MAYFLY POPULATION DENSITY, PERSISTENCE AND GENETIC STRUCTURE IN FRAGMENTED HEADWATER HABITATS

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Abstract

We assessed the effects of stream habitat loss and fragmentation on the density, genetic diversity and persistence of a mayfly (*Ephemerella inconstans* Traver, 1932) in 24 first-order streams across nine headwater stream networks in Maryland and Virginia. We present differences in population density and local extinction in forested versus deforested headwater streams, as well as a preliminary analysis of genetic diversity in populations of *E. inconstans* and three closely related species. Because the sampling period spanned two years of drought (2001–2002) followed by two years of recovery (2003–2004), we predicted that mayfly density would be higher and population extinction rates lower at forested sites compared with deforested (agricultural and residential) sites. We found no difference in initial density at forested and deforested sites and no difference in the level of population decline across all sites by the end of the drought. However, one year after the drought had ended, population density was significantly higher in forested streams compared with streams flowing through agricultural and residential areas. Further, while only 1 of 11 populations at forested sites remained extirpated in 2004, populations in 4 of the 13 deforested streams were extirpated at the end of the study. These results suggest that recovery and recolonization following a major regional disturbance was more successful in the intact, forested stream networks than in the altered networks. To examine the population genetic effects of the demographic decline, extinction events and post-drought recovery, we sequenced a region of mitochondrial DNA in 10 populations. However, we found very low polymorphism across the entire 200 km range of the study, suggesting that a prehistoric bottleneck occurred in this species.

Key words: Ephemeroptera; *Ephemerella*; dispersal; population extinction; first-order streams; population density; genetic diversity.

Introduction

Extensive deforestation of small watersheds in the Mid-Atlantic Piedmont region of North America has altered the structure and function of headwater streams by reducing their number, disrupting critical ecosystem processes, and fragmenting surviving headwaters into isolated or semi-isolated habitat patches. Predicting the

impact of habitat loss on aquatic insect species depends, in part, on understanding how individuals move among resource patches and population units (Turner et al. 2001, Goodwin and Fahrig 2002). Some movements, such as annual migration, are predictable; others, such as stream drift by insects, are responses to random events or local conditions (Humphries 2002, Ledger et al. 2002, Anholt 1995). Whatever the reason and mechanism, the movements of individuals shape the spatial structure of populations and the species, and play an important role in their persistence (Hanski and Ovaskeinen 2002, Lowe 2002).

Headwaters are naturally patchy habitats that support diverse communities of aquatic insects. Individual headwater streams may be only a few hundred meters in length and flow through watersheds less than one square kilometer in area. However, in their natural state in the Mid-Atlantic Piedmont region of North America, headwaters rarely exist in isolation; rather, they form complex networks covering large areas over which flow is diffused through a dendritic network of many small channels. Preserving natural connections for movement of individuals can significantly affect the population extinction probabilities in habitats with this particular geographic structure (Fagan 2002).

Small as they are, headwater streams and their associated wetlands perform multiple ecological functions of critical importance to the larger ecosystem. Headwaters retain sediment and slow runoff; recharge groundwater sources; take up chemicals and excess nutrients that would otherwise be transported to bays, lakes and oceans; process and transport beneficial organic matter to downstream ecosystems; and provide refuge and habitat for mayflies and other aquatic organisms (Peterson et al. 2001, Wallace et al. 1997). Recent surveys estimate that headwater streams comprise at least 80% of total stream miles in the United States (Meyer et al. 2003) and at least 66% of stream miles in Maryland (MDNR 1997, 2001). However, because of their small size and ubiquitous presence in areas with high dollar-value real estate, headwater networks in the Central Piedmont are highly susceptible to development. Viewed as nuisances to some property owners, the wet lowlands and small channels associated with headwaters are filled, diverted or piped underground to prevent flooding of roads, fields, lawns and buildings. In recent years, simplification and degradation of small stream networks in Maryland and Virginia has greatly reduced the quantity and quality of habitats available to headwater-specific organisms (MDNR 1997, 2001).

We tracked 24 populations of the mayfly *Ephemerella inconstans* Traver, 1932, over two to four generations to ask how recent changes in the landscape structure of headwater steam networks have affected the interaction and persistence of mayfly populations living in them. Here we present results of a study of population density and patch extinction, an initial study of genetic diversity among the sampled populations of the species *E. inconstans* and two other species recently synonymized with *Ephemerella invaria* (Walker), 1853 (Jacobus and McCafferty 2003), and plans for the continuing the population genetic and phylogenetic analyses in the coming year.

