/1.0	0.47	0.46	0.68	0.49	0.94	0.94	0	<0.01
1.0/0.81	0.43	0.44	0.04	0.42	0.06	0.06	0.02	0.02
0.81/0.81	0.10	0.10	0.28	0.09	<0.01	<0.01	0.98	0.98
Departure from Hardy-Weinberg equilibrium (χ^2)	NS[b]		$P < 0.005$		NS[b]		NS[b]	

[a] Delaware River populations NY1–7 and PA6 pooled for this analysis. Delaware River populations are shown two ways: as *"verisimilis"* (unsorted, including *E. verisimilis* and *E. verisimilis*-B) and as the same individuals sorted into *verisimilis* and *verisimilis*-B by morphological characters described in text. DE3 population of *E. verisimilis* is used for comparison because allele frequencies for *G6pdh* were similar to unsorted *"verisimilis"* data for Delaware River sites.

[b] Not significant.

ignated types. Unless or until morphological characters are found to distinguish reliably all members of the *E. verisimilis* complex in the adult stage (see Discussion), we cannot be certain of this association. Lacking evidence to the contrary, we assume McDunnough (1930) was correct and that these specimens represent the true *E. verisimilis*. McDunnough's (1930) specimens have well-developed head tubercles in both sexes, whereas *E. verisimilis-A* individuals are characterized by lack of head tubercles in male larvae and relatively small head tubercles in female larvae. We also found *E. verisimilis-A* at four other sites (Table 1).

After removing *E. verisimilis-A* individuals from the data set for all sites on the Delaware River, there was still a severe deficiency of heterozygotes at the *G6pdh* locus for *E. verisimilis* (χ^2 test, $P < 0.005$, see Table 3). This was especially notable because none of 12 other *E. verisimilis* populations distributed throughout eastern North America (Georgia to Quebec) exhibited significant departures from Hardy-Weinberg equilibrium at this locus, even though allele frequencies differed significantly from site to site (Sweeney et al. 1987). Examination of the preserved larval exuviae associated with these adults revealed that individuals from the Delaware River that were homozygous for allele 0.81 at *G6pdh* had smaller head tubercles (hereafter referred to as *E. verisimilis-B*) than individuals homozygous for allele 1.00 or heterozygous for alleles 1.00 and 0.81. Subsequent tests on individuals sorted according to head tubercle size prior to electrophoresis confirmed this correlation for the Delaware River population as well as populations in Maine, Vermont, and Pennsylvania (sites ME4, VT3, and PA2, respectively). Thus, 96% of *E. verisimilis-B* individuals were homozygous for the 0.81 allele compared with less than 1% of the *E. verisimilis* individuals found sympatrically. Of 171 *E. verisimilis-B* individuals tested, none was homozygous for the common *E. verisimilis* allele (1.00) at the *G6pdh* locus.

Although factors such as inbreeding, presence of null alleles, and assortative mating can cause heterozygote deficiencies, in this case a mixture of individuals from two species that had significantly different allele frequencies (referred to as the Wahlund effect) caused the apparent deficiency. No significant deficiencies of heterozygotes were observed at the *G6pdh* locus once the two species were distinguished (Table 3). The concordance of electrophoretic and morphological evidence justifies our treatment of these two groups as distinct species—*E. verisimilis* and *E. verisimilis-B* (the latter comprised largely of individuals homozygous for allele 0.81 at *G6pdh*). Significant and consistent differences in allele frequencies at three other loci (*Mpi, Acp, Est-5*) give additional support to our conclusions (Table 2). Compared with *E. verisimilis-A*, *E. verisimilis-B* was nearly fixed for alternate alleles at four loci (*Mdh-1, Pgm, 6pgd, G6pdh*) and shared no common alleles at two others (*Sod-1, Est-5*). Although larvae of *E. verisimilis-A* have relatively longer submedian tubercles on abdominal tergites five through seven than *E. verisimilis-B*, we have been unable to distinguish the two species consistently except by electrophoresis.

Another cryptic species resembling *E. verisimilis* was discovered in Sinking Creek, Va. (VA5), and is here referred to as *E. verisimilis-C*. The common allele for *E. verisimilis* was absent in this population at six loci (*Sod-1, Pgm, G6pdh, Ald, Est-5*, and *Est-4*), and there were large differences in allele frequency at another four loci (*Sod-2, Mpi, G3pdh*, and *Lap-2*; Table 2). As with *E. verisimilis-A* and *E. verisimilis-B*, *E. verisimilis-C* larvae could be distinguished morphologically from *E. verisimilis* on the basis of their smaller head tubercles. Compared with *E. verisimilis-A*, *E. verisimilis-C* had fixed allelic differences at three loci (*6pgd, G6pdh, Ald*), shared no common alleles at three loci (*Pgm, Est-5, Est-4*), and had large differences in frequency at four others (*Sod-1, Acp, G3pdh, Lap-2*). Compared with *E. verisimilis-B*, *E. verisimilis-C* was fixed for an alternate allele at one locus (*Ald*), shared no common alleles at three loci (*Sod-1, Pgm, Est-4*), and had large frequency differences at two loci (*G3pdh, Lap-2*). *E. verisimi-*

lis-C showed minor morphological differences from both *E. verisimilis-A* and *E. verisimilis-B*, with closer similarities to the former, but so far we have found no reliable morphological characters to separate these three species from each other.

Eurylophella aestiva. *E. aestiva* was sampled from seven locations (Table 1; Fig. 1). Allele frequencies were reasonably uniform among populations except at PA4. This population, hereafter referred to as *E. aestiva-A*, shared no alleles with other populations of *E. aestiva* at two loci (*G6pdh* and *Isdh-1*) and had unique alleles that were fairly common (0.19) at two other loci (*Mdh-2* and *Lap-2*; Table 2). Morphologically, *E. aestiva-A* larvae from PA4 appear identical to *E. aestiva* from the other six localities.

Eurylophella temporalis. We found three electrophoretically distinct groups of individuals resembling *E. temporalis* (Table 2; Fig. 1). Four populations from streams in Delaware, North Carolina, Georgia, and South Carolina (sites DE2, NC2, GA1, and SC4, respectively), hereafter referred to as *E. temporalis-B*, formed a coherent group electrophoretically and morphologically. Two populations from lakes in Pennsylvania (sites PA5 and PA7), hereafter referred to as *E. temporalis-A*, shared no alleles with the four *E. temporalis-B* populations at three loci (*Mdh-1*, *Pgm*, and *Mpi*) and were fixed for an allele that was rare (<0.05%) in *E. temporalis-B* at one other locus (*6pgd*). Morphologically, larvae of *E. temporalis-A* can be distinguished from *E. temporalis-B* by their relatively short posterolateral projections on abdominal segments 2 and 3, and their smaller (but variable) head tubercles. A third group, *E. temporalis-C*, was found co-existing with *E. temporalis-A* at lake sites PA5 and PA7, although it was far less abundant. Compared with *E. temporalis-A*, *E. temporalis-C* was fixed for an alternate allele at loci *Gpi* and *Pgm*, shared no alleles at loci *Mpi* and *Est-5*, and had large differences in allele frequencies at loci *Lap-2* and *Est-4*. Compared with *E. temporalis-B*, *E. temporalis-C* was fixed for an alternate allele at locus *Mdh-1*, fixed for an allele that was rare (<0.05%) in *E. temporalis-B* at loci *Gpi* and *6pgd*, shared no alleles at locus *Est-5*, and differed significantly in allele frequency at loci *Mpi* and *Est-4*. We have not been able to distinguish *E. temporalis-C* morphologically from *E. temporalis-A*.

McDunnough (1931) described *E. temporalis* larvae from lakes in Ontario and Quebec. Site NC2 is the type locality for *Eurylophella doris* (Traver), which was synonymized with *E. temporalis* (McDunnough) by Berner (1984). Morphologically, *E. temporalis-B* individuals bear a closer resemblance to McDunnough's (1931) material than do *E. temporalis-A* or *E. temporalis-B*. However, it is unclear which, if any, of the three taxa in this study represents McDunnough's (1924) original *E. temporalis*.

