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Population genetic structure of 3 alpine stream insects: influences of gene flow, demographics, and habitat fragmentation

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Abstract. Estimating scales of dispersal for benthic macroinvertebrates using neutral genetic markers requires consideration of genetic, demographic, and historical influences on population genetic structure. We used allozyme electrophoresis to investigate the population genetic structure of 3 species of alpine stream insects among major drainages of the Swiss Alps (Rhine, Inn, and Ticino rivers), among streams within each drainage, and within single streams. Within streams we examined reaches that were fragmented by lakes or resevoirs and unfragmented reaches. *Rhithrogena loyolaea* (Heptageniidae) exhibited little genetic differentiation (θ) within (θ = 0.01–0.03) and among (θ = 0.02–0.03) streams but significant differentiation among drainages (θ = 0.08), suggesting that dispersal occurs among stream fragments and among stream valleys. *Allogamus auricollis* (Limnephilidae) did not exhibit genetic differentiation at any scale, suggesting that dispersal occurs throughout the geographical range of the study. In contrast, *Baetis alpinus* (Baetidae) showed moderate to substantial differentiation both within (θ = 0.08–0.39) and among (θ = 0.06–0.09) streams. However, a distinct lack of genetic differentiation for *B. alpinus* among major drainages of the Alps (θ = 0.01) suggests that low θ values reflect historical rather than present-day levels of gene flow. We suggest that genetic population structure reflects a lack of equilibrium between gene flow and genetic drift, resulting from historical gene flow that continues to mask reduced dispersal and from recurring processes of recruitment that lead to random changes in genetic signatures. We conclude that demographic processes affect small-scale patterns and historical processes affect large-scale patterns. The simultaneous study of multiple spatial scales helps determine the relative importance of each. A synthesis of our results and data from published studies indicated that 4 consistent patterns of genetic differentiation emerged when multiple spatial scales were investigated. These patterns are indicative of taxon-specific dispersal ability within and among streams and whether taxa are in gene flow–genetic drift equilibrium.

Key words: habitat fragmentation, dispersal, genetic diversity, equilibrium, inbreeding, alpine streams, macroinvertebrates, allozymes, insects.

Dispersal can be an important factor maintaining populations of species in fragmented habitats. Dispersal can counteract local extinction directly via immigration (Stacey and Taper 1992) and can ensure population persistence at larger spatial scales by maintaining a metapopulation structure (Hanski 1998). Dispersal among habitat fragments also may provide sufficient gene flow to maintain the genetic diversity within fragments, thereby indirectly reducing the probability of local extinction (Saccheri et al. 1998). Dispersal of organisms among habitat fragments often is studied using population genetics, where levels of gene flow are inferred from the spatial distribution of neutral alleles (Slatkin 1985). Such an approach can circumvent some of the practical difficulties involved with directly measuring the dispersal of organisms. Population genetic techniques also directly estimate genetic diversity, which can be reduced as a consequence of habitat fragmentation (e.g., Morden and Loeffler 1999). On the other hand, genetic structure can be influenced by historical and demographic processes. These processes can confound patterns interpreted as present-day levels of gene flow because they may result in nonequilibrium between genetic drift (random loss of alleles within a population) and gene flow (movement of alleles among populations).

Historical and demographic processes likely affect population genetic signatures at large and small scales, respectively. For some species, Pleistocene climate changes have resulted in large-scale habitat changes such that populations have become increasingly fragmented. Large-scale population genetic signatures may continue to reflect the older, more continuous habitat distribution, whereas small-scale population genetic structure may better reflect pre-

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sent-day levels of gene flow (Barber 1999). Different patterns at different spatial scales can result from the fact that equilibrium between genetic drift and gene flow is more rapidly reached at smaller than larger spatial scales (Hellberg 1994). On the other hand, rapid population turnover within and between generations can lead to temporal variation in genetic signatures at small spatial scales (e.g., Piertney and Carvalho 1995). These rapid, small-scale changes in genetic structure may confound large-scale estimates of gene flow because of the resulting nonequilibrium at small scales (Wade and McCauley 1988).

Stream benthic macroinvertebrates face habitat fragmentation at a variety of spatial scales, and so understanding the spatial scale of dispersal and the processes that may affect population genetic signatures at different spatial scales are important for predicting potential consequences of habitat fragmentation. River systems often traverse several biomes, effectively isolating their headwaters by biogeographic barriers downstream (Minshall 1988). At smaller spatial scales, rapid changes in longitudinal habitat characteristics may isolate species locally (Ward 1994) and drainage divides may limit dispersal among streams. Within individual streams, lakes and reservoirs create discrete flowing reaches separated by unsuitable habitat for many stream macroinvertebrates.

Evidence from genetic studies of stream benthic macroinvertebrates suggests that both historical and demographic processes may influence their population genetic signature. In an earlier study of the mayfly, *Baetis alpinus* Pictet (Baetidae), we found that populations were genetically differentiated among habitat fragments in alpine streams and concluded that dispersal over lakes was limited (Monaghan et al. 2001). Genetic differentiation was unrelated to lake size, but occurred only if lakes were situated in valleys that were ice-free throughout the Holocene. We concluded that the low levels of genetic differentiation observed between fragments separated by reservoirs (100 y old) and more recently formed lakes (100s–1000s y old) did not indicate high levels of gene flow but rather indicated that nonequilibrium between genetic drift and gene flow has prevented genetic differentiation since fragmentation. With regard to demographic processes that may affect population genetic signatures, many studies of stream macroinvertebrates have observed pronounced levels of reduced heterozygosity (inbreeding) and attribute this finding to oviposition by a few females (Schmidt et al. 1995, Bunn and Hughes 1997). Such recurring demographic processes may confound large-scale genetic structure because of rapid fluctuations in the spatial distribution of alleles at small scales.

The aim of our study was to investigate how habitat fragmentation at multiple spatial scales affected 3 species of stream insects. We examined larval population genetic structure of *B. alpinus*, *Rhithrogena loyolaea* Navàs (Heptageniidae), and *Allogamus auricollis* Pictet (Limnephilidae) using allozyme electrophoresis. We estimated levels of gene flow at multiple spatial scales: among major drainages of the Swiss Alps, among streams, and within streams. Our 1st objective was to examine whether habitat fragmentation within streams had similar effects on genetic diversity and gene flow of *R. loyolaea* and *A. auricollis* as was previously observed for *B. alpinus* (Monaghan et al. 2001). Our 2nd objective was to determine explicit scales of dispersal by examining whether or not genetic patterns were consistent across multiple spatial scales. We hypothesized that a lack of consistency among scales indicates that different processes affect patterns at different scales.

Methods

Study sites

The study was conducted in 3 major drainages in the Swiss Alps, constituting the headwaters of the Rhine, Inn, and Ticino rivers (Fig. 1). Within each major drainage we sampled either 3 or 4 streams and within each stream we sampled either 2 or 3 sites (Table 1), resulting in a total of 25 sampling sites. At the within-stream scale, we sampled 2 types of streams in each major drainage: those that contained potential dispersal barriers (a lake or reservoir) and those that did not (unfragmented). Upstream sampling sites were numbered 1 and downstream sites 2 (Fig. 1) for purposes of data presentation. Sites located between lakes were called site 2 of the upper lake. For example, Upper Jöri-2 was the lower site of Upper Jöri lake and was the upper site of Lower Jöri lake (Fig. 1). Upstream sites ranged in elevation from 1100 m to 2525 m, with 19 of 25 locations occurring

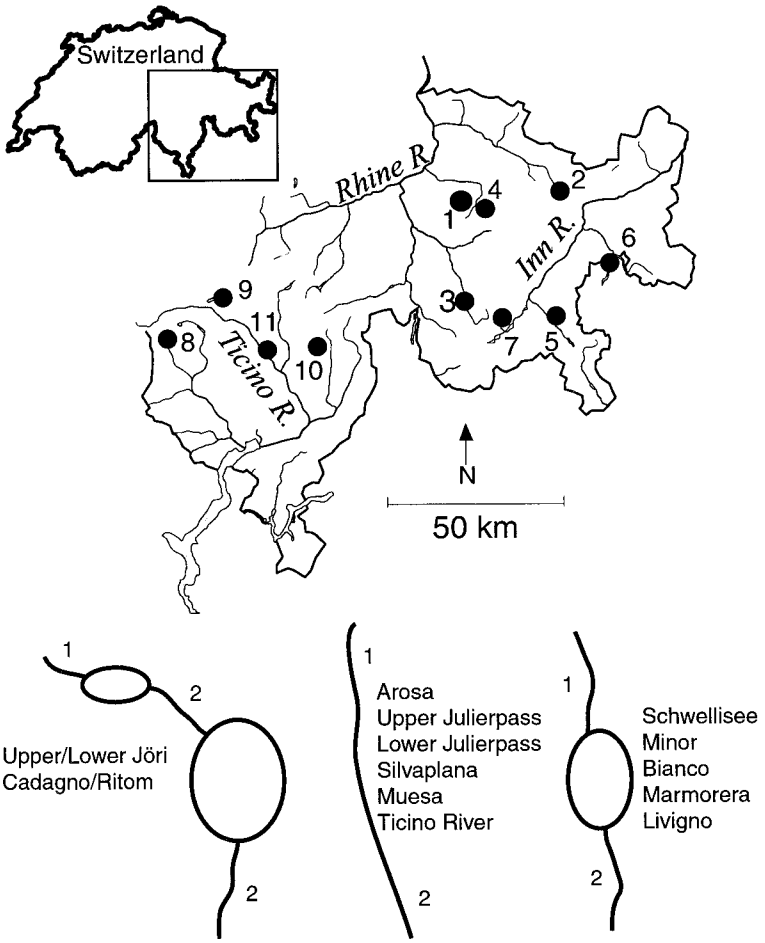

FIG. 1. The 11 study streams in the Swiss Alps (top) and schematic representations of sampling sites within each stream (bottom). Streams on the map are designated as: 1–Schwellisee, 2–Upper/Lower Jöri, 3–Marmorera, 4–Arosa, 5–Minor, 6–Livigno, 7–Upper/Lower Julierpass, 8–Bianco, 9–Cadagno/Ritom, 10–Muesa, 11–Ticino River. Ovals in the schematics represent lake or reservoir habitat and numbers indicate sampling sites along the stream. Distance between sampling sites in a stream ranged from 280 m to 10 km (see Table 1).

above 1900 m (Table 1). Sites within streams were 280 m to 10 km apart. The change in elevation within streams ranged from 4 to 250 m (Table 1).

Study animals

Baetis alpinus is a widespread and abundant alpine species (Humpesch 1979, Breitenmoser-Würsten and Sartori 1995). Larvae are eurythermal and occur between 200 and 2600 m in elevation (Sartori and Landolt 1999). The *B. alpinus* life cycle is plastic, ranging from bivoltine to semivoltine depending upon elevation (Humpesch 1979, Lavandier 1988). Adults exhibit pronounced upstream flight bias (Thomas 1975, Lavandier 1982) and our previous finding indicated dispersal was limited within fragmented streams. *Rhithrogena loyolaea* also is widespread but occurs within a more limited elevation range of 1300 to 2600 m (Sartori and Landolt 1999). Larvae are cold stenotherms (Vincon and Thomas 1987) with semivoltine (2–3 y) development (Lavandier 1981, Olechowska 1981). Its flight behavior is not as well studied as *B. alpinus*, although Thomas (1975) observed upstream bias and considerable altitude gains over *B. alpinus*, suggesting it may be a stronger flier.

TABLE 1. Geographical characteristics of the study streams and sampling sites in the 3 major drainages (see Fig. 1). Lake and reservoir names are from maps. Unfragmented reference streams, with the exceptions of Muesa and the Ticino River, were unnamed and therefore designated by location. Elevation data are for the upper location at each site. asl = above sea level.

Major drainage and stream	Dispersal barrier	Distance between fragments (m)	Elevation (m asl)	Elevation change (m)
Rhine				
Schwellisee	Lake	350	1935	5
Upper/Lower Jöri	Lakes (2)	550/975	2525/2495	30/175
Marmorera	Reservoir	7750	1700	250
Arosa	None	375	1940	10
Inn				
Minor	Lake	375	2340	15
Livigno	Reservoir	10,000	1910	155
Upper/Lower Julierpass[a]	None	625/3225	2310	105/220
Ticino				
Bianco	Lake	525	2080	4
Cadagno/Ritom[b]	Lakes (2)	1075/4500	1940/1090	40/120
Muesa	None	280	2225	25
Ticino River[c]	None	8000	1100	110

[a] Julierpass is a single stream with 3 sampling sites. For Upper Julierpass, the uppermost site was compared with a site ~0.6 km downstream. For Lower Julierpass, the uppermost site (= upstream site of Upper Julierpass) was compared with a site ~3.2 km downstream

[b] One stream fragmented by a lake (Cadagno) and a reservoir that is an enlarged lake (Ritom)

[c] The Ticino River was sampled only for *Allogamus auricollis* (see text)

Lavandier (1981) also documented upstream flight bias but gave no estimates of distance. *Allogamus auricollis* is locally very abundant and is univoltine (Waringer 1986, Graf et al. 1993). Limnephilids are considered strong fliers (Svensson 1974) but we know of no studies that have investigated the flying ability of *A. auricollis*.

Sample collection and allozyme electrophoresis

Late-instar larvae were collected using a 250-μm mesh kicknet, kept alive for 1 to 2 h in stream water, flash-frozen in liquid N, and stored for between 4 and 8 mo prior to allozyme electrophoresis in the laboratory. Collection of animals occurred on a single day at each site in summer 1999. Initially, all sites except the Ticino River were sampled for *B. alpinus* and *R. loyolaea*. *Rhithrogena loyolaea* was not observed at all sites, including both streams that were fragmented by a single reservoir (Livigno, Marmorera). Thus, *A. auricollis* was collected at these sites for the purpose of comparing it with *B. alpinus*. The Ticino River was then sampled only to provide an unfragmented reference stream for *A. auricollis*.

In the laboratory, larvae were thawed, identified, and ground in ~80 μL of crushing buffer (diH_2O, NADP, β-mercaptoethanol). Cellulose acetate electrophoresis (Hebert and Beaton 1989) was used to screen 25 enzyme systems for each species using individuals from a subset of sampling sites to identify polymorphic loci. Five and 6 polymorphic loci were identified for *R. loyolaea* and *A. auricollis*, respectively, and Monaghan et al. (2001) reported on 6 polymorphic loci for *B. alpinus* (Table 2). Data analysis was based on at least 25 animals from each sampling location when possible.

Data analysis

Mean number of alleles per locus (A) and expected Hardy–Weinberg heterