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Population genetic structure of two mayflies (*Ephemerella subvaria, Eurylophella verisimilis*) in the Delaware River drainage basin

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Abstract. The population genetic structure of two species of mayflies was studied using protein electrophoresis at eight locations in the headwater region of the Delaware River drainage basin. Study sites were located upstream and downstream from two large reservoirs. The working hypothesis was that little or no genetic differentiation occurs between conspecific populations within and among tributaries of the same drainage basin. A total of 24 and 28 loci were examined for *Eurylophella verisimilis* and *Ephemerella subvaria* respectively. Geographic differentiation was significant for three of nine polymorphic loci (average F_{ST}=0.028) for *Ep. subvaria* when all populations were compared. Spatial variation in allele frequencies among populations of *Ep. subvaria* was substantially higher in the West Branch than in the East Branch of the Delaware River. The spatial cline of allele frequencies for certain loci of *Ep. subvaria* paralleled environmental gradients in the vicinity of the two reservoirs but no cause-effect relationship can be implied without more extensive data. Regardless, the results for *Ep. subvaria* failed to support the hypothesis. In contrast, geographic differentiation for *Eu. verisimilis* was low, being limited to one locus and caused by allelic abnormalities at one site (average F_{ST}=0.008). No significant spatial clines were observed for *Eu. verisimilis* among study sites. These data seem to be consistent with the hypothesis. There was no indication of disrupted gene flow caused by the reservoirs acting as barriers to dispersal by larval drift or adult flight in either species. However, additional data on species in drainage basins without potential barriers to gene flow are needed to adequately test the hypothesis.

Key words: reservoir, electrophoresis, genetic structure, Ephemeroptera, gene flow, heterozygosity, polymorphism.

Recent studies on stream populations of waterstriders (Zera 1981), black flies (Snyder and Linton 1984), and mayflies (Sweeney, Funk, and Vannote, unpublished data) show that significant allelic differentiation occurs among conspecific populations inhabiting different drainage systems. However, the amount of spatial variability in the genetic structure of insect populations within river drainage basins is presently unknown. We hypothesize that little or no genetic differentiation occurs between conspecific populations within and among tributaries of the same drainage basin. This hypothesis seems reasonable a priori because of the prevailing viewpoint that population sizes, dispersal, and gene flow are high enough within drainage basins to prevent differentiation caused by genetic drift and/or natural selective processes.

Since many species have extensive geographic ranges and are distributed throughout large drainage networks, it is clear that viable mechanisms exist for individual dispersal. The two principal mechanisms for dispersal of aquatic insects are larval drift and adult flight. Although larval drift seems to be an important mechanism for dispersing within drainage basins (Minshall and Petersen 1985). The extent of larval movement and subsequent reproductive success of individual immigrants are unknown (Sheldon 1984). There is some indication from recent studies that larvae may not drift very far in streams (Brussock 1986) and the viability of drifting invertebrates may be low owing to a relatively high incidence of parasitism (Statzner and Bittner 1983, Wilzbach et al. 1986).

It is generally assumed that adult flight is the most important means of long distance dispersal for aquatic insects (Sheldon 1984). However, few studies have actually measured absolute flight distances or estimated the number of individuals moving significant distances within or among drainage basins. Adult flights up to 0.6 km have been documented for the stonefly *Brachyptera risi* (Madsen and Butz 1976) and Coutant (1982) documented movements of about 16 km by adult caddisflies on the Colum-