Methods and Materials

Study Organism. The genus *Ephemerella* is Holarctic in distribution, encompassing a range of Europe and nontropical Asia, Africa north of the Sahara and North America south to the Mexican desert region. Until recently it was thought that the species *E. inconstans* was found only in the southeast United States, in the Central Piedmont and Southern Appalachian regions (Allen and Edmunds 1965). A revision by Jacobus and McCafferty (2003) recognizes eight previously distinct species, including *E. inconstans*, as morphological synonyms of the more widely distributed species *E. invaria*. For clarity in the presentation of molecular data for *E. invaria* synonyms, and because the project described herein was well underway before the 2003 revision, thus we use the historical species names.

The naturally patchy distribution of headwater stream habitat is reflected in the population distribution of *E. inconstans* in the states of Maryland and Virginia, USA. In a preliminary survey of first-to-third order streams in the Maryland Piedmont in 2001, *E. inconstans* was common throughout the area but was found only in small, relatively undisturbed streams (unpublished data). Based on the 2001 survey, the preference of *E. inconstans* for headwater habitat over larger streams appears to depend more on flow regime and substrate than on nutrient levels or chemical composition of the stream water (unpublished data). This species was selected for the project because its distribution, habitat preferences, univoltine life cycle, synchronized emergence, equal sex-ratio (determined through emergence trapping) and limited flight period make it a good model for examining the role of dispersal in the patch dynamics of insects in headwater stream networks.

Study Sites. A total of 24 headwater streams in 9 headwater stream networks were sampled. Ten streams were sampled in 2001, 20 streams in 2002 and 24 streams in both 2003 and 2004. The names and locations of the study sites, which fall within four major river watersheds in Maryland and Virginia, are provided in Table 1. Each stream network consists of one to four adjacent headwater streams containing one or more populations of *E. inconstans*. All study sites are located within the region of Central Piedmont between 37ϒ20' and 39ϒ20' latitude, bounded to the west by the Appalachian mountains and to the east by the Coastal Plain. Although lengths and flow regimes vary among the streams due to differences in local topography, groundwater sources and land use, a typical stream in this study drains an area < 1 km^2 with baseflow discharge < 0.03 m^3/s.

Sampling. In 2001, 2002 and 2004, nymph samples were collected using moss-packs (colonizing samplers) consisting of a fixed amount of dried moss enclosed in plastic mesh bags and tied with string to roots or stakes along the stream margin for a period of three weeks in March and April, when late instar *E. inconstans* nymphs are present in the stream margins. Moss-packs are designed to move freely with stream flow to

imitate natural moss or root-wad habitats. They are readily colonized by *E. inconstans* and other aquatic invertebrate taxa. Eight moss-packs were placed in each stream, positioned in pairs along a 75 m reach so that a total of four subsamples were taken in each stream.

Table 1. Sample sites and years sampled.

County and State (USA)	8-Digit Watershed (HUC#)	Headwater stream network identifier	No. of streams sampled			
			2001	2002	2003	2004
Baltimore MD	Patapsco River Lower North Branch (02130906)	Daniels Creek	-	3	3	3
		MPEA	3	3	3	3
	Middle Patuxent River (02131106)	Homewood	-	1	2	2
Howard MD		UMD Dairy Farm	1	3	3	3
	Rocky Gorge Dam (02131107)	Rocky Gorge	1	1	1	1
	Brighton Dam (02131108)	Cattail Creek	2	3	3	3
Montgomery MD	Seneca Creek (02140208)	Little Seneca	1	3	3	3
Appomattox VA	Appomattox River (02080207)	Saunders Creek	2	3	3	3
Buckingham VA	Slate River (0208020)	Jamison Creek	-	-	3	3
		Total per year	10	20	24	24

Samples were bagged in stream water and sorted while specimens were still alive. Ephemerellid mayflies were identified and sorted by species using Allen and Edmunds' key (1965), counted and stored in 100% ethyl alcohol at –20ϓC. When a stream sample contained fewer than 16 individuals of *E. inconstans*, that stream was resampled with a D-frame net to increase the size of the sample available for population genetic analysis. The extra samples were labeled appropriately, stored separately from the moss-pack samples and excluded from the population density counts.

