HOLARCTIC ECOLOGY 10: 52–59. Copenhagen 1987

Geographic parthenogenesis in the stream mayfly Eurylophella funeralis in eastern North America

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Sweeney, B. W. and Vannote, R. L. 1987. Geographic parthenogenesis in the stream mayfly *Eurylophella funeralis* in eastern North America. – Holarct. Ecol. 10: 52–59.

Unisexual populations were found largely at the periphery of the geographic range of the mayfly *Eurylophella funeralis* in eastern North America. Bisexual populations generally had a normal sex ratio but at least two populations were observed with only about 2–11% males. Hatch success for unfertilized eggs depended on temperature but in general averaged > 61% in unisexual populations and < 14% in bisexual populations. Eggs took about 65 d to hatch at 10°C and 19 d at 25°C; no hatching occurred at 5 or 30°C. Successful oviposition was observed for about 97% and 20% of unmated female adults from unisexual and bisexual populations respectively. The hatch success of unfertilized eggs for three geographically distinct bisexual populations was inversely correlated with the proportion of males in the populations. The genetic structure of bisexual and unisexual populations was very similar. Unisexual populations consists of clones, with as few as four recognizable phenotypes being present at a given location. All offspring had the same phenotype as the mother. Tychoparthenogenesis is suggested as the most reasonable working hypothesis concerning the origin of unisexual populations in *E. funeralis*.

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1. Introduction

Parthenogenesis in a North American mayfly (Ephemeroptera) was first demonstrated in 1922 when Clemens confirmed the successful hatching of parthenogenetic eggs for *Ameletus ludens* Needham. Other mayfly species have subsequently been shown to have varying degrees of parthenogenetic reproduction (Mingo 1978). For many mayfly populations containing males (hereafter referred to as bisexual), a low (< 10%) percentage of unfertilized eggs have been shown to hatch successfully (Huff and McCafferty 1974). Facultative parthenogenesis occurs in an array of families and genera and does not seem to be associated with a unique lineage within the phylogenetic hierarchy of mayflies.

Some mayfly populations are unisexual, and a few North American species have been described from only female specimens because males were not collected. In general, however, unisexual mayfly populations in North America have been associated taxonomically with populations of a bisexual species and only a few instances remain where bisexual conspecifics have not been described. In Europe, males are known for all mayfly species exhibiting parthenogenetic reproduction (Degrange 1960, Humpesch 1980).

Although mayflies are widespread and comprise a significant portion of the fauna in many streams and rivers, unisexual populations are often overlooked because mayfly specimens are usually collected as larvae, which can be difficult to sex, or in swarms (predominantly males). It is now evident that parthenogenetic reproduction (both facultative and obligatory) in mayflies is fairly common. Existing data, however, are highly descriptive and no attempt has been made to experimentally assess the ecological implications or significance of parthenogenesis in any mayfly species.

This paper focuses on a mayfly, *Eurylophella funeralis* (McDunnough), which commonly occurs in small

Accepted 9 May 1986

woodland spring seeps and first and second order spring brooks throughout eastern North America. We present data concerning the geographic distribution of bisexual and unisexual populations, the occurrence of parthenogenesis in bisexual populations, oviposition behavior, cytological aspects of parthenogenesis and the genetic structure of unisexual and bisexual populations.

2. Methods

The effects of temperature on developmental rate and hatch success of eggs was determined by dissecting eggs out of live subimagines collected from White Clay Creek, and placing them in glass jars (Ø 5.5 cm, 6.5 cm deep) containing 100 ml of filtered (0.45 µm) stream water. Each jar contained only eggs from a single female. All jars were partially submerged in water baths kept at various temperatures (5, 10, 15, 20, 25, 30°C and ambient creek temperatures). Jars containing eggs were inspected daily for newly hatched larvae. Percent hatch success for a given clutch was determined by counting the number of hatched and unhatched eggs. We also used similar techniques to determine the hatch success of unfertilized eggs for other mayfly species (see Tab. 1). However, eggs were only incubated at ambient creek temperatures for these additional studies.

The hatch success of eggs collected from various geographic populations was determined by allowing adult females to oviposit on their own into glass jars that are described above. We generally observed better hatch success for egg batches oviposited relative to those dissected from females.

Artificial insemination of eggs involved removing the eggs from a live female imago, putting them on a depression microscope slide, and adding sperm by removing the posterior half of a male abdomen and macerating the seminal vesicle and testes with forceps while in contact with the eggs. After about 2–3 min contact with sperm, eggs were placed in glass jars (Ø 5.5 cm, 6.5 cm deep) containing 25 ml filtered (0.45 µm) stream water. Jars containing eggs were incubated at ambient White Clay Creek temperatures.

Oviposition behavior of unmated females was studied for various bisexual and unisexual populations located in eastern North America. Following the molt from subimago to imago, each unmated female was placed on the water surface (0.45 µm filtered stream water) in a glass jar (Ø 5.5 cm, 6.5 cm deep) and given 10 min to oviposit. If no oviposition occurred, the female was removed and placed in a screened cage covered with damp paper towels. After 3 h, the female was placed back onto the water surface for a second attempt. If oviposition still did not occur, the female was removed to a cage and the third attempt was performed 3 h later. Females generally either oviposited during the first three attempts or never oviposited and eventually died.

Electrophoretic analysis of proteins was performed on subimagines which were obtained by laboratory rearing of mature larvae collected from various geographic locations. Each subimago was frozen separately in a 5 ml analyzer cup containing about 2 ml of tris tissue buffer (0.05 M tris, adjusted to pH 7.4 with phosphoric acid) and stored at −65°C. A reference number was assigned to each frozen adult and corresponded to the preserved (80% ETOH) larval exuvium that was collected after metamorphosis and kept as a taxonomic voucher. Allozymes were separated by horizontal starch gel electrophoresis using methods similar to those described by Selander et al. (1971). Fourteen enzymes (18

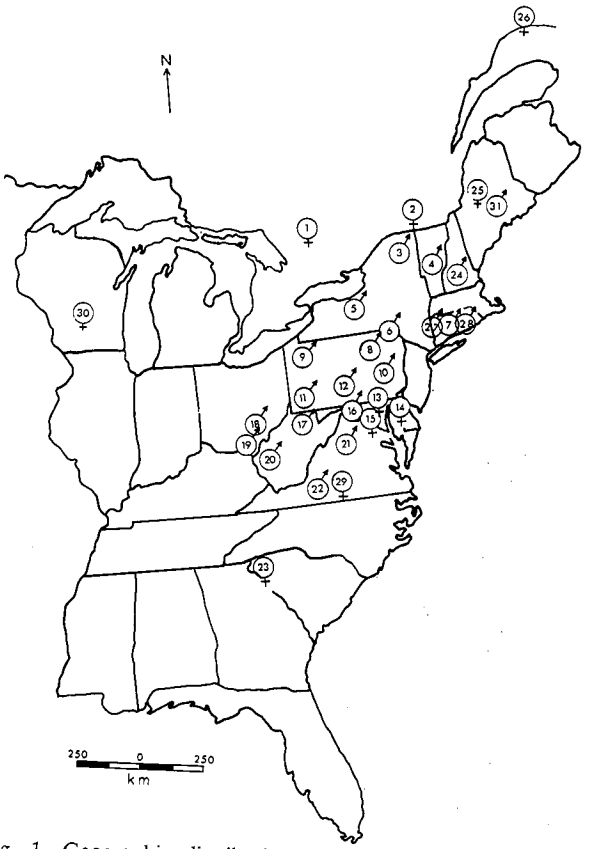

Fig. 1. Geographic distribution of unisexual (circles with crosses) and bisexual (circles with arrows) populations of *E. funeralis* in eastern North America. Specific collection sites are as follows: (1) Mud Creek, ONT; (2) Allen's Brook, QUE; (3) Unnamed tributary near Tupper Lake, NY; (4) Goodmans Brook, VT; (5) Fall Creek, NY; (6) Starucca Creek, NY; (7) Indian Well Creek, CT; (8) Meshoppen Creek, PA; (9) Unnamed tributary of Kinzua Creek, PA; (10) Hickory Run, PA; (11) Roaring Run, PA; (12) Fortune Teller Creek, PA; (13) White Clay Creek, PA; (14) White Clay Creek, DE; (15) Unnamed tributary in Gabrill State Park, MD; (16) Unnamed tributary in Green Ridge State Forest, MD; (17) Unnamed tributary of Tygert Lake, WV; (18) Baker Run, OH; (19) Storms Creek, OH; (20) Hisey Fork, WV; (21) Jordan River, VA; (22) Big Otter Creek, VA; (23) Cranes Creek, SC; (24) Hubbard Brook, NH; (25) Rocky Brook, ME; (26) Beaver Creek, QUE; (27) Unnamed tributary near Betheny, CT; (28) Fenton River, CT; (29) Slate Creek, VA; (30) Otter Creek, WI; (31) Nesowadnehunk River, ME.

loci) were studied on each population. The enzymes were: ACP = acid phosphatase; AO = aldehyde oxidase; FUM = fumarase; G3PDH = glyceraldehyde-3-phosphate dehydrogenase; GPI = glucose phosphate isomerase; HEX = hexakinase; ISDH1, ISDH2 = two loci of isocitrate dehydrogenase; LAP1, LAP2 = two loci of leucine aminopeptidase; MDH1, MDH2 = two loci of malate dehydrogenase; ME = malic enzyme; MPI = mannose phosphate isomerase; PGM = phosphoglucomutase; SOD1, SOD2 = two loci of superoxide demutase; aGPDH = alpha-glycerophosphate dehydrogenase.

3. Results

3.1. Geographic parthenogenesis

Facultative parthenogenesis has been demonstrated for 18 mayfly species including *E. funeralis* in North America (Tab. 1). Unisexual populations have been described at one or more locations for at least four species in North America (*Ephemera varia, Eurylophella funeralis, Baetis macdunnoughi* and *B. hageni*). Two mayfly taxa are presently known only from unisexual populations (viz. *Ameletus ludens, Cloeon triangulifer*).

For *E. funeralis,* unisexual populations seem to occur largely at the periphery of its geographic range (Fig. 1). Collections of mature larvae and adults at most sites containing males were large enough to confirm a normal sex ratio. However, in Goodmans Brook, Vermont only 16% of 87 adults reared were male in 1980 and 13% of 53 adults were males in 1981. The sex ratio of mature larvae was also skewed (12% of 89 larvae were males in 1981; 11% of 99 larvae were males in 1984). Fiance (1978) also reported skewed sex ratios for *E. funeralis* in Hubbard Brook, New Hampshire. We have verified these findings with additional field collections that indicate 2% of the population are males.

3.2. Adult oviposition behavior

Parthenogenetic reproduction by individuals usually requires behavioral changes such that unmated females will oviposit. We compared females from bisexual and unisexual populations for differences in the propensity to oviposit without mating. Results indicate that an average of 97.2% (range: 86.4–100%) of unmated females from unisexual populations oviposited successfully, with most females ovipositing immediately when placed on the water surface for the first time (Tab. 2). In

Tab. 1. Mean hatch success for unfertilized eggs of 22 species of mayflies in North America. Bisexual and unisexual populations indicate the presence or absence of males respectively for a particular population, but not necessarily for all populations of a given species. Missing data for the range of hatch success usually indicates that eggs from several females were studied collectively and only an overall percent hatch for the combined egg total was calculated.

Species	Mean hatch success (%)	Range (%)	Reference
	Unisexual populations		
Ameletus ludens Needham	90	89–91	present study
Baetis hageni Eaton	97	97–98	Bergman & Hilsenhoff 1978
Baetis macdunnoughi Ide	87	57–100	Bergman & Hilsenhoff 1978
Cloeon triangulfier McDunnough	86	–	Gibbs 1977
Cloeon triangulifer McDunnough	78	50–96	present study
Ephemera varia Eaton	99	–	present study
Eurylophella funeralis (McDunnough)	61	0.1–96	present study
	Bisexual populations		
Ameletus sp. near *tertius*	20	20–22	present study
Ameletus cryptostimulus Carle	0.1	<0–0.1	present study
Baetis frondalis McDunnough	31	10–60	Bergman & Hilsenhoff 1978
**Baetis propinquus* (Walsh)	40	12–63	Bergman & Hilsenhoff 1978
Baetisca rogersi Berner	1	0.3–1.6	Pescador & Peters 1974
Centroptilum rufostrigatum (McDunnough)	<0.1	0–<0.1	present study
Ephemera varia Eaton	10	0.7–42	present study
Ephemerella dorothea Needham	<0.1	0–<0.1	present study
Ephoron album (Say)	8	8–10	Britt 1962
Eurylophella funeralis McDunnough	14	1.7–69	present study
Hexagenia rigida McDunnough	9	–	Friesen & Flannagan 1976
Isonychia bicolor (Walker)	<0.1	0–<0.1	present study
Leptophlebia cupida (Say)	13	–	present study
Stenonema pulchellum (Walsh)	<0.1	–	Huff & McCafferty 1974
Stenonema vicarium (Walker)	2	–	Huff & McCafferty 1974
Stenonema femoratum (Say)	8	0.2–40.2	Huff & McCafferty 1974
¶ Stenacron interpunctatum (Say)	0.1	–	Huff & McCafferty 1974
Stenacron interpunctatum frontale (Burks)	9.1	–	Mingo 1978

* as *Baetis spinosus* McDunnough
¶ subspecific form was not recognized

Tab. 2. The relative oviposition performance of unmated females and subsequent hatch success (at 25°C) of the unfertilized eggs for various bisexual and unisexual populations in eastern North America. SC = Cranes Creek, Oconee County, South Carolina; DE = White Clay Creek, New Castle County, Delaware; PA-S = White Clay Creek, Chester County, southeastern Pennsylvania; QU-B, QU-T = local populations in tributaries of the Matamek River, Quebec; NH = tributary of Hubbard Brook, Grafton County, New Hampshire; VT = tributary of the Battenkill River, Bennington County, Vermont; PA-N = Nine Partners Creek, Susquehanna County, northeastern Pennsylvania.

			Relative oviposition performance of unmated females (%)*				Hatch success (%) of unfertilized eggs			
Study Population	Sexuality (% male)	n	first attempt	second attempt	third attempt	never oviposited	n	$\bar{x}$	S.D.	range
SC	0	9	100	–	–	–	8	88.5	10.0	74.0–97.1
DE	0	52	96.1	3.9	–	–	10	80.9	14.5	54.2–98.4
PA-S	0	27	92.5	7.5	–	–	10	84.9	15.1	48.0–97.5
QU-B	0	9	88.8	11.2	–	–	7	86.7	11.7	65.9–97.6
QU-T	0	37	72.9	10.8	2.7	13.6	10	83.4	24.3	17.7–97.2
NH	2	38	31.5	28.5	8.5	31.5	9	86.8	9.5	66.9–97.9
VT	11	17	5.8	5.8	–	88.4	7	62.8	25.2	23.2–87.4
PA-N	50	35	22.8	2.8	2.8	71.6	10	6.1	5.2	0.3–15.8

* several hours elapsed between attempts to induce ovipositing by placing the adult on a water surface.

contrast, only 36.1% (range: 11.6–68.5%) of the unmated females from populations containing males oviposited successfully. The New Hampshire population, which has a very low proportion (2%) of males, had an exceptionally high oviposition rate (68.5%) for unmated females.

3.3. Egg development

For *E. funeralis,* the average percentage of eggs that hatch parthenogenetically was significantly higher (t-test, $p < 0.05$) for eggs from females of unisexual populations (mean 84.5%, range: 17.7–98.4%) relative to those obtained from populations containing males (mean 49.3%, range: 0.3–97.9%; Tab. 2). However, hatch success for eggs taken from bisexual populations was inversely related to the proportion of males in the population. Thus, eggs from females of bisexual populations having 2, 11, and 50% males had hatch success frequencies for unfertilized eggs of 86.8, 62.8, and 6.1% respectively. These data suggest that populations containing a low frequency of males probably contain some females that were produced parthenogenetically.

Hatch success for eggs obtained from a unisexual population in White Clay Creek, Pennsylvania varied with incubation temperature, being greatest at 20°C (76.9%) and decreasing at both higher and lower temperatures (Tab. 3). This is similar to the response pattern observed for fertilized eggs of a number of other mayfly species (Elliott and Humpesch 1980). The effect of temperature on hatch success is probably not related directly to the duration of embryonic development because, like most other insects, *E. funeralis* exhibited an inverse relationship between development time and temperature (Tab. 3).

We also examined the development rate of unfertilized eggs taken from several geographic populations, both unisexual and bisexual (Tab. 4). We initially compared development of eggs from three unisexual populations (PA-R, PA-W, PA-P) and two bisexual populations (WV, OH) at ambient White Clay Creek (Chester County, Pennsylvania) temperatures. Significant differences in mean development time were observed among the five study populations (ANOVA, $p < 0.05$), with eggs from the bisexual populations taking longer (5–6 days) than eggs from the unisexual populations (Scheffe

Tab. 3. Development time (no. days to first hatch) and hatch success for unfertilized eggs of *E. funeralis* obtained from site PA-S in White Clay Creek, southeastern Pennsylvania.

		Days to first hatch			Hatch success (%)		
Temperature (°C)	No. replicates	$\bar{x}$	(S.D.)	range	$\bar{x}$	(S.D.)	range
5	7	–	(–)	–	0	(–)	–
10	7	64.5	(8.3)	59–82	66.1	(15.4)	42.3–79.6
15	7	32.8	(4.9)	22–36	71.6	(22.5)	35.0–93.7
20	7	20.1	(1.2)	18–21	76.9	(22.7)	33.5–99.2
25	7	18.8	(2.8)	17–24	62.4	(27.3)	16.0–90.1
30	7	–	(–)	–	0	(–)	–

Tab. 4. Development time (no. days to first hatch) of unfertilized eggs obtained from various bisexual and unisexual populations of *E. funeralis* in eastern North America. PA-R, PA-W, PA-S = local populations in tributaries of White Clay Creek, Chester County, southeastern Pennsylvania; PA-P = tributary to Brandywine Creek, Chester County, Pennsylvania; WV = Hisey Fork of Four Pole Creek, Cabell County, West Virginia; OH = Storms Creek, Lawrence County, Ohio; SC = Cranes Creek, Oconee County, South Carolina; DE = White clay Creek, New Castle County, Delaware; QU-B, QU-T = local populations in tributaries of the Matamek River, Quebec; NH = tributary of Hubbard Brook, Grafton County, New Hampshire; VT = tributary of Battenkill River, Bennington County, Vermont; PA-N = Nine Partners Creek, Susquehanna County, northeastern Pennsylvania. In exp. 1 eggs were incubated at ambient White Clay Creek temperatures which gradually increased during the experiment from an average of 12°C on day 1 to about 19°C at hatching. In exp. 2 eggs were incubated at 14.5 ± 1°C during the experiment.

	Study population	Sexuality (% male)	Days to first hatch n	$\bar{x}$	S.D.	range
Experiment 1	PA-R	0	5	28.0	1.0	27–29
	PA-W	0	15	26.6	0.6	26–28
	PA-P	0	11	26.8	0.6	26–28
	WV	50	15	32.2	3.1	28–40
	OH	50	4	34.2	3.7	29–37
Experiment 2	SC	0	9	37.4	2.4	33–41
	DE	0	50	33.7	1.9	30–39
	PA-S	0	27	33.9	2.4	29–38
	QU-B	0	8	36.1	2.2	34–39
	QU-T	0	30	34.6	1.8	31–38
	NH	2	26	38.1	4.2	30–53
	VT	11	6	36.8	3.6	32–40
	PA-N	50	39	44.1	4.5	36–57

multiple range test, $p < 0.05$, Tab. 5). Thus, unfertilized eggs from unisexual populations not only had a higher hatch frequency but they also developed significantly faster than unfertilized eggs from individuals of bisexual populations.

We repeated this experiment at a constant temperature (14.5°C), using eggs from a different set of geographic populations – viz. five unisexual populations (SC, DE, PA-S, QU-B, QU-T) and three bisexual populations (NH, VT, PA-N; Tab. 4). This experiment revealed a significantly longer development time (ANOVA, $p < 0.05$) for eggs from the PA-N bisexual population relative to the other populations (Scheffe multiple range test, $p < 0.05$, Tab. 5). Unfertilized eggs obtained from the two other bisexual populations (NH, VT) developed at the same rate as those taken from unisexual populations. However, since the proportion of males in the NH and VT populations is low (2 and 11% respectively), it is quite probable that the females used in the experiments had originated from parthenogenetic and not sexual reproduction. Thus, the results of both experiment strongly suggest that differences in egg development rate among geographic populations are related more to the type of reproduction than to geographic location.

3.4. Artificial insemination

We attempted to fertilize eggs from a unisexual population of *E. funeralis* with sperm from conspecific males and, for control purposes, with sperm from a related species, *Ephemerella subvaria* McDunnough. The degree of fertilization was assessed by statistically comparing hatch success of inseminated and non-inseminated eggs. In addition, we also compared hatch success for unfertilized and artificially inseminated eggs taken from females of bisexual populations (Tab. 6). Results show statistically significant differences in mean hatch success between artificially inseminated eggs (i.e. PA × WV and PA × OH crosses) and non-inseminated eggs (i.e. PA) of females from unisexual populations (ANOVA, $p < 0.003$; Scheffe test, $p < 0.05$). No significant difference in hatch success was observed for eggs inseminated by Ohio (i.e. PA × OH cross) or West Virginia (i.e. PA

Tab. 5. Results of Scheffe multiple range test comparing mean development time (days) for unfertilized eggs obtained from various geographic populations of *E. funeralis*. Abbreviations for study populations are described in Tab. 4.

	Study sites							
Experiment 1	PA-W	PA-P	PA-R	WV	OH			
	26.5	26.7	27.7	32.5	34.3			
Experiment 2	DE	PA-S	QU-T	QU-B	VT	SC	NH	PA-N
	33.7	34.0	34.7	36.1	36.9	37.4	38.1	44.2

Tab. 6. Mean hatch success for *E. funeralis* eggs obtained from females of various geographic populations. Test crosses lacking males indicate unfertilized eggs. PA-S = unisexual population from White Clay Creek, Chester County, Pennsylvania; WV = bisexual population from Hisey Fork of Four Pole Creek, Cabell County, West Virginia; OH = bisexual population from Storms Creek, Lawrence County, Ohio.

Test cross female × male	Number of replicates	Mean hatch success (%)	S.D.
PA-S	17	61.0	32.8
PA-S × WV	30	83.4	15.8
PA-S × OH	10	83.7	12.6
WV	25	14.2	15.1
WV × WV	16	19.1	19.3
WV × *E. subvaria**	4	11.0	8.8
OH × WV	4	18.1	25.9
OH	9	18.5	15.9

* *E. subvaria* were obtained from White Clay Creek, Chester, County, Pennsylvania.

× WV cross) males. Although it appears that we successfully inseminated eggs from females of unisexual populations, results from control crosses indicate that further experiments ought to be performed. Specifically, we did not obtain significant differences in mean hatch success when artificially inseminated and noninseminated females from bisexual populations were compared. For example, artificially inseminated WV females had only a slightly higher mean hatch success (19.1%) relative to non-inseminated WV females (14.2%).

3.5. Genetic structure of unisexual and bisexual populations

The genetic distance (Nei 1972) between populations averaged 0.005 (n = 28, S.D. = 0.008, range = 0.0–0.02), for all possible pairwise comparisons. Thus, on the average, individuals from any two populations differed from one another by only about five electrophoretically detectable allelic substitutions per 1000 loci (Ayala 1978). Three populations, including one bisexual (VT) and two unisexual (QU-B, QU-T), were fixed for the same allele at all 5 polymorphic loci (Tab. 7). Two unisexual populations (VA, ME) exhibited slight differences in allele frequencies at two loci but still shared the same dominant alleles as the other populations. The Delaware (DE) and South Carolina (SC) unisexual populations exhibited the most significant differences in allele frequencies. For example, allele 1.25 of the SOD1 locus occurred in these two populations at frequencies of 0.15 and 0.19 but was generally not observed at all in the other populations. Similarly, allele 1.07 was the dominant allele at the ISDH1 locus for the DE population and was unique to that location. The existence of clones in unisexual populations was further evident from the data on polymorphic loci. For example, at the SOD1 locus, the DE population did not contain any heterozygotes for alleles 0.96 and 1.25 and the SC population contained no homozygotes for allele 1.25 even though heterozygotes between alleles 0.96 and 1.25 were common.

We have also used electrophoresis to gain some insight into the cytological mechanism of parthenogenesis for *E. funeralis*. Thus, a unisexual population in White Clay Creek consists of four coexisting clones, each with a distinct biochemical phenotype. We have reared the offspring of females representing each of these phenotypes in isolated chambers in the laboratory and found that each daughter shares the exact phenotype of the mother.

4. Discussion

The proportion of mayfly species that have a propensity towards parthenogenesis is difficult to estimate because only successful attempts to hatch unfertilized eggs of a bisexual species are reported. Unsuccessful attempts are probably considered normal and not mentioned in the literature. In North America, Bergman and Hilsenhoff (1978) tested six species of *Baetis* and found four of them to exhibit parthenogenetic egg development to varying degrees. Huff and McCafferty (1974) observed parthenogenesis in all three species of *Stenonema* that were tested. In Europe, Degrange (1960) found that eggs from 26 of 51 mayfly species tested exhibited some parthenogenetic development, while Humpesch (1980) observed low levels of parthenogenesis in all seven mayfly species that he examined. Clearly, facultative par-

Tab. 7. Geographic variation in allele frequencies for the polymorphic loci of *E. funeralis*. Sample size is indicated in parentheses below each geographic location. QU-T, QU-B = tributaries of Matamek River, Quebec; ME = Nesowadnehunk River, Piscataquis County, Maine; VT = tributary of the Battenkill River, Bennington County, Vermont; PA-N = Nine Partners Creek, Susquehanna County, Pennsylvania; DE = White Clay Creek, New Castle County, Delaware; VA = tributary of Slate River, Buckingham County, Virginia; SC = Cranes Creek, Oconee County, South Carolina.

Locus	Allele	Geographic location							
		QU-T (30)	QU-B (30)	ME (30)	VT (30)	PA-N (55)	DE (59)	VA (30)	SC (34)
GPI	1.42			0.05					
	1.28								
	1.03	1.00	1.00	0.95	1.00	0.01			
	0.77					0.99	1.00	1.00	0.04
SOD1	1.25								0.88
	0.96	1.00	1.00	1.00	1.00	0.01	0.15		0.08
	0.48					0.97	0.85	1.00	0.19
ME	1.00	1.00	1.00	1.00	1.00	0.02			0.81
	0.80					1.00	1.00	0.93	1.00
ISDH1	1.07							0.07	
	0.80	1.00	1.00	1.00	1.00	1.00	0.53		
LAP2	0.93						0.47	1.00	1.00
	0.89	1.00	1.00	0.05					
	0.79			0.87	1.00	1.00	0.26	0.08	0.04
				0.08			0.73	0.85	0.90
							0.01	0.07	0.06

thenogenetic reproduction is generally widespread among mayfly species.

For *E. funeralis,* parthenogenesis appears to be thelytokous or female producing. From a cytological standpoint, two types of thelytoky can be distinguished: (1) automixis, in which meiosis still occurs in the oocyte but is compensated for by a doubling of the chromosome number either before meiosis or at some later stage; and (2) apomixis, in which meiosis is entirely suppressed. Practically all instances of occasional or rare parthenogenesis (i.e. tychoparthenogenesis) in bisexual insect species has involved automixis (White 1977). Our electrophoretic results for *E. funeralis* indicate that each daughter shares the same phenotype as the mother. This suggests that parthenogenetic development in eggs taken from females of unisexual populations involves either apomixis or a type of automixis known as central fusion without recombination (May and Holbrook 1978).

We have not attempted to determine the mechanism for parthenogenetic development of eggs taken from females of bisexual populations. We have shown, however, that under the same conditions parthenogenetic eggs obtained from females of bisexual populations take longer to develop than eggs from unisexual populations. It is also known that parthenogenetic eggs generally take longer to develop than fertilized eggs in bisexual populations (Degrange 1960, Huff and McCafferty 1974, Pescador and Peters 1974, Humpesch 1980). These data suggest that offspring produced parthenogenetically in a bisexual population will always be at a competitive disadvantage to other conspecific larvae due to delayed hatching if food resources and/or space are limited in a given habitat and early colonizers are able to successfully defend optimal habitat.

Although we do not know the exact mechanism for parthenogenetic development in *E. funeralis,* we do know that it successfully occurs in < 14% of the eggs for females taken from bisexual populations and in 60–80% of the eggs for females from unisexual populations. Thus, a certain percentage of unfertilized eggs in all females do not develop. If we assume that eggs fail to hatch because they are haploid, then it seems feasible that fertilization could occur to a limited extent in unisexual populations. Supporting evidence is provided by Koss and Edmunds (1974) who report a well developed micropyle (structure for allowing sperm penetration) in eggs from unisexual populations of the mayfly *Cloeon triangulifer* McDunnough. We certainly cannot rule out the possibility, based on results of our artificial insemination experiments, of sexual reproduction in females from unisexual populations of *E. funeralis.* More data are clearly needed concerning the possibility of gene flow between males and thelytokous females because it appears that some stream sites containing males also contain parthenogenetically produced females. We reach this conclusion based on our observations that populations containing a low frequency of males (e.g. < 11%) characteristically exhibit a greater tendency for females to oviposit without mating and have a higher hatch success for unfertilized eggs than in populations with a normal sex ratio.

Geographic studies on other invertebrates in temperate regions have shown that unisexual populations generally occur at the periphery of the species range, usually to the north of the bisexual populations (Glesener and Tilman 1978). This pattern seems to result from a gradual dispersal northward following glaciation, with parthenogenetic populations preceding the bisexual populations (Hubbell and Norton 1978). Our data on *E. funeralis* are not completely consistent with this view because unisexual populations also occur at southern and southeastern peripheral areas (e.g. Pennsylvania, Delaware, Maryland, South Carolina). It is possible, however, that dispersal in all directions by *E. funeralis* occurs initially by parthenogenetic females, with sexually reproducing individuals dispersing at a much slower rate. Thus, populations with skewed sex ratios in Vermont and New Hampshire may represent a mixture of parthenogenetic and sexually reproducing individuals.

Cuellar (1977) suggests that parthenogenesis is more likely to occur at the periphery of the geographic range where hybridization and competition with the bisexual population would not impede establishment and expansion respectively of unisexual populations. Alternatively, unisexual populations may arise gradually by selection from tychoparthenogenesis (accidental or rare parthenogenenesis) in bisexual populations and situations that cause virgin females to lay eggs probably arise more frequently near the periphery of a species range (Templeton 1982). For example, founder events involving only virgin females are more likely to occur at the periphery because population densities may be low enough to make finding a mate difficult, or a local shortage or even total absence of males could occur in small populations just by chance (Stalker 1956, Templeton 1982).

Whether unisexual populations arise as single macromutations, gradually from bisexual populations, or through hybridization of two closely related species is still an area of considerable debate. Cuellar (1977) and Templeton (1982) suggest that many unisexual populations arise largely from tychoparthenogenesis in bisexual populations. Wright (1978) and many other workers feel that hybridization provides a better explanation for the origin of unisexual populations, especially since many parthenogenetic vertebrate species have the morphology, karyotype, and allozymes of hybrids. Many workers seem to assume that there is one all inclusive answer concerning the origin and value of parthenogenesis in vertebrate and invertebrate animals. This assumption appears generally to be unwarranted.

For *E. funeralis,* we cannot rule out completely any of the above hypotheses concerning the origin of parthenogenetic individuals. However, hybrid origin seems

unlikely because it usually results in a high degree of fixed heterozygosity at many loci and we observed very low levels of heterozygosity for unisexual populations. Although only two bisexual populations have been studied, the predominance of monomorphic loci suggest that polymorphisms observed in unisexual populations may have arisen as mutations within the unisexual populations. An equally plausible but less appealing interpretation in view of the data is to postulate multiple founder events for unisexual populations consisting of several distinct clones. Although more data are clearly needed for *E. funeralis,* it appears that individuals from both bisexual and unisexual populations were fixed or nearly fixed on the same allele frequencies of polymorphic loci. Thus, tychoparthenogenesis is probably the best working hypothesis for the origin of unisexual populations of *E. funeralis* because it occurs at substantial levels in practically every female tested from bisexual populations and the genetic structure of unisexual and bisexual populations seems to be very similar.

Acknowledgements – This work was supported by the U.S. Department of Energy (contract no. DE-AC-02-79 EV 10259), the National Science Foundation (contract no. DAR 78-18589), the Stroud Foundation, and the Francis Boyer Research Endowment. We thank the following individuals for invaluable field and laboratory assistance: B. Anderson, J. Bardsley, G. Davis, P. Dodds, S. Duczkowski, D. Funk, A. Graham, B. Green, M. Griffith, C. Hesterman, M. Horikawa, S. Hyatt, K. Kral, L. Petersen, J. Pierson, D. Rebuck, J. Richardson, and E. Wickersham.

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