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Genetic differentiation among populations of the rare mayfly *Siphlonisca aerodromia* Needham

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Abstract. We used 5 species-specific microsatellite DNA markers to assess genetic differentiation among 6 populations (4–226 km apart) of the rare mayfly *Siphlonisca aerodromia* Needham in Maine. All populations, even those 4 km apart and from the same river drainage, showed significant differences in allele frequencies, and all subpopulation fixation indices based on infinite allele mutation models (F_{st}) and stepwise mutation models (R_{st}) were significantly different from 0. However, a hierarchical analysis of F_{st} and R_{st} values among sets of populations indicated that the most substantial pattern of differentiation was between the 2 populations from the Kennebec drainage in western Maine and the 4 populations from the Penobscot and St. Croix drainages in central and eastern Maine. We found no evidence for significant linkage disequilibrium among alleles at any locus in any population, suggesting that parthenogenesis is not an important mode of reproduction in these insects. Our results confirm previous findings that local populations of mayflies may not form panmictic breeding units. Nevertheless, the minimal differentiation among populations located >100 km apart in the St. Croix and Penobscot drainages suggested that adult flight may be an important mode of dispersal and sufficiently frequent to maintain substantial levels of gene flow between these populations. We hypothesize that the differentiation between eastern and central, and western populations may be caused by geographic isolation and long-term reproductive isolation. The later occurs because climatic differences between these areas result in a substantial gap (~20 d) in emergence times of the reproductively active, short-lived adults.

Key words: mayfly, genetic structure, microsatellite DNA loci, migration, parthenogenesis.

A major unresolved issue in the dispersal of stream insects is the relative importance of adult flight versus nymphal drift (Sheldon 1984). Genetic analysis of geographically separate populations provides an indirect method to determine the extent of movement within and between streams and the possible mechanisms involved. If instream movement by immatures is the primary form of dispersal, then most differentiation will occur between populations in different drainages. However, if dispersal by adults is important, then populations in nearby but independent drainages will show little or no genetic differentiation. A recent review by Bunn and Hughes (1997) of genetic studies of differentiation between aquatic insect populations concluded that, in general, adult flight is the principal mode of dispersal because only small genetic differences exist between geographically widespread populations (e.g., Sweeney et al. 1986, 1987, Jackson and Resh 1992). However, Bunn and Hughes (1997) emphasized that some species showed differentiation within streams or drainages over very small distances, suggesting that dispersal by either immatures or adults may be limited (e.g., Sweeney et al. 1987, Schmidt et al. 1995) and hence that local populations may be small and spatially restricted in distribution. More studies of species with diverse biological features would be valuable to establish the generality of these findings, and to determine what biological and environmental factors structure stream insect populations.

With few exceptions (e.g., Sweeney and Funk 1991, Plague and McArthur 1998), all genetic studies to date have focused on common, widespread species. It is unknown whether the above conclusions also apply to rare aquatic in-

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TABLE 1. Collection sites, dates of collection, and sample sizes used for genetic analyses of *Siphlonisca aerodromia* from Maine.

Location	Latitude, Longitude	Date	Sample size
St. Croix River drainage			
Upper Tomah Stream (UTS)	45°26'42"N, 67°34'50"W	13 May 1993	28
		26 May 1996	30
Lower Tomah Stream (LTS)	45°26'03"N, 67°36'23"W	11 May 1994	33
Penobscot River drainage			
Macwahoc Stream (MAS)	45°42'09"N, 68°12'07"W	14 May 1993	33
Passadumkeag River (PAR)	45°10'33"N, 68°34'04"W	27 May 1993	21
Kennebec River drainage			
Upper Dead River (UDR)	45°11'49"N, 70°27'33"W	14 May 1994	34
Lower Dead River (LDR)	45°11'37"N, 70°27'45"W	14 May 1994	35

sects with limited distributions caused by restricted habitat requirements and/or human disturbances. From a conservation perspective, genetic analyses of differentiation between populations of rare species is valuable because it can define appropriate management units for these taxa by identifying demographically independent and possibly locally adapted sets of populations (Moritz 1994). In addition, no past studies have assessed genetic differentiation in stream insects using recently developed, highly variable DNA-based genetic markers, which may be more suitable than allozymes for assaying differentiation on a local scale (Quellar et al. 1993). Here, we address both needs by using microsatellite DNA markers to examine fine-scale genetic differentiation in a rare mayfly.