In 2003, nymph samples were collected with a D-frame net. From a comparison of samples taken using both methods in one stream, active search with a D-frame net produced larger sample counts and thus would overestimate the density relative to the mosspack samples. To make the D-frame samples comparable to the moss-pack samples for categorical estimates of population density, D-frame sampling for 2003 season was constrained to three 25-m sections selected at random from a 150-m stream reach. All suitable habitats in the substrate and stream margins within the three randomly selected sections were sampled extensively. Processing of samples in 2003 was done as in 2001, 2002 and 2004, described above.

Population Density and Persistence. Nymph sample counts were converted to a categorical variable with four levels: none (sample count=0), rare (0 < sample count < 10), common (10 ≤ sample count < 20) and abundant (sample count ≥ 20). The counts in each density category were plotted to visually check for trends in the density distribution in forested versus deforested streams within each year. The density categories were then combined to create two broader density categories: low (sample count < 10) and high (sample count ≥ 10), to compare the densities in forested and deforested streams using Fisher's Exact Test. A separate statistical test was conducted for each of the last three years (2002, 2003, 2004). The results of 2002 reflect density during the drought; the results of 2003 reflect the population response to the final year of drought (summer 2002); and the results of 2004 reflect the population recovery one year after the end of the drought.

Genetic Analysis: E. inconstans. We sequenced a portion of the mitochondrial DNA (mtDNA) cytochrome oxidase (CO) I gene in 10 populations in along a north-south gradient in Maryland and Virginia to determine the scale at which regional genetic variation occurs in this species. Mitochondrial DNA markers were selected because a high mutation rate and maternal inheritance result in a smaller effective population size, so that mtDNA can accumulate population genetic variation at a faster rate than nuclear DNA. This process, which occurs over long periods of time, provides the background of genetic variation against which reductions in diversity that occur in more recent time-frames may become visible. Also, mtDNA haplotypes make it possible to separate population history from current population structure through the use of gene genealogies. This is a powerful factor in separating effects from historical events, such as geographic isolation due to formation of major land barriers, from recent events, such as habitat fragmentation. Mitochondrial DNA is also relatively easy to use, providing results more rapidly than methods that require development of new markers (e.g., microsatellites), and has been used successfully in other studies of small scale aquatic insect population genetics (e.g., Hughes et al. 2003, Galacatos et al. 2002, Myers et al. 2001)

Genetic Analysis: E. invaria. To estimate genetic similarity among the historical species recently synonymized with *E. invaria,* we also sequenced samples of *Ephemerella rotunda* Morgan, 1911, *Ephemerella floripara* McCafferty, 1985 and *E. invaria*. Tissue specimens for this analysis were obtained from the primary author of the 2003 revision.

Results

Population Density and Persistence. The population density distribution for each year in (a) all streams; (b) forested streams only; and (c) deforested streams only, is described and plotted in Fig. 1. The plot of combined sites (Fig. 1a) shows the general trend of population decrease during the drought, followed by population increase during recovery. The apparent symmetry of the combined response is a composition of inverse patterns of response by forested streams and deforested stream populations (Figs. 1b and 1c). One year after the drought (2004), 91% of forested streams were classified as having high (= abundant + common) mayfly density, none were classified rare and the population in one of the 11 streams (9%) was extinct. In the deforested streams, 46% of streams were classified as high density, 23% were rare and populations in four of 13 streams (31%) were extinct. Fisher's Exact Tests of categorical density in forested and deforested sites within each year (Table 2) show that the population density does not differ between forested and deforested sites in years 2002 or 2003 (p=0.4 and p=0.6), indicating that streams could not be distinguished by site (forested or unforested) at the start of the study or at end of the drought. However, one year after the drought had ended (spring 2004) the proportion of streams with high population density was significantly greater in forested than in deforested streams (p<0.04).

Table 2. Fisher's Exact Test of density across sites, within years. Test of Site (Forested, Unforested) by Density (Low=None + Rare, High=Common +Abundant).

a. 2002	Low	High	b. 2003	Low	High	c. 2004	Low	High
Forested	1	7	Forested	5	6	Forested	1	10
Deforested	4	8	Deforested	9	4	Deforested	7	6

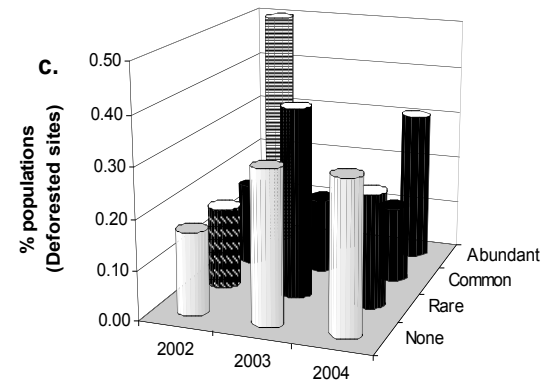| a) 2002: No difference in density by site during drought (p=0.4).	b) 2003: No difference in density by site at the end of the drought (p=0.6).	c) 2004: Significant difference in density one year after drought (p<0.04).
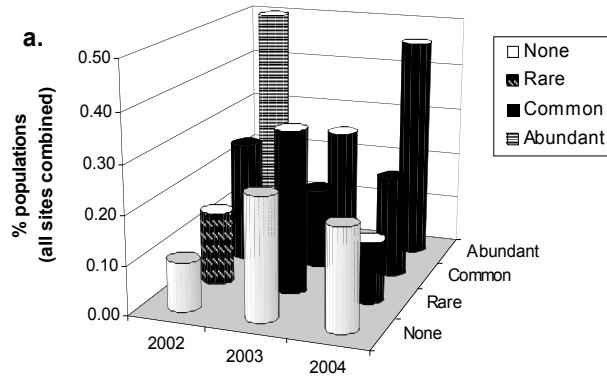
a. All Sites

	2002	2003	2004
None	2	6	5
Rare	3	8	3
Common	5	4	5
Abundant	10	6	11
Total	20	24	24
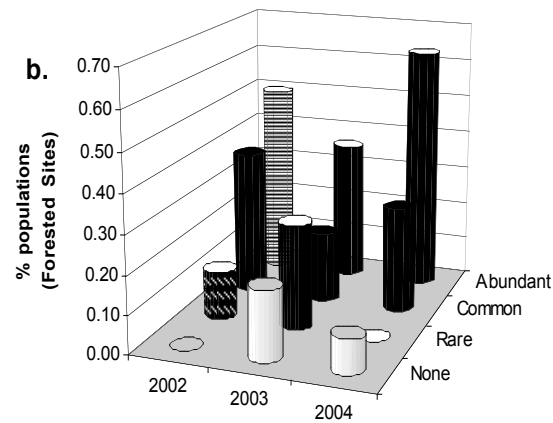
b. Forested Sites

	2002	2003	2004
None	0	2	1
Rare	1	3	0
Common	3	2	3
Abundant	4	4	7
Total	8	11	11

c. Deforested Sites

	2002	2003	2004
None	2	4	4
Rare	2	5	3
Common	2	2	2
Abundant	6	2	4
Total	12	13	13

Figure 1. Density Distribution by Year. Counts represent the density distribution for each year in (a) all streams, (b) forested streams and (c) deforested streams.

Genetic Analysis: E. inconstans. We found extremely low variation among the 10 study populations of *E. inconstans* across the ~200 km range of sites in Maryland and Virginia. There were two haplotypes, a "southern" haplotype and a "northern" haplotype, which differed at only two loci (2 alleles per locus) in the sequenced region of mtDNA (~450 bp). This lack of polymorphism in mtDNA is probably the result of a bottleneck following recolonization of the Central Piedmont at the end of the last Ice Age. Thus, isolation that might be occurring now from current ecological processes (e.g., habitat fragmentation) is not detectable in these populations using mtDNA.

Genetic Analysis: E. invaria.   The estimated genetic distances among five populations of *E. invaria* synonyms from Maryland (MD), Virginia (VA), Tennesee (TN) and North Carolina (NC) are given in Table 3. *E. inconstans* and *E. invaria* specimens had the lowest among-population genetic distances (0.053 to 0.059), whereas populations of *E. rotunda* (VA) and *E. floripara* (NC) showed the equally high genetic divergence in all pairwise comparisons (mean distance = 0.12 for both species). Within-population distance ranged from .005 to .022 in all samples, with mean within-population genetic distance < 0.01. The genetic divergences among these populations could be the result of speciation, geographic distance, founder effect, or local adaptation. Since the amount of genetic distance among species varies considerably among taxonomic orders and families, interpretation of the observed differences among these populations will require the addition of molecular characters from other, morphologically-distinct *Ephemerella* species.

Table 3. Mean genetic distance among 5 populations of *Ephemerella invaria* synonyms.

spp	A	B	C	D	E		Species Key	
A	-	0.059	0.121	0.058	0.105	A	*E. inconstans,* TN	n=2
B		-	0.111	0.053	0.116	B	*E. inconstans,* D+VA	n >100
C			-	0.123	0.124	C	*E. rotunda,* VA	n=2
D				-	0.137	D	*E. invaria,* NC	n=6
E					-	E	*E. floripara,* NC	n=1

Discussion

The results of this study show that population recovery and habitat recolonization following a major regional disturbance was more successful in the intact, forested headwater stream networks than in the altered, deforested networks. This could be because dispersal from surviving populations to uninhabited patches was more effective in forested stream networks, resulting in a higher probability of recolonization as well as a larger founding population in these streams. Or, it could be that refugia in the forested sites (e.g., in the hyporheic zone) provided protection to a small number of individuals who were able to regenerate large population sizes in a single generation. These findings are consistent with studies that have found habitat type to be a significant predictor of local extinction, even after the effect of regional distribution has been removed (e.g., Korkeamaeki and Suhonen 2002).

We would like to know what role mayfly dispersal plays in maintaining population abundances and population genetic diversity in the face of local and regional disturbances, and how changes to the structure of headwater stream networks may affect demographic processes. To that end, we are now using Amplified Fragment Length Polymorphism (AFLP) to evaluate nuclear DNA diversity in *E. inconstans*. This technique samples the entire nuclear genome for polymorphisms at restriction enzyme sites, and thus provides high temporal and spatial sensitivity for detecting changes in genetic composition of populations at small scales (Mueller and Wolfenbarger 1999). If fragment length polymorphism exists in this region and species, then our samples of four generations of mayflies (spanning a period of drought, local extinction and recovery) will provide a good opportunity to relate observed events in the demographic history of these populations to changes in their genetic composition through space and time (Barrett et al. 2005).

We are also developing a spatially explicit model of population growth with migration to model different theoretical scenarios of genetic variation in subdivided mayfly populations (as in Johst et al. 2002) and, when AFLP data become available, to test alternative hypotheses of dispersal against the observed changes in allele frequencies (Felsenstein 1982, Takami et al. 2004). The theoretical implications of alternative dispersal mechanisms in a spatially structured insect habitat are interesting and may have applications to conservation of headwater species. For example, Fagan (2002) showed that the geometry of dendritic stream systems affects the persistence of interacting populations, especially in landscapes subjected to fragmentation or natural disturbance. Thus, a specific landscape structure, interacting with mayfly dispersal behavior, could increase or decrease the environmental pressures faced by small populations of these insects.

Lastly, we are working in collaboration with Luke Jacobus at Purdue University and David H. Funk at the Stroud Water Research Center in a continuing molecular analysis of species in the genus *Ephemerella*. Samples of 9 additional species and subspecies: *Ephemerella excrucians* Walsh, 1862*; Ephemerella aurivillii* (Bengtsson), 1908; *Ephemerella alleni* Jensen & Edmunds, 1966; *Ephemerella*

hispida Allen & Edmunds, 1965; *Ephemerella catawba* Traver, 1932*; Ephemerella subvaria* McDunnough, 1931; *Ephemerella dorothea dorothea* (Needham), 1908; *Ephemerella dorothea infrequens* (McDunnough), 1924; *Ephemerella rossi* Allen and Edmunds, 1965; from populations broadly distributed across the United States and Canada have been added to *E. inconstans*, *E. floripara*, *E. rotunda* and *E. invaria* Walker, for analysis. This work will provide molecular characters for constructing a partial phylogeny of the genus based on both morphological and molecular data. In combination with the study of population diversity and dispersal, it may also provide a small link from local genetic processes by which populations become structured and differentiated over relatively short periods of time, to the long-term, large-scale genetic processes underlying the evolution of new characters and species.

Acknowledgments

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