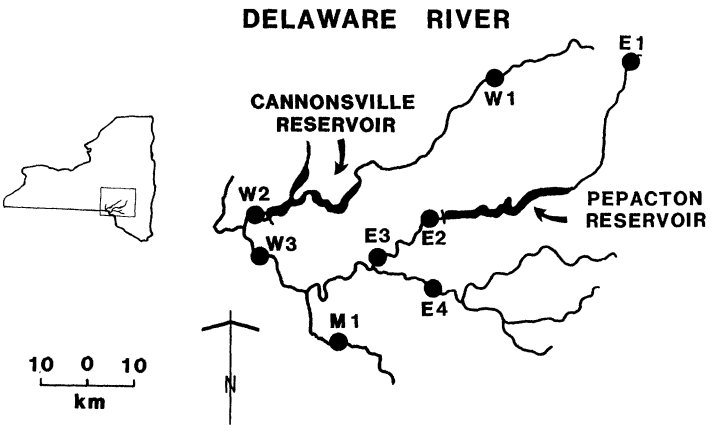

FIG. 1. The relative location of collecting sites near the Cannonsville and Pepacton reservoirs on the upper Delaware River. Inset map shows location of study area in New York State.

bia River, but few data are available for other aquatic insects. For some species, long distance movement by adults may be too rare to be measured by mark and recapture or similar techniques. The population genetic structure of a species reflects in part such movement. Theoretically, individuals sampled from two populations will be genetically similar if the populations have been connected by substantial gene flow. If gene flow is reduced, individuals from the two populations will begin to differentiate (through random genetic drift or selection) and genetic structuring will be higher. The extent of differentiation will depend on how long and to what extent the populations have been isolated and on the size of the populations (Pashley 1986).

This paper focuses on the population genetic structure of *Ephemerella subvaria* McDunnough and *Eurylophella verisimilis* (McDunnough) (Insecta:Ephemeroptera) at various locations throughout the upper drainage basin of the Delaware River, including sites above and below reservoirs on the two principal tributaries. With regard to our hypothesis that conspecific populations within drainage basins lack genetic differentiation, this basin is a less than ideal test site because: (i) the presence of reservoirs could disrupt larval and adult dispersal between some sites; and (ii) physical and chemical alteration of the downstream area might differentially affect survival and/or reproductive success of individuals in the population.

We chose this basin to test our hypothesis for two reasons. First, we have found significant differences in certain life history parameters (timing of adult emergence, adult size, fecundity) for both *Ep. subvaria* and *Eu. verisimilis* from various localities in this basin, especially at sites immediately downstream from the reservoirs. We wanted an independent assessment of whether these species were panmictic in the basin. This would help us evaluate the probability that observed life history differences were largely ecophenotypic.

Second, data supporting the hypothesis that were collected in a river system containing substantial barriers to dispersal would be irrefutable and readily extrapolated to natural basins. The risk, of course, is that the hypothesis would not hold in the perturbed system and would remain essentially untested for natural drainage basins.

Methods

Study sites

Eight sampling sites were selected in the upper Delaware River basin (Fig. 1). The Cannonsville and Pepacton dams, which are located on the West and East Branches of the Delaware River respectively, release hypolimnetic water that significantly alters the annual temperature regime of downstream reaches, especially during the summer months (Fig. 2). During the late 1970s, New York State established a program to assure that enough water

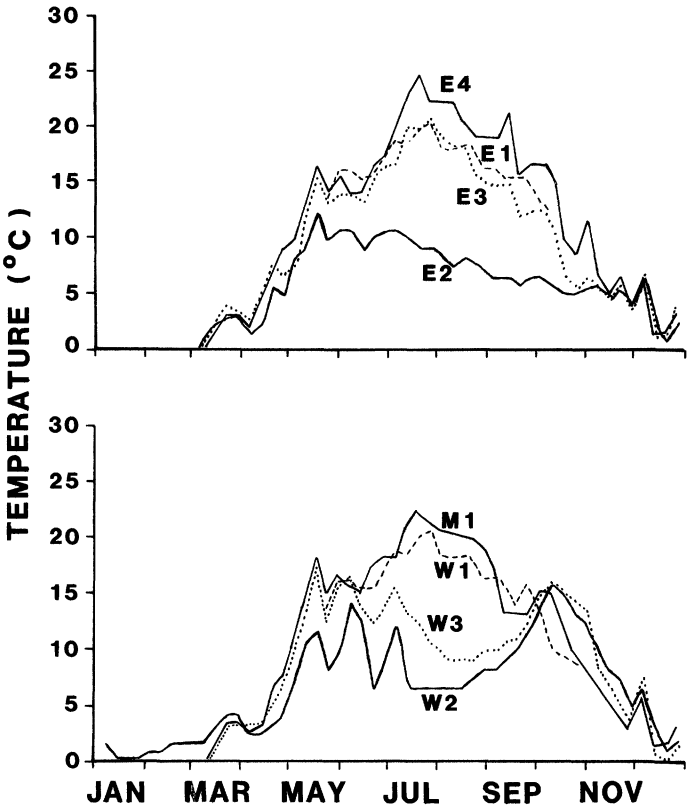

FIG. 2. Seasonal pattern of temperature at six study sites on the Delaware River. No data were collected for sites E1 and W1 during the November-May period.

was released from both reservoirs during all seasons to keep the river bottom wetted from bank to bank in all downstream areas. This program has greatly increased the amount of habitat available for insect colonization and has made the seasonal temperature cycles in downstream reaches more consistent from year to year.

The West Branch of the Delaware River has a higher average discharge than the East Branch (11.8 versus 5.6 m^3/s). In addition, the New York State release program mandates that the Cannonsville reservoir on the West Branch be the principal source of makeup water if the discharge on the mainstem falls below a critical level needed for recreation and consumptive use. Thus, the thermal gradient below the Cannonsville reservoir generally extends farther downstream than on the East Branch below the Pepacton reservoir.

Sampling sites included: (i) two stations (W1, E1) located about 45 and 35 km upstream from the Cannonsville and Pepacton reservoirs respectively; (ii) two stations located about 7 km below the dams on the West (W2) and East (E2) Branches; (iii) two stations located about 14 and 22 km below the dams on the West (W3) and East (E3) Branches respectively; (iv) one station on a large unimpounded tributary (Beaverkill River: E4) of the East Branch that is about the same size as E3 and enters downstream of E3; and (v) one station on the mainstem of the Delaware river (M1) about 13.8 km downstream from the confluence of the West and East Branches (i.e., about 43 and 73 km downstream from the Cannonsville and Pepacton dams respectively).

Study species

Ephemerella subvaria larvae inhabit rocky areas of riffles in medium size streams to large rivers

(ca. 3rd–9th order) in eastern North America. *Eurylophella verisimilis* larvae occur in rivers of similar size in the same region but are found largely on organic debris in areas of slow current. Both species feed on algae (diatoms) and fine particles of organic detritus (Sweeney and Vannote 1981). The two species occur at all eight sampling locations but *Eu. verisimilis* was too rare at the mainstem river site (M1) to obtain a sufficient number of specimens for electrophoresis.

Electrophoresis

Mature larvae from the various collection sites were returned alive to the laboratory, separated according to species, and reared until metamorphosis to the winged true adult stage (Sweeney and Vannote 1981 describe methods for rearing mayflies). Each adult 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% ethanol) larval exuviae collected soon after metamorphosis. Larval exuviae served as a taxonomic reference collection for the study because they contain most of the important morphological characteristics used for identifying *Ep. subvaria* and *Eu. verisimilis*.

Allozymes were separated by horizontal starch gel electrophoresis using methods similar to those described by Selander et al. (1971) with recipes from Shaw and Prasad (1970) and Harris and Hopkinson (1976). A maximum of 23 enzymes including 28 presumptive gene loci were assayed for each specimen. Bands were interpreted as alleles and frequencies were calculated for each allele. All presumed allelic homologies were verified in subsequent tests by running a few individuals from each of several populations of a given species on the same gel. A locus was considered polymorphic if the frequency of the most common allele was <0.95.

The F-statistics (Wright 1978) for inbreeding (F_{IS}) and geographic differentiation (F_{ST}) of populations were calculated using formulae described for f and θ, respectively (Weir and Cockerham 1984). These formulae contain correction factors for small number of populations and unequal and small sample sizes. For these calculations, multiallelic data were reduced to diallelic form by combining all of the less common alleles. The significance of observed f and θ values was tested using chi-square statistics described in Baker (1981).

The relative levels of gene flow between conspecific populations were determined from the spatial distribution of private alleles as described by Slatkin (1985). This method assumes that high levels of gene flow will result in a large number of shared alleles among populations and a low average frequency for private alleles (i.e., those that occur in only one population). The following formula (taken from Slatkin 1985) was used to estimate Nm, the number of immigrant individuals in a population, where N equals population size and m the fraction immigrating:

$$\ln[p(1)] = a[\ln(Nm)] + b$$

with a=0.505, b=−2.440, and p(1) = average frequency of alleles occurring in only one population. Values of a and b are for sample size of 25. The estimate of Nm can be corrected for different sample sizes using Caccone's (1985) method. For this analysis, the evolutionary importance of gene flow depends on both m and Nm as well as on the selection intensities at different loci. Although it is not possible to estimate m separately without knowing the effective population size, Slatkin's (1985) estimate of Nm yields a quantitative estimate of average gene flow in a subdivided population that can be compared among different groups of organisms. Nm values >1.0 indicate relatively high gene flow.

Results

Ephemerella subvaria

Nine of the 28 study loci were polymorphic in at least one population of *Ep. subvaria* (Table 1). The inbreeding coefficient (F_{IS}) was significant and positive for two loci (*Aat1, Lap2*), indicating a deficiency of heterozygotes across the eight study populations. None of the negative F_{IS} values was statistically significant.

Geographic differentiation (F_{ST}) was significant for three of the nine polymorphic loci (*Aph, Gdh, Est4*) when all eight populations were compared (Table 1). Allele 0.97 of *Aph* was generally rare (frequency <0.1) for populations

TABLE 1. Allele frequencies of polymorphic loci for populations of *Ephemerella subvaria* sampled at various locations in the Delaware River. Wright's (1978) F-statistics describe geographic variation (F_{ST}) and inbreeding (F_{IS}) for all eight populations. An asterisk indicates statistical significance ($p<0.05$).

		Study Populations on Delaware River									
		East Branch				West Branch			Main-stem	F-statistics	
Poly-morphic Loci[a]	Allele	E1 n=25	E2 n=25	E3 n=18	E4 n=25	W1 n=25	W2 n=25	W3 n=25	M1 n=25	F_{ST}	F_{IS}
Mdh1	1.24		0.04				0.04		0.08	0.024	−0.042
	0.94	1.00	0.96	1.00	1.00	1.00	0.96	1.00	0.92		
Sod1	1.21	0.02		0.06	0.06				0.02		
	1.09	0.98	1.00	0.94	0.94	1.00	1.00	1.00	0.98	0.010	−0.018
Aph	1.00	0.92	0.88	0.78	0.88	0.98	0.84	0.72	0.64	0.054*	0.086
	0.97	0.08	0.12	0.22	0.12	0.02	0.16	0.28	0.36		
Gdh	1.00	0.34	0.30	0.39	0.35	0.26	0.20	0.32	0.10	0.052*	−0.008
	0.75	0.66	0.70	0.61	0.65	0.74	0.80	0.68	0.90		
Mpi	1.31		0.02								
	1.26	1.00	0.98	1.00	1.00	1.00	0.94	0.96	0.98	0.007	−0.023
	1.17						0.06	0.04	0.02		
Aat1	1.36				0.02	0.06			0.10		
	1.00	1.00	1.00	1.00	0.98	0.94	1.00	1.00	0.90	0.032	0.415*
Lap2	0.93	0.94	0.90	1.00	0.94	0.82	0.92	1.00	0.84	0.026	0.303*
	0.86	0.06	0.10		0.06	0.18	0.08		0.16		
Est4	0.68	0.04	0.12	0.06	0.14	0.08	0.18	0.20	0.06		
	0.65	0.90	0.86	0.88	0.80	0.86	0.72	0.64	0.92	0.048*	0.076
	0.59	0.04	0.02	0.06	0.06	0.06	0.10	0.14			
	0.54	0.02						0.02	0.02		
Est6	0.34	0.22	0.28	0.08	0.20	0.18	0.14	0.18	0.08		
	0.28	0.34	0.38	0.53	0.52	0.40	0.56	0.38	0.40	0.004	0.091
	0.23	0.38	0.32	0.33	0.26	0.40	0.30	0.36	0.38		
	0.15	0.06	0.02	0.03	0.02	0.02		0.08	0.14		
	0.12			0.03							

[a] Monomorphic loci were as follows: *Mdh2, aGpdh, Hex, Gpi, Ao, Sod2, G6pdh, Me, 6Pgd, Pgm, Xdh, Isdh1, Isdh2, Ald, Ldh, Aat2, G3pdh, Lap3, Fum.*

upstream from both dams but gradually increased to significantly higher frequencies (arcsine test; $p<0.05$) in a downstream direction. Conversely, the frequency of allele 0.68 of *Est4* was greatest immediately below the dams and generally decreased in frequency in both an upstream and downstream direction.

Spatial variation in allele frequencies seemed greater among populations in the West Branch relative to those in the East Branch of the Delaware River (Table 1). For example, the range of frequencies observed among sites within a given branch was generally larger for West Branch populations relative to the East Branch for the following alleles: allele 0.97 of *Aph* (range: 0.02–0.36 vs. 0.08–0.22), allele 0.75 of *Gdh* (range: 0.68–0.90 vs. 0.61–0.70), allele 0.86 of *Lap2* (range: 0–0.18 vs. 0–0.10), allele 0.59 of *Est4* (range: 0–0.14 vs. 0.02–0.06), allele 0.15 of *Est6* (range: 0–0.14 vs. 0.02–0.06). This tendency was also reflected by differences in F_{ST} values when various subsets of populations were compared (Table 2). When these comparisons were arranged in descending order according to their respective F_{ST} values, the highest levels of geographic differentiation were associated with comparisons involving West Branch and mainstem populations. For example, four times more variation was observed among the three sites on the West Branch (F_{ST}=0.029 for sites W1, W2, W3) than on the East Branch (F_{ST}=0.006 for sites E1, E2, E3). The amount of variation

TABLE 2. Average geographic differentiation (F_{ST}) in allele frequencies and estimated gene flow (*Nm*) between various subsets of populations of *Ep. subvaria* and *Eu. verisimilis* in the Delaware River. Horizontal lines indicate those sampling sites included in the analysis of F_{ST} and *Nm*. ND = no data because the species occurs rarely at site M1.

Comparison	Study Populations on Delaware River: East Branch E1	E2	E3	E4	West Branch W1	W2	W3	Mainstem M1	*Ep. subvaria* Avg. F_{ST}	*Nm*	*Eu. verisimilis* Avg. F_{ST}	*Nm*
1					—	—	—	—	0.029	4.6	0.003	6.8
2		—	—	—		—	—	—	0.029	12.4	ND	ND
3					—	—	—	—	0.028	18.4	ND	ND
4	—	—	—	—	—	—	—	—	0.028	11.8	ND	ND
5	—	—	—		—	—	—	—	0.021	11.8	ND	ND
6	—	—	—					—	0.021	4.2	ND	ND
7	—	—	—		—	—	—		0.018	5.8	0.003	18.4
8	—	—	—	—	—	—	—		0.017	11.8	0.008	10.9
9	—			—	—	—			0.016	18.4	0.007	11.8
10	—	—	—						0.006	10.2	0.008	8.2
11	—	—	—	—					0.005	11.8	0.002	10.9

among all study sites (comparison 4; F_{ST}=0.028) was not different from that observed when sites upstream from the reservoirs were deleted (comparison 2; F_{ST}=0.029).

Eurylophella verisimilis

The proportion of study loci that was polymorphic (11 of 24) was higher in *Eu. verisimilis* than in *Ep. subvaria* in the Delaware River. Heterozygote deficiency was also observed for three loci (*aGpdh, Mpi, Est5*) when F_{IS} values were calculated over all seven study populations (Table 3). Geographic differentiation (F_{ST}) was significant for only one locus (*Ao*) and was caused by allelic abnormalities at one site (E4). There was a tendency for the frequency of allele 0.80 of locus *Me*, 0.70 of locus *Ald*, and 0.37 of *Est4* to decline with downstream distance in both the East and West Branches of the Delaware River. However, these trends were not statistically significant. Genetic differentiation for *Eu. verisimilis* was low throughout the study area and no differences were observed between the East and West Branches (Table 2).

Discussion

The hypothesis that little or no genetic differentiation occurs between conspecific populations within and among tributaries of the same drainage basin was not fully supported by this study. For *Ep. subvaria*, significant subdivision among populations (F_{ST}) was indicated at three of nine polymorphic loci studied. Although the overall amount of genetic differentiation among local populations was low (F_{ST}=0.028), it was substantially higher than we would have predicted a priori based on levels of differentiation for populations distributed throughout the geographic range of *Ep. subvaria* (F_{ST}=0.064; unpublished data). The apparent cline in allele frequencies for loci *Aph* and *Est4* on the West Branch correlates well with known environmental gradients associated with the Cannonsville reservoir (e.g., Fig. 2). However, the geographic scope of the study, which was chosen arbitrarily owing to the general lack of data on population structure for stream biota, was inadequate, and data from additional sampling sites (especially further downstream) are needed to clearly resolve the nature of the cline and any possible relationship with the reservoir. The fact remains for *Ep. subvaria*, however, that allele frequencies at certain loci vary significantly at sites on the Delaware River and most of the variance in population structure is associated with sites below the reservoirs, especially on the West Branch.

Genetic differentiation among local populations of *Eu. verisimilis* was substantially lower than for *Ep. subvaria* (F_{ST}=0.008 vs. 0.028). It was

TABLE 3. Allele frequencies of polymorphic loci for populations of *Eurylophella verisimilis* sampled at various locations in the Delaware River. Wright's (1978) F-statistics describe geographic variation (F_{ST}) and inbreeding (F_{IS}) for all seven populations. An asterisk indicates statistical significance ($p<0.05$). ND = no data.

		Study Population on Delaware River								
		East Branch				West Branch			F-statistics	
Polymorphic Loci[a]	Allele	E1 n=30	E2 n=59	E3 n=18	E4 n=29	W1 n=36	W2 n=30	W3 n=30	F_{ST}	F_{IS}
aGpdh	1.00	1.00	0.99	1.00	0.95	0.92	0.95	0.98	0.015	0.255*
	0.78		0.01		0.05	0.08	0.05	0.02		
Hex	1.06	0.02	0.02			0.03	0.07	0.03		
	1.00	0.98	0.98	1.00	1.00	0.97	0.93	0.97	0.004	−0.026
Ao	1.00	1.00	1.00	1.00	0.93	1.00	1.00	1.00	0.056*	−0.058
	0.85				0.07					
Pgm	1.08		0.01		0.02	0.02		0.03		
	1.04				0.02					
	1.00	0.98	0.99	0.97	0.94	0.94	0.98	0.95	0.000	−0.024
	0.95	0.02		0.03	0.02	0.02	0.02	0.02		
	0.93					0.02				
G6pdh	1.00	0.93	0.97	0.94	1.00	0.96	0.98	0.98	0.000	0.107
	0.81	0.07	0.03	0.06		0.04	0.02	0.02		
Me	1.17		0.01	0.03	0.03					
	1.00	0.88	0.91	0.94	0.88	0.86	0.85	0.97	0.002	0.029
	0.80	0.12	0.08	0.03	0.09	0.14	0.15	0.03		
Mpi	1.10		0.02	0.03						
	1.00	0.93	0.97	0.92	0.98	0.94	0.98	0.98	0.000	0.209*
	0.89	0.02								
	0.81	0.05	0.01	0.05	0.02	0.06	0.02	0.02		
Ald	1.00	0.85	0.90	0.97	0.97	0.90	0.90	0.95	0.006	−0.043
	0.70	0.15	0.10	0.03	0.03	0.10	0.10	0.05		
Lap2	1.06				0.02			0.02		
	1.00	0.90	0.89	0.92	0.81	0.89	0.92	0.85	0.000	0.077
	0.93	0.08	0.11	0.08	0.17	0.11	0.08	0.13		
	0.89	0.02								
Est4	0.55						0.02	ND		
	0.52	0.02								
	0.50	0.45	0.29	0.33	0.38	0.50	0.54			
	0.47	0.03	0.09	0.03	0.05	0.05	0.05			
	0.45	0.35	0.29	0.47	0.22	0.24	0.18			
	0.42	0.02				0.03				
	0.40	0.03	0.24	0.17	0.21	0.11	0.18			
	0.37	0.10	0.09		0.14	0.07	0.03			
Est5	0.38				0.02					
	0.36		0.02			0.01				
	0.32	0.13			0.12					
	0.30	0.47	0.46	0.53	0.50	0.56	0.52	0.60	0.000	0.216*
	0.25				0.03					
	0.23	0.40	0.50	0.47	0.33	0.43	0.48	0.40		
	0.17		0.02							

[a] Monomorphic loci were as follows: *Mdh1, Mdh2, Sod1, Sod2, Gpi, 6Pgd, Isdh1, Isdh2, Xdh, Acp, Aph, G3pdh, Est1.*

also significantly lower than observed levels of differentiation among populations throughout the geographic range of *Eu. verisimilis* (F_{ST}=0.008 vs. 0.12; unpublished data). No loci showed any clinal tendency and the presence of a unique, rare allele (frequency 0.07) at one locus (*Ao*) for one study site was the only significant deviation among populations. Many polymorphic loci showed little variation in allele frequencies among study sites. Thus, despite potential barriers to gene flow within the study area, the population genetic structure of *Eu. verisimilis* seems to approach a panmictic situation as predicted by the hypothesis.

Any factor that alters migration rates and the effective size of the populations could conceivably affect population structure through genetic drift or by compounding the effects of selective mortality. We propose that reservoirs can reduce both migration rates and population size of aquatic insects in several ways. First, the physical presence of the reservoir can act as a barrier to migrating individuals. For our species, this barrier is probably complete for larval migrants because their flow and habitat requirements are not met by the lake-like reservoir environment. Although the barrier may not be complete for adult migrants, dispersal between populations separated by the reservoir could be affected by the increased flight distance. A general lack of information concerning movement of aquatic insects within and among basins makes it difficult to put our results into proper perspective. We found no evidence of an indirect effect of the Delaware River reservoirs on our study populations. The amount of genetic differentiation for either *Ep. subvaria* or *Eu. verisimilis* did not increase when sites upstream from the reservoirs were added to the analysis (Table 2, comparison 2 vs. 4). Thus, most variation was associated with the six sites below the reservoirs which are not separated by any physical barrier.

Differences in the magnitude and seasonal pattern of water temperature among study sites, which result from reservoir discharge (e.g., Fig. 2), can also affect the relative success of migration in terms of actual gene flow. For example, the timing and duration of adult emergence in the spring for both *Ep. subvaria* and *Eu. verisimilis* (and other aquatic insects) is altered by several weeks in river reaches near the cold-water discharge of the reservoirs. This lack of temporal synchrony among river reaches increases the probability that an individual (especially males) will disperse to a site either too early or too late to find a mate and reproduce successfully. This phenomenon could significantly subdivide the Delaware River population, with each subpopulation having a substantially smaller effective size.

Estimates of gene flow (Slatkin 1985) among study populations were high (i.e., $Nm>1.0$) for both *Ep. subvaria* and *Eu. verisimilis* (Table 2) despite the potential barriers discussed above. High gene flow seems consistent with the nearly panmictic population structure observed for *Eu. verisimilis* but is inconsistent with the extent of genetic differentiation observed for *Ep. subvaria*. However, any conclusions drawn from the gene flow analysis are tentative because the broad applicability of Slatkin's (1985) model for estimating gene flow has not been substantiated (Pashley and Johnson 1986). For example, Slatkin's (1985) model, which is based on the occurrence and frequency of private alleles, assumes selective neutrality of all study loci. Two results make this assumption suspect for our study on *Ep. subvaria*: (1) the clinal pattern of allele frequencies for two loci (*Aph, Est4*); and (2) the significant deficiency of heterozygotes ($+F_{IS}$ values) for some but not all loci. Although our data are not rigorous enough to argue that a selection gradient exists for any of our study loci in the vicinity of the reservoirs, others have implicated selection as a plausible explanation for spatial clines in allele frequencies and/or differentiation in F_{IS} values among loci (Barker et al. 1986, Watt 1985).

Although we do not have quantitative data concerning the density or size of populations at any of the study sites, we have observed changes in certain life history characteristics of both species below the reservoirs (unpublished data). For example, adult size and fecundity of *Ep. subvaria* gradually increases almost two-fold at sites downstream from the reservoirs. Conversely, adult size and fecundity of *Eu. verisimilis* exhibits a gradual two-fold decrease in a downstream direction from the dams. Consistent with these trends are qualitative observations that *Ep. subvaria* are rare at sites (E2, W2) immediately below the dams, whereas *Eu. verisimilis* are least abundant at the most downstream site (M1). A key question with respect to this type of life history variation is whether

or not there is a heritable basis to it. The most useful quantitative genetic technique to assess this question is a full-sib or half-sib breeding design (Falconer 1981). Thus, the life history parameters of interest are studied on individuals collected from separate geographic locations but kept under common conditions. This method allows direct estimation of genetic variability in fitness traits. However, the logistics of this approach for benthic aquatic organisms, especially univoltine species are substantial (e.g., problems ranging from inducing successful mating and reproduction in the laboratory to rearing lotic species for long periods of time).

Enzyme electrophoresis, as described in this paper, is an alternate method of assessing the genetic structure of populations. It is most commonly used to evaluate: genetic similarity among populations separated by various geographic distances, levels of gene flow between conspecific populations, the breeding structure of local populations, and the level of gene diversity in natural populations; it is also used to assess the possibility of selection as a result of environmental variation. The effects of selection on allozyme divergence must be carefully assessed, and the contribution of selection to observed differences can be somewhat controversial (Kimura 1983, Pashley 1986). However, Cavener and Clegg (1981) have clearly shown that natural selection can discriminate among allozymes of a given genetic locus.

We cannot use the lack of allozyme variation among Delaware River populations of *Eu. verisimilis* to prove that observed variation in life history characteristics among sites is ecophenotypic. However, we can use the electrophoretic data to: (i) assess (independent of morphology) the systematic relationship of study populations (i.e., assess whether the observed variation in life history characteristics is intraspecific); (ii) confirm the presence of (or lack of) spatial clines in allele frequencies for enzyme loci with a proven genetic basis that parallel spatial variation in life history characteristics; (iii) yield a quantitative estimate of the amount of overall genetic divergence that has occurred between the study populations; and (iv) estimate the relative amount of gene flow between the study populations. Thus, although we cannot use electrophoresis to unequivocally determine what proportion of the variability in fitness characters is genetic versus phenotypic, we can use it to provide ancillary information that bears directly on the issue.

Enzyme electrophoresis is a valuable tool that should be used more extensively by benthic ecologists in their studies of natural populations. Here we used it to assess the degree of genetic structuring among populations within a drainage basin. The results were somewhat ambiguous (i.e., little structuring in one species and substantially more in another). However, the ambiguity of our results was related largely to inadequacies of the test basin and spatial arrangement of sampling sites rather than to the experimental approach using electrophoresis. The data presented in this paper do not provide an adequate test for our initial hypothesis concerning the lack of genetic differentiation within drainage basins. They do, however, provide a starting point that we (and, we hope, others) will build on to test the hypothesis and broaden our fundamental understanding of the population structure of benthic organisms.

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