***Eurylophella* sp. 1 (near *E. coxalis*).** McDunnough (1926) described *E. coxalis* from male and female adults collected in southern Quebec. Larvae have never been definitely associated, but McDunnough (1931) tentatively assigned a single larva collected in Ontario to this species. We collected larvae from two localities in North Carolina and South Carolina that appear morphologically to be conspecific with McDunnough's (1931) specimen. Because adults reared from these larvae clearly do not fit McDunnough's (1926) description of *E. coxalis*, we refer to these specimens here as *E.* sp. 1. Our electrophoretic characterization of this species is based on a single individual from site NC3 (Fig. 1). As with morphological data, conclusions based on electrophoresis of a single specimen must be considered tentative. However, a reasonable estimate of genetic distance can be made from even one individual, provided the genetic distance is large and average heterozygosities of the species being compared are low (Nei 1978). The individual that we electrophoresed satisfied these requirements, having an average genetic distance of 0.84 when compared to the other 14 species (range = 0.74–1.25) and an expected heterozygosity of 0.026. Because heterozygosities in *Eurylophella* are generally low, it is unlikely that our estimate of genetic distance between *E.* sp. 1 and the other *Eurylophella* species would change significantly with increased sample size.

Gene Diversity and Intraspecific Differentiation

Expected heterozygosity values of *Eurylophella* averaged 0.11 and ranged from 0.03 in *E. lutulenta* to 0.21 in *E. verisimilis-C* (Table 4). Several parthenogenetic populations of *E. funeralis*, not included here, had heterozygosity values of zero using a different group of loci (Sweeney & Vannote 1987). Means of most *Eurylophella* species were between 0.10 and 0.12.

All 18 loci used for this study were polymorphic in at least one species. Although allele frequencies at some loci varied significantly among populations within a species, there were no fixed allelic differences among conspecific populations.

Intraspecific genetic distances (Nei 1978) were low, averaging 0.016 overall (range = 0–0.119; Table 4). Highest values were found among some of the 16 *E. verisimilis* populations sampled and resulted from a few aberrant populations. A UPGMA phenogram generated from Nei's (1978) genetic distances for *E. verisimilis* is shown in Fig. 2. Except for the group including VT3, NY2, NY1, NY3, and PA2, the populations generally do not sort out according to geographic proximity. However, the most electrophoretically distinctive population was DE3 located on the Delmarva Peninsula. This site is somewhat isolated from streams in the mountain and piedmont areas of eastern North America where *E. verisimilis* is most commonly found (Fig. 1). Allele frequencies at three loci (*Mdh-1*, *Hex*, and *Est-5*) in particular were markedly different at site

Table 4. Intraspecific genetic distances (Nei 1978) and expected heterozygosity for 14 species of *Eurylophella*. Only samples ≥five individuals were used to calculate average heterozygosities

Species	Nei's distance			Heterozygosity		
	No. of populations	$\bar{x}$	Range	No. of populations	$\bar{x}$	Range
E. verisimilis	16	0.032	0–0.119	16	0.12	0.10–0.17
E. verisimilis-A	2	0.019		2	0.11	0.10–0.12
E. verisimilis-B	5	0.006	0–0.014	5	0.13	0.12–0.14
E. verisimilis-C	1			1	0.21	
E. minimella	3	0.010	0–0.027	1	0.10[a]	
E. bicolor	4	0.016	0.009–0.021	2	0.13[b]	0.11–0.15
E. funeralis	8	0.009	0–0.026	1	0.07[c]	
E. temporalis-A	2	0.011		2	0.06	0.06
E. temporalis-B	4	0.004	0–0.010	4	0.10	0.07–0.11
E. temporalis-C	2	0.037		1	0.05[d]	
E. prudentalis	4	0.021	0.008–0.028	4	0.14	0.12–0.16
E. aestiva	6	0.011	0–0.026	4	0.13[e]	0.11–0.14
E. aestiva-A	1			1	0.16	
E. lutulenta	1			1	0.03	

[a] Population from PA2 only.
[b] Populations from PA2 and PA6 only.
[c] Population from PA1 only.
[d] Population from PA5 only.
[e] Populations from PA6, PA2, VA2, and DE2 only.

DE3 from those of populations elsewhere (see Table 10 in Sweeney et al. [1987]. Although there were no clear macrogeographic clines with regard to allele frequencies (Sweeney et al. 1987), there does appear to be a microgeographic cline at the *Hex* and *Est-5* loci between DE3 and the mainland. The 1.03 allele at the *Hex* locus has a frequency of 77% at DE3. This allele is absent from 12 populations, rare (<3%) at two others, but present at 18% at DE2, the nearest site to DE3 (distance = 44 km). A similar pattern is evident for the 0.30 allele at *Est-5*, which averages 47% (SE = 4.2) for the 14 populations from the mainland, but decreases to 7% at DE2 and 3% at DE3.

Interspecific Relationships

All 18 loci used for this analysis varied significantly among species. A lack of shared alleles was evident for at least one locus for all pairwise interspecific comparisons except between *E. verisimilis* and *E. verisimilis-B*. In general, a single individual of any species can be positively identified by its electromorphic phenotype.

The phylogenetic relationship among *Eurylophella* species was assessed by comparing allele frequencies at 18 loci for 52 populations of 15 species. For this analysis, *E. funeralis* was represented by a single population (PA1); data for nine other populations of *E. funeralis* could not be included because some of the 18 loci were not studied. The average interspecific genetic distance (D) for *Eurylophella* was 0.74 (range = 0.11–1.87; Table 5). A UPGMA phenogram generated from the Nei's (1978) genetic distances for 52 populations is presented in Fig. 3. Individual populations are not

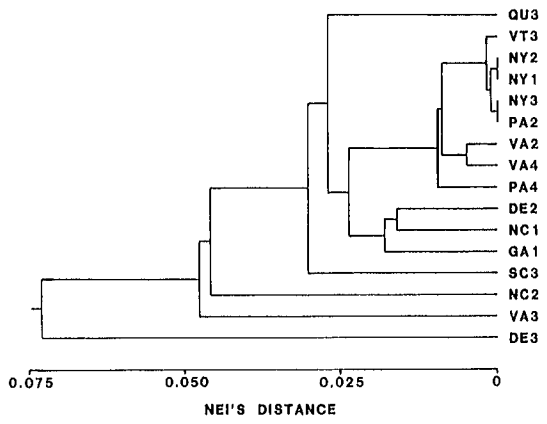

Fig. 2. Phenogram of 16 populations of *E. verisimilis* based on a cluster analysis (UPGMA) of Nei's (1978) genetic distance (D) coefficients. Site descriptions for the population codes are given in Table 1.

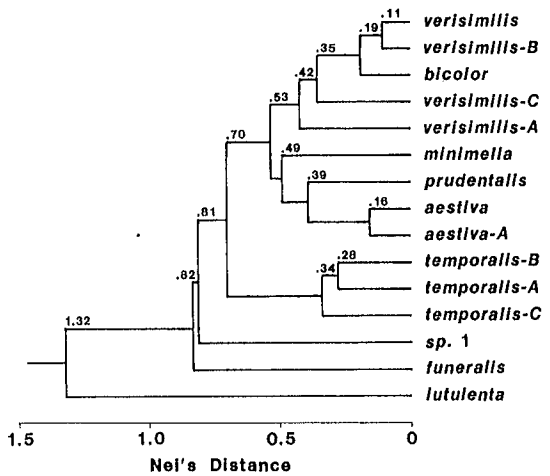

Fig. 3. Phenogram of 15 *Eurylophella* species based on a cluster analysis (UPGMA) of Nei's (1978) genetic distance (D) coefficients.

Table 5. Average Nei (1978) distance (above diagonal) and identity (below diagonal) among 15 species of *Eurylophella*

	EV	EVA	EVB	EVC	EM	EB	EF	ETA	ETB	ETC	EP	EA	EAA	EL	ES1
E. verisimilis (EV)	—	0.43	0.11	0.36	0.56	0.16	0.81	0.48	0.70	0.67	0.42	0.58	0.61	1.24	0.84
E. verisimilis-A (EVA)	0.65	—	0.46	0.61	0.50	0.29	0.97	0.62	0.58	0.75	0.58	0.52	0.43	1.24	0.74
E. verisimilis-B (EVB)	0.90	0.63	—	0.33	0.60	0.28	0.99	0.44	0.64	0.69	0.47	0.65	0.58	1.45	0.75
E. verisimilis-C (EVC)	0.70	0.55	0.72	—	0.87	0.36	0.83	0.47	0.61	0.66	0.56	0.83	0.73	1.59	0.80
E. minimella (EM)	0.57	0.60	0.55	0.42	—	0.46	0.60	0.86	0.99	0.87	0.42	0.51	0.62	1.49	0.79
E. bicolor (EB)	0.85	0.75	0.76	0.70	0.63	—	0.73	0.48	0.60	0.58	0.36	0.44	0.47	1.12	0.74
E. funeralis (EF)	0.45	0.38	0.37	0.44	0.55	0.48	—	1.04	1.01	0.96	0.61	0.68	0.94	1.71	0.82
E. temporalis-A (ETA)	0.62	0.54	0.64	0.63	0.42	0.62	0.35	—	0.28	0.29	0.74	1.03	0.93	1.43	0.78
E. temporalis-B (ETB)	0.49	0.56	0.53	0.54	0.37	0.55	0.36	0.75	—	0.37	0.73	0.85	0.75	1.20	0.74
E. temporalis-C (ETC)	0.51	0.47	0.50	0.52	0.42	0.56	0.38	0.75	0.69	—	0.63	1.02	0.93	1.87	0.96
E. prudentalis (EP)	0.66	0.56	0.62	0.57	0.66	0.70	0.54	0.48	0.48	0.53	—	0.35	0.56	1.38	0.81
E. aestiva (EA)	0.56	0.60	0.52	0.44	0.60	0.64	0.50	0.36	0.43	0.36	0.70	—	0.15	1.20	0.85
E. aestiva-A (EAA)	0.55	0.65	0.56	0.48	0.54	0.63	0.39	0.39	0.47	0.40	0.57	0.86	—	1.55	0.91
E. lutulenta (EL)	0.29	0.29	0.23	0.20	0.23	0.33	0.18	0.24	0.30	0.15	0.25	0.30	0.21	—	1.25
E. sp. 1 (ES1)	0.43	0.48	0.47	0.45	0.45	0.48	0.44	0.46	0.48	0.38	0.45	0.43	0.40	0.29	—

shown because all intraspecific lengths were less than the smallest interspecific branch length (range = 0–0.07) and were generally too short to resolve at this scale. The species cluster into the following five groups, all of which branch at $D > 0.6$:

- *E. verisimilis, E. verisimilis-A, E. verisimilis-B, E. verisimilis-C, E. bicolor, E. minimella, E. prudentalis, E. aestiva, E. aestiva-A;*
- *E. temporalis*-A, *E. temporalis*-B, *E. temporalis*-C;
- *E.* sp. 1;
- *E. funeralis;* and
- *E. lutulenta.*

Discussion

A previous study of geographic variation in the population genetic structure of five ephemerellid species, including *E. verisimilis* and *E. funeralis*, revealed significant genetic differentiation among conspecific populations but no geographic clines in allele frequencies (Sweeney et al. 1987). In this study, we compared the levels of genetic differentiation among local populations, morphologically cryptic species, and morphologically distinct congeners of *Eurylophella* mayflies. Again we found no evidence of macrogeographic clines of allele frequencies among conspecific populations. However, we observed a microgeographic cline for the *Hex* and *Est-5* loci in *E. verisimilis* populations from the Delmarva Peninsula. Assuming long-distance flight by mayflies to be rare or nonexistent because of their short adult life (<48 h for most species), we hypothesize that gene flow between peninsular and mainland populations involves stepwise overland processes rather than direct dispersal across the Chesapeake Bay. Thus, these local clines probably reflect genetic drift associated with low gene flow rather than a selectional gradient. Consistent with this hypothesis is the fact that populations of all five *Eurylophella* species sampled from the Delmarva Peninsula had the highest average genetic distances from their respective conspecific (mainland) populations (namely, *E. verisimilis* from DE3, *E. temporalis-B and E. aestiva* from DE2, *E. prudentalis* from DE4, and *E. funeralis* from DE1).

E. verisimilis exhibited the highest genetic differentiation among the 15 species examined (Table 4). This finding might be an artifact resulting from our survey of a substantially higher number of individuals and populations of *E. verisimilis* compared with the other species (i.e., about 800 individuals from 25 localities ranging from Quebec to Georgia). Nevertheless, the geographic variation in allele frequencies reported for *E. verisimilis* (Sweeney et al. 1987, Table 10) probably represents the typical range of variation one might expect within a species of *Eurylophella*. Although most comparisons between conspecific populations of *Eurylophella* revealed significant allelic variation, there were never any fixed allelic differences between them. In contrast, almost all interspecific comparisons revealed at least one and usually several fixed allelic differences. Similarly, there was virtually no overlap in the range of genetic distance values measured between conspecific populations (range = 0–0.119; Table 4) and the range of average values estimated between congeners (range = 0.11–1.87; Table 5). Our data suggest that although gene flow may be relatively low among conspecific mayfly populations, genetic differences between them are still small compared to those found between closely related species. This pattern has also been observed for horseflies (Sofield et al. 1984), pine beetles (Stock et al. 1984), crickets (Howard 1983), and other insect species (see review by Brussard et al. [1985]).

We found no electrophoretic evidence for hybridization among any of the 15 *Eurylophella* species. Although the possibility of some hybridization between *E. verisimilis* and *E. verisimilis-B* cannot be ruled out, the fact that *E. verisimilis-B* was always found coexisting with *E. verisimilis* suggests that reproductive barriers are effective and that hybridization is negligible. In fact, many of the most closely related *Eurylophella* species are sympatric in the narrowest sense (i.e., present in

the same habitat at the same locality; see Table 1), and have broadly or even completely overlapping adult emergence periods (unpublished data). In their study of *Leptophlebia marginata* in Finland, Saura et al. (1979) found electrophoretic evidence for reproductive isolation between two sympatric, morphologically indistinguishable forms of this species that had previously been found to orient to different (and predictable) swarm markers even after being captured and transported to another area. *E. verisimilis* and *E. verisimilis-B* are the most closely related pair of *Eurylophella* species based on electrophoretic evidence, and at the seven localities where they coexist (Table 1), their emergence periods appeared to be identical. We hypothesize that they too may avoid interbreeding by differences in swarming behavior, whether by orienting to different swarm markers or swarming at different times of day.

Of the 15 electrophoretically distinct species reported here, 7 are morphologically distinct and separable from one another as full-grown larvae. These species include *E. verisimilis, E. bicolor, E. minimella, E. prudentalis, E. funeralis, E.* sp. 1, and *E. lutulenta.* The other 8 species fall into three morphologically distinct groups:

- *E. aestiva* and *E. aestiva-A;*
- *E. temporalis-A, E. temporalis-B,* and *E. temporalis-C;* and
- *E. verisimilis-A, E. verisimilis-B,* and *E. verisimilis-C.*

Within these groups, we presently cannot separate species (except by electrophoresis). A more thorough morphological study may reveal characters to distinguish these cryptic species. We found as many as five species of *Eurylophella* to be common at a given locality (e.g., PA2; Table 1), sometimes including more than one representative of the second or third group above, and all from the same habitat. For these reasons, accurate identification of *Eurylophella* from benthic samples may be difficult.

According to Allen & Edmunds (1963), many *Eurylophella* species are morphologically variable, especially in the size and spacing of tubercles on the head and abdomen. However, our examination of larval exuviae associated with individuals tested electrophoretically revealed at least some of this variation to be interspecific. For example, the *E. verisimilis* complex, shown here to contain at least four species, can be divided into two groups based on the size of head tubercles on larvae. One group consisting of *E. verisimilis* has distinct, well-developed head tubercles in both sexes. In the other group, male larvae have only small roughened areas or lack head tubercles altogether, and female larvae have relatively small head tubercles. This group includes three species that are referred to here as *E. verisimilis-A, E. verisimilis-B,* and *E. verisimilis-C.* We examined more than 1,000 individual larval exuviae from 25 localities ranging from Quebec to Georgia and South Carolina (associated with adults that were compared electrophoretically) and found the size of head tubercles to be consistent in each of the *E. verisimilis* complex species throughout its range. Allen & Edmunds (1963) described head tubercle size in male larvae of *E. verisimilis* as "barely discernible to moderately well developed" based on material they examined from the area delineated in our Fig. 1. All of our collections of *E. verisimilis-A, E. verisimilis-B,* and *E. verisimilis-C* are from that area. Of the specific localities they listed, two in particular are likely to have included what we consider *E. verisimilis-B* and possibly *E. verisimilis-A:* "Beaverkill" (New York) probably corresponds with our site NY3, where *E. verisimilis-B* is slightly more abundant than *E. verisimilis* (Table 1), and "Scranton" (Pennsylvania) is near our sites PA2 and PA8, where *E. verisimilis-B* and *E. verisimilis-A* are common (Table 1). It seems likely that their concept of *E. verisimilis* was based on a mixture of at least two, and possibly all four, of the species we regard as the *E. verisimilis* complex.

In comparing McDunnough's (1938) *E. bicoloroides* with *E. verisimilis,* Allen & Edmunds (1963) found only one distinguishing feature, a lack of head tubercles in the male larvae from McDunnough's *E. bicoloroides* type series. They concluded that "having regard to geographic distribution and morphological characters in all stages, we consider the nominal *E. bicoloroides* to be a junior synonym of *E. verisimilis.*" But they suggested a large series of specimens from the type locality would be needed to be certain whether the head tubercle character was important enough to merit specific or subspecific status. This argument assumes that such a series (i.e., a group of similar individuals collected at the same time and place) would consist of a single species. Our data show this assumption may be risky; we have found *E. verisimilis, E. verisimilis-A,* and *E. verisimilis-B* (all of which would be included in their concept of *E. verisimilis*) at the same locality. Thus, a large series from the type locality of *E. bicoloroides* could include several species. However, based on our present knowledge of this group, we believe that either *E. verisimilis-A* or *E. verisimilis-B* of this study is *E. bicoloroides* of McDunnough (1938).

A phylogeny of the *Eurylophella* species has not been proposed to date. Allen & Edmunds (1963) considered

- *E. bicolor* and *E. minimella* to be "near cognate" and to form a "complex of closely related species with *E. aestiva* and *E. verisimilis*";
- *E. prudentalis* to be similar to *E. verisimilis* and *E. aestiva* in some respects, but quite distinct overall;
- *E. temporalis* (including *E. doris* and *E. trilineata,* which they considered to be of "questionable taxonomic status") and *E. funeralis* to each be quite distinct; and
- *E. lutulenta* and "*E. coxalis*?" to be similar to each other based on larval characters.

Our phenogram also groups *E. verisimilis, E. bicolor, E. aestiva,* and *E. minimella,* but pairs them differently (*E. verisimilis* with *E. bicolor,* and *E. aestiva* with *E. minimella*) and includes *E. prudentalis* with the *E. aestiva* and *E. minimella* subgroup. *E. temporalis, E. funeralis,* and *E. lutulenta* represent distinct groups in our phenogram, but *E.* sp. 1 does not group with *E. lutulenta* as would be indicated by morphological evidence (assuming it to be conspecific with the larva tentatively assigned to *E. coxalis*).

Brussard et al. (1985) reviewed genetic similarity (*I*) values from 14 studies of various insect taxa with regard to different levels of evolutionary divergence. The average literature value at the local population level corresponds to a genetic distance (*D*) of 0.03 (where $D = -\ln I$), which compares well with our data for *Eurylophella* (average D = 0.02; Table 4). Average literature values for sibling species ranged from 0.06 to 0.57, and for nonsibling species from 0.15 to 1.04. *Eurylophella* species in our phenogram range from 0.11 to 1.32, and the average value was 0.74 (Table 5). *E. lutulenta* has a branch length of 1.32, which is outside the range of literature values for nonsibling species, and would be considered representative of genera within subfamilies (reported literature range = 0.84–1.66). If we exclude *E. lutulenta,* our range of *D* values for nonsibling species was 0.11–0.82 (average from Table 5 = 0.64). Nevertheless, the average distance between *Eurylophella* species is in the upper range of literature values for nonsibling species. We believe Allen's (1979) elevation of the subgenus *Eurylophella* to generic status is supported by these data. We did not sample the western North American *E. lodi* or the European *E. karelica,* but according to Allen & Edmunds (1963) they are morphologically similar to *E. lutulenta.* These three species may represent a distinct lineage within the *Eurylophella* worthy of subgeneric status.

This study represents the first of its kind on a genus of mayflies. In the case of *Eurylophella,* electrophoretic techniques have proven to be more sensitive than classical morphological methods, enabling the resolution of what appear to be 15 species included in what is recognized presently as 8 or 9. Similar studies on other groups of Ephemeroptera are clearly needed, but our results suggest that the diversity of Ephemeroptera (or at least the Ephemerellidae) has been underestimated.

Acknowledgment

We thank G. M. Davis, C. Hesterman, and T. M. Uzzell for valuable assistance in developing electrophoretic techniques for mayflies. J. D. Newbold and T. W. Condon helped considerably with data analysis. We thank B. C. Kondratieff, R. W. Lake, and D. I. Rebuck for help in locating and collecting specimens and J. E. H. Martin and L. L. Pechuman for the loan of type specimens. We also thank the following individuals for technical assistance: P. J. Dodds, S. C. Duczkowski, B. L. Green, A. C. Graham, J. A. Gustin, W. D. Kintzer, and J. W. Pierson. This work was supported by the U.S. Department of Energy (Contract No. DE-AC-0279EV-10259) and the National Science Foundation (Grant No. DAR 78-18589).

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Received for publication 5 March 1987; accepted 23 September 1987.