Siphlonisca aerodromia Needham is a mayfly with a limited distribution in eastern North America. Despite extensive search efforts, only 13 populations have been located, mostly in Maine, but also in New York, Quebec, and Labrador (Gibbs 1993, Gibbs and Siebenmann 1996). The restricted, patchy distribution of this species is likely a result of its specialized habitat requirements: nymphs are only found in rivers that have sedge-dominated floodplains that are seasonally flooded (Gibbs 1993). Its limited distribution in Maine may be a result of habitat fragmentation caused by extensive construction of dams on rivers and streams (Gibbs 1993). *Siphlonisca aerodromia* is listed as a Threatened Species in Maine (State of Maine Inland Fisheries and Wildlife Laws 1997) and has been listed as a candidate for the US Federal Endangered Species List (Category 2) (US Department of the Interior, Fish and Wildlife Service 1991).

The ecology and life-history characteristics of this species in Maine are described by Gibbs and Mingo (1986), Gibbs (1993), and Gibbs and Siebenmann (1996). The female-biased sex ratios of late-instar nymphs and the fact that up to 94% of the eggs from unmated females hatched or showed embryonic development, suggests that parthenogenesis may occur, although the relative importance of this mode of reproduction is unclear (Gibbs and Siebenmann 1996). A major biological feature that remains unknown is to what extent interbreeding occurs among populations of *S. aerodromia* within the same drainage or among different drainages. In other words, do populations of *S. aerodromia* in Maine form a single large panmictic population or a series of smaller demographically isolated populations? We address this question by comparing the degree of genetic differentiation among 6 populations of *S. aerodromia* from 3 separate river drainages.

Methods

Population samples

Nymphs for DNA analyses were collected from 6 sites in Maine (Table 1). Sites spanned the east-west range of *S. aerodromia* in the state and formed a hierarchical sampling design made up of 2 populations from each of 3 major river drainages. Sites were in the Kennebec drainage in western Maine (Upper [UDR] and Lower Dead River [LDR], 4 km apart), the Pe-

nobscot drainage in central Maine (Macwahoc Stream [MAS] and Passadumkeag River [PAR], 65 km apart), and the St. Croix drainage in eastern Maine (Upper [UTS] and Lower Tomah Stream [LTS], 4 km apart). At each site ~30 nymphs (Table 1) were collected with a D-frame net from a restricted area of <25 m^2. Nymphs were returned to the laboratory where they were held to allow gut clearance and frozen at −80°C. An additional sample of nymphs was collected at UTS in 1996 from the same area in which they were collected in 1993 to test for the effects of sampling on estimates of population differentiation.

DNA-based genetic analyses

We used the procedure described by Hunt and Page (1992) to isolate DNA from individual nymphs. Individual nymphs were snap frozen in liquid nitrogen, homogenized using a mortar and pestle, and then combined with 500 μL of CTAB buffer (1% hexadecyltrimethyl ammonium bromide; 0.75 M NaCl; 50 mM Tris-HCl, pH 8.0; 10 mM EDTA) and 15 μL of proteinase K solution (0.8 units/μL). The homogenate was then incubated at 55°C for 3 h and the DNA extracted with 2 phenol/choroform (70:30) and 1 choroform extractions. DNA was precipitated from this solution by adding 1/10 volume of sodium acetate and 2 volumes of 100% ethanol, followed by a 70% ethanol wash and resuspension in 15–100 μL of Tris-EDTA buffer (10 mM Tris-HCL, pH 8.0; 1 mM EDTA), depending on the size of pellet. RNAse (1 μL of 10 mg/μL) was then added and the solution incubated at 37°C for 1 h. Finally, the DNA concentration of the solution was determined using a fluorometer.

Despite successful use of this protocol to isolate DNA in other insects (e.g., Hunt and Page 1992), we were unable to amplify mayfly DNA isolated as described above. Repurification of extracted DNA with up to 3 additional phenol-chloroform extractions and/or silica-based extraction procedures (Qiagen) failed to produce consistently amplifiable samples, suggesting that a contaminant that inhibited the activity of the Taq polymerase was being co-purified with the mayfly DNA. We finally achieved consistently successful amplifications when, following the suggestion of Akane et al. (1993), we added bovine serum albumin (BSA) to the polymerase chain reaction (PCR) mixture (see below). This additive is widely used in forensic applications to bind contaminants, mainly heme-related products, which prevent amplification of DNA extracted from human blood stains. It apparently performs a similar function in mayfly DNA, although the identify of the contaminant in our samples remains unknown.

We isolated microsatellite DNA sequences from a *S. aerodromia* genomic library and designed primers to amplify those sequences to obtain highly variable mayfly-specific genetic markers. We used a modification of the procedure of Rassman et al. (1991) as described by Dawson et al. (1997) to isolate microsatellite loci. We constructed an enriched plasmid library by digesting 10 μg of DNA from a single mayfly with 10 units each of Alu I, Hae III, and Rsa I and then cloned genomic fragments 250–450 base pairs in length into the Sma I site of pUC18 plasmid vector. Colonies were screened by hybridizing filters with probes made by labeling 3 dinucleotide polymer tracts $(TG)_n$, $(TC)_n$, and $(GC)_n$ (Pharmacia) with alpha ^{32}P-dCTP using random priming. Primary and secondary screening of ~5000 colonies yielded a total of 172 positive clones. A subset of these clones were then cycle sequenced (AmpliTaq), and the Primer program (Lincoln et al. 1991) was used to identify suitable primers and appropriate annealing temperatures for amplifying regions containing a microsatellite repeat.

To assay variation among individuals, we performed PCRs using a Perkin-Elmer 480 DNA Cycler in 10 μL volumes that contained 50 ng of genomic DNA, 0.2 pmoles of the forward primer end-labeled with ^{33}P-dATP, 0.3 pmoles of unlabeled forward primer, 0.5 pmoles of unlabeled reverse primer, 300 μM dNTPs, 0.25 units AmpliTaq, 0.1 M Tris- HCl pH 8.3, 0.5 M KCl, 2.5 mM $MgCl_2$, and 1 μL of BSA solution (900 mg/μL). Following an initial denaturing step at 94°C for 3 min, 30 cycles of PCR were carried out, each cycle consisting of 30 s at 94°C, 30 s at the T_m in Table 2, and 30 s at 72°C. Amplification products were resolved on 6% polyacrylamide denaturing gels containing 7.7 M urea. Gels were run at 55W for 2–3 h. Where possible, products were double-loaded. Dried gels were exposed to BIOMAX (Dupont) X-ray film overnight. Product sizes were determined by reference to 1 or more reference reactions consisting of 1) a sequencing reaction of a known template,

2) a clone of known size for each locus run every 5 lanes, and 3) amplifications of reference individuals. It was sometimes necessary to re-run individual reactions on different gels for varying periods of time because of the large size range in the alleles at particular loci.

Analysis of variation

We estimated levels of non-random association of alleles within mayfly populations by 1) testing for both locus-specific and overall heterozygote deficiencies within populations using the exact tests in GENEPOP (Raymond and Rousett 1995), and 2) calculating values for the inbreeding coefficient (F_{is}) as described by Weir and Cockerham (1984) for each locus and then an overall F_{is} value using data pooled across all loci using FSTAT (Goudet 1995). The significance of the overall F_{is} value for each population was tested using the permutation procedure in FSTAT. Linkage disequilibrium between alleles at different loci within particular populations was tested using the DIS subroutine in GENEPOP.

We quantified differentiation among populations by testing for differences in allele frequencies and by calculating fixation indices to estimate the extent of differentiation between populations. Some loci used in this study have disjunct distributions of alleles within populations (H. L. Gibbs, unpublished data), and the large number and range of sizes of alleles at some loci suggest that some of the variation among alleles is not simply a result of variation in the numbers of a given repeat motif that are present. These disjunct distributions raised the possibility that mutations at these loci may not follow the stepwise mutation model assumed by recent measures of population differentiation developed for microsatellites (e.g., Slatkin 1995), although we have not explicitly tested for this possibility (e.g., Estoup et al. 1995). Therefore, we present subpopulation fixation indices based on both infinite allele mutation models (F_{st}, estimated as described by Weir and Cockerham 1984) and stepwise mutation models (R_{st}, estimated as described by Slatkin 1995). We also assume that same size alleles are evolutionarily homologous. This assumption is reasonable given the short time scale that populations have been separated, making it unlikely that substantial amounts of convergent evolution occured among alleles. If the assumption is violated it will mean that our estimates of differentiation are minimum estimates because we would score same size alleles with different histories (i.e., indicative of differentiation) as identical. We used several analytical software packages to calculate these measures: GENEPOP to test for differences in allele frequencies, FSTAT to calculate and test F_{st} values, and RSTCALC (Goodman 1997) to calculate and test R_{st} values.

A hierarchical analysis of differentiation (Holsinger and Mason-Gamer 1996) was done to assess patterns of differentiation between different sets of populations. This method uses pairwise estimates of differentiation (e.g., in our case, F_{st} or R_{st} values) between different combinations of populations to construct a dendrogram, which identifies patterns of hierarchical population structure in the data. The 2 localities showing the smallest F_{st} or R_{st} value (the least genetic divergence) are grouped together, and pairwise-fixation indices are then recomputed between the remaining localities and the new cluster. The analysis proceeds until all locations have been added, and a tree of the relationships among them is constructed. Statistical significance of F_{st} or R_{st} values at each node is then tested by creating a null distribution of values generated by random resampling and comparing the observed value with this null distribution. This approach allows any patterns to emerge naturally from the data rather than having a priori hypotheses about structure imposed prior to the analyses. The topology of the tree can then be interpreted as reflecting patterns of gene flow between sets of populations (Holsinger and Mason-Gamer 1996). Our method differs slightly from the original approach in that the measures of differentiation that we used are adjusted for differences in sample size between populations compared (single and/or pooled).

Results

Microsatellite loci

We developed 5 primer sets that successfully amplified microsatellite loci in these mayflies (Table 2). Many of the darkest positive clones did not provide useful sequences for developing loci because the microsatellite repeats were too long, leaving little or no single copy sequence in the insert in which to locate primers, or the in-

TABLE 2. Genetic characteristics and primer sequences for 5 *Siphlonisca aerodromia* microsatellite loci. Sequence motifs are based on the sequenced allele. T_m = annealing temperature (°C), N = the number of adults pooled across all populations that were genotyped for a particular locus, H_{obs} = proportion that were heterozygotes, bp = base pairs.

Locus	Repeat motif	Primer (5′–3′)	T_m	Number of alleles (N)	Allele size range (bp)	Frequency of most common allele	H_{obs}
Saμ 65	$(GA)_{20}GGA$	F-GCGTTCTCACTTCTGTATGC R-ACTCGAATTTTCTGCCAATAC	57	19 (184)	163–207	0.24	0.908
Saμ 107	$(TC)_{40}$	F-GTGATCCTTTTGTAAGC R-AGGAGATTCTAGATGA	50	62 (180)	136–234	0.11	0.872
Saμ 108	$(TC)_{11}AT\ (TC)_4$	F-GAGTTGTGACGTCACTGGAGAA R-TGGATACCCATCAAATCAATGA	57	63 (179)	180–378	0.08	0.422
Saμ 111	$(TC)_{44}$	F-AACCCAATAAACCACCA R-GCTACTGATTGGATACC	60	54 (170)	178–334	0.07	0.839
Saμ 122	$(GA)_{12}$	F-AGACTTCCTAGAAAATATCG-AGG R-ACTCAACACTCTTTGATTAA-ACCA	57	11 (185)	113–149	0.75	0.853

sert could not be sequenced, presumably because of the presence of large and complicated GC-rich satellite sequences. Contrary to the usual criteria with vertebrate microsatellites (e.g., Dawson et al. 1997, Gibbs et al. 1998), we were most successful when we chose only moderately dark positive clones for sequencing. Surveys of the levels of variability in the 5 loci selected showed that these loci are highly variable: there were large numbers of alleles (range: 19–63), high levels of observed heterozygosity (0.42–0.91), and a low frequency of alleles present (Table 2). Thus, these markers should be ideal for detecting differentiation on fine spatial scales in these mayflies.

Non-random associations of alleles

Four of the 6 populations had small (mean = 0.073), positive overall F_{is} values that were significantly different from 0, suggesting small heterozygote deficiencies in some populations (Table 3). However, these positive values were mainly a result of deficiencies at a small number of loci. Only 8 of 30 locus-by-population values were significantly different from 0; 6 of these 8

TABLE 3. Inbreeding coefficient (F_{is}) values for each microsatellite locus/population combination. Significance of individual and overall F_{is} values tested using the permutation procedures in FSTAT (Goudet 1995). * indicates values that are significantly different from 0 after adjusting significance levels for multiple comparisons using a sequential Bonferroni correction (Rice 1989). Population locations as in Table 1.

	Population					
Locus	UDR	LDR	MAS	PAR	UTS	LTS
Saμ 65	−0.164	0.017	0.014	−0.193	−0.188	−0.015
Saμ 107	0.269*	−0.023	0.004	0.081	0.300*	0.078
Saμ 108	0.100*	0.116*	0.305*	−0.040	−0.036	0.003
Saμ 111	0.062	0.124*	0.256*	0.071	0.155*	−0.032
Saμ 122	−0.050	0.062	−0.081	0.064	−0.288	0.070
Combined	0.064*	0.059*	0.116*	0.002	0.053*	0.013

TABLE 4. Subpopulation fixation indices based on infinite allele mutation models (F_{st}) or stepwise mutation models (R_{st}) for all pairwise combinations of mayfly populations. F_{st} values pooled across all loci are shown above the diagonal, whereas R_{st} values pooled across all loci are shown below the diagonal. Probabilities that a particular value is significantly different from 0 are given in parentheses. Population locations as in Table 1.

	Population					
	UDR	LDR	MAS	PAR	UTS	LTS
UDR	—	0.0085 (0.005)	0.046 (<0.001)	0.062 (<0.001)	0.054 (<0.001)	0.041 (<0.001)
LDR	0.027 (0.031)	—	0.024 (<0.001)	0.046 (<0.001)	0.031 (<0.001)	0.028 (<0.001)
MAS	0.22 (<0.001)	0.088 (<0.001)	—	0.014 (0.004)	0.0046 (0.039)	0.0083 (<0.001)
PAR	0.33 (<0.001)	0.16 (<0.001)	0.019 (0.042)	—	0.015 (<0.001)	0.023 (<0.001)
UTS	0.24 (<0.001)	0.15 (<0.001)	0.056 (0.042)	0.009 (0.189)	—	0.0067 (0.008)
LTS	0.31 (<0.001)	0.15 (<0.001)	0.056 (0.005)	−0.0014 (0.54)	0.012 (0.10)	—

involved alleles at just 2 loci (Saμ 108 and 111). This pattern, combined with the large number (11 of 30) of negative (albeit non-significant) locus-by-population values suggests that the positive F_{is} values are more likely a result of the presence of null or non-amplifying alleles (e.g., Brookfield 1996) at low frequencies at specific loci, rather than genome-wide inbreeding.

Only 3 of 51 locus-by-locus comparisons within single populations showed any significant non-random associations of particular alleles. Thus, genotypic disequilibria between alleles at different loci were absent, which suggests that parthenogenesis rarely, if ever, occurs in these populations.

Population differentiation

Allele frequency distributions and overall fixation indices (F_{st}, R_{st}) showed the same pattern: all populations were genetically distinct from each other, even those located on the same river drainage. However, the magnitude of these differences varied. In 10 of 15 pairwise comparisons, at least 1 locus showed a significant difference in allele frequency distributions between 2 populations (Table 4). Three of the 5 comparisons not showing differences for any locus were between populations from the same drainage (UTS and LTS, MAS and PAR, and UDR and LDR). However, when significance probability values were combined across loci (Sokal and Rohlf 1981), all pairwise comparisons were significantly different from 0.

Fixation indices showed a similar pattern: both overall F_{st} (0.031) and R_{st} (0.13) values were significantly different from zero ($p < 0.001$) indicating significant genetic structure among these mayfly populations, which ranged up to a maximum of 226 km apart. The greater magnitude of the R_{st} value indicates that weighting for the degree of genetic differentiation between alleles increased the degree of differentiation detected between populations. Pairwise comparisons of F_{st} values showed differentiation between all populations, including those from the same drainage (Table 5). Although values for the 2 sets of populations on Tomah Stream (UTS and LTS) and on the Dead River (UDR and LDR) were small (0.0067 and 0.0085, respectively) they were nonetheless both significantly different from 0. Most (9 of 15) pairwise R_{st} values were also significantly different from 0; however, unlike the F_{st} values, populations from the same drainage were no longer significantly different from 0. Despite this difference, there is a strong correlation between the magnitude of F_{st} and R_{st} values for the same pairwise population comparison, suggesting they detect similar patterns of differentiation ($r = 0.90$; $p < 0.01$).

The hierarchical analysis of differentiation showed that, at a state-wide scale, the major

TABLE 5. Numbers of significant pairwise comparisons between mayfly populations of allele frequencies for 5 microsatellite loci. Values are for exact tests described in GENEPOP (Raymond and Rousset 1995). Significance levels were adjusted for multiple tests (Rice 1989). Combined significance of p-values pooled across all 5 loci (Sokal and Rohlf 1981) is shown by * (<0.05), ** (<0.01), or *** (<0.001). Population locations as in Table 1.

	Population					
	UDR	LDR	MAS	PAR	UTS	LTS
UDR	—	0/5*	4/5***	5/5***	5/5***	5/5***
LDR		—	3/5***	4/5***	4/5***	3/5***
MAS			—	0/5***	2/5***	0/5**
PAR				—	0/5***	1/5***
UTS					—	0/5**

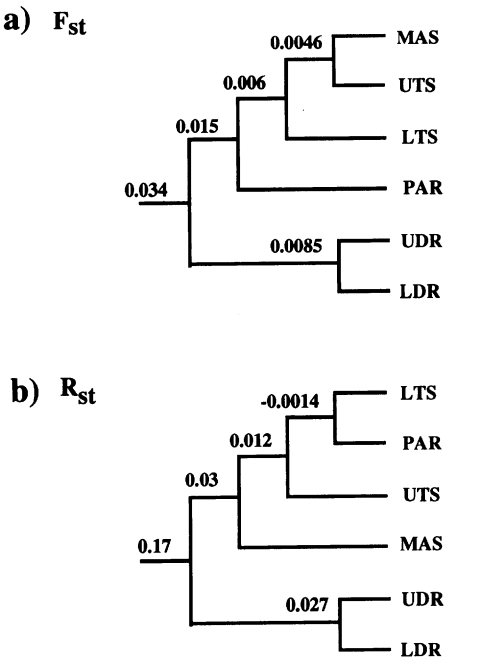

FIG. 1. Phenograms showing the results of hierarchical analyses of population differentiation (Holsinger and Mason-Gamer 1996) using subpopulation fixation indices based on both (a) infinite allele mutation models (F_{st}) and (b) stepwise mutation models (R_{st}) for 6 *Siphlonisca aerodromia* populations in Maine. See text for details of how the analysis was done. Values at each node represent the level of differentiation (either F_{st} or R_{st} value) between sister clusters. All such values are significantly different from 0 ($p < 0.007$) except for the negative R_{st} value between LTS and PAR. Abbreviations of specific populations as given in Table 1.

pattern of differentiation was between the 2 western populations (UDR and LDR) and a group made up of the 2 central and 2 eastern populations (MAS and PAR, UTS and LTS) (Fig. 1). Phenograms based on both F_{st} (Fig. 1a) and R_{st} (Fig. 1b) revealed that the largest values (F_{st}: 0.034, R_{st}: 0.17) existed between these 2 clusters of populations. Within the eastern-central clade there were minor differences between the F_{st}- and R_{st}-based trees in terms of which populations clustered; most strikingly, populations from the same drainage did not necessarily group together (e.g., UTS and LTS, MAS and PAR) in either tree. This result indicates that the 4 populations, despite being from different drainages, have similar levels of differentiation between them, and that the magnitude of this differentiation is less than that observed between the western and eastern-central groups.

Sampling Effects

The 1996 and 1993 UTS samples were significantly different from each other in terms of overall F_{st} value (0.026, $p < 0.05$) but not their overall R_{st} value (−0.01, $p = 0.90$). The significant F_{st} value was a result of differences in allele frequencies between samples at a single locus (Maμ 122), because there were no differences in allele frequencies between the samples at the other 4 loci (all $p > 0.05$). This result indicates the possibility of sampling effects attributable to either biological or statistical causes. However, this variation has only a minimal impact on the patterns of differentiation observed among the original samples. All F_{st} and R_{st} values for paired comparisons between 1996 UTS and other sites remained significant as before, with the exception that 1996 UTS and LTS populations were no longer significantly different ($p = 0.38$). Mean absolute differences in F_{st} values for 6 comparisons (1996 UTS with all other original populations) equaled 0.0074, whereas the same R_{st} value was higher (0.0164). These values may provide an estimate of the variation to be expected in fixation indices when multiple samples are taken from single sites. This variation is equivalent in magnitude to the F_{st} and R_{st} values observed between UDR and LDR, and UTS

and LTS. Thus, any differentiation observed between populations as close as these may have as much to do with sampling effects as with limited migration between sites.

Discussion

Local patterns of differentiation

Mayfly populations only 4 km apart along the same stream showed significant differences in allele frequencies. This result is surprising, because at least 1 of these sets of populations (UTS and LTS) are point samples taken from a continuous distribution of *S. aerodromia* along this section of the river (K. E. Gibbs, unpublished data). However, these small-scale differences may be ephemeral, given that no genetic differentiation was shown in comparisons between samples from the same 2 sites collected in different years (LTS and 1996 UTS).

Other genetic studies of aquatic insects have found evidence for fine-scale differentiation between populations within the same river drainage (Sweeney et al. 1986, Jackson and Resh 1992, Schmidt et al. 1995), and differentiation between samples collected at the same site in different years (Preziosi and Fairburn 1992). Several researchers (Preziosi and Fairburn 1992, Bunn and Hughes 1997) have emphasized the importance of stochastic factors in determining fine-scale patterns of structure in stream insects. These factors include sampling effects in which temporal and spatial patterns of differentiation are strongly affected by how well the collected samples reflect the genetic variation present in the sampled populations. In addition, biological effects could also be important: Bunn and Hughes (1997) suggest microgeographic differentiation may result because successful reproduction at any point in the stream only occurs by very few females, and such founder effects lead to differences in allele frequencies between nearby sites. Alternately, larval dispersal may be very limited in these streams, possibly because of ecological advantages of philopatry. However, the potential distance that adults could fly in a lifespan of up to 9 d (Gibbs and Siebenmann 1996), and the extent of movement of nymphs of other mayfly species (e.g., Hershey et al. 1993) both suggest that high rates of migration between sites only 4 km apart are possible.

Multiple samples collected from a number of closely spaced sites in the same year are necessary to determine the relative importance of sampling effects versus biological explanations (e.g., limited dispersal and founder effects) for within-site, across-year genetic variation. Direct study of movement patterns of individuals (e.g., Hershey et al. 1993) and variance in reproductive success among individual females are needed to evaluate these possibilities.

Regional patterns of differentiation

Similar, limited levels of differentiation occurred between the 2 central (MAS and PAR) and 2 eastern (LTS and UTS) populations, each set of which was in a different river drainage. This result suggests that adult flight is a genetically effective mode of dispersal, maintaining the genetic cohesiveness of populations over distances of up to 100 km, because dispersal by nymphs between watersheds is unlikely. However, this explanation assumes that the populations are in genetic equilibrium with respect to the evolutionary forces (migration, drift, and mutation) that mold neutral variation within and between populations, and that low levels of differentiation are a result of high levels of contemporary gene flow (Slatkin 1985). If the populations are not in genetic equilibrium then current migration between populations may not be occurring. The observed present-day similarity may be a result of gene flow that happened in the past but that no longer takes place (e.g., Strand et al. 1996) because of, for example, human-caused destruction of intermediate populations. Discriminating between these possibilities requires non-genetically based measurements of flight migration between populations in different drainages.

The greatest differentiation was between the 2 western populations and a group made up of the 2 central and 2 eastern populations. Several factors may limit interbreeding between these sets of populations. First, the western populations are geographically isolated. Most populations of *S. aerodromia* occur in eastern and central Maine with only 1 small population on the Sebasticook River known to occur between the sets of populations examined in this study. This intermediate population is small, with only 1–2 individuals collected during visits to the site (K. E. Gibbs, unpublished data). Thus, there is little opportunity for gene flow be-

tween western and other populations via intermediate populations.

Second, the 2 sets of populations are found in different climatic regions within the state. Sites in western Maine were in an area with lower mean maximum temperature, higher annual snowfall, lower potential evapotranspiration, and lower annual heat accumulation than the area in which the 4 other sites were located (Boone 1997). Cooler temperatures may account for the delayed development of nymphs from the western site relative to that of nymphs in the central and eastern sites. On 30 May 1994 all nymphs at UDR were in the penultimate and antepenultimate instars, whereas those from UTS, LTS, and MAS were in the final instar (20 nymphs per site examined, M. Siebenmann, unpublished data). We estimate that this delay in development would cause a difference in 1st-emergence times of adults of ~20 d. If a similar difference in seasonal emergence patterns of short-lived adults occurs in most years, then the western populations would be temporally as well as geographically isolated from other *S. aerodromia* populations; hence, the differentiation we observed could evolve via genetic drift.

Third, the differentiation also could reflect local adaptation to the cold climatic conditions of the western region through selection on emergence times. Differences in the length of egg diapause of other mayflies are heritable (D. Funk, Stroud Water Research Center, personal communication). If this condition is true of *S. aerodromia,* then different populations may have evolved to have genetically distinct nymphal development and emergence patterns. This hypothesis could be tested by rearing nymphs from the different populations under the same conditions in the laboratory.

Conservation implications

Our results suggest that the *S. aerodromia* populations analyzed here form at least 2 demographically independent units composed of populations in the Dead River drainage and populations in the Penobscot and St. Croix River drainages. We hypothesize that these populations also may form evolutionarily distinct lineages (sensu Moritz 1994) that are locally adapted in terms of having genetically distinct emergence times, but we have no evidence to support this possibility. Management plans for this species should account for the presence of at least 2 distinct sets of populations within the state of Maine, particularly in terms of 1) the spatial scale of impacts on populations of this species, 2) maximizing the preservation of genetic variation within this species as a whole in the state, and 3) the significance of the genetic distinctiveness of different populations on potential plans for translocating individuals for supplementation programs. Our results also suggest that climatic regions may be an effective way to identify demographically independent populations of individuals in other aquatic insects with short adult life spans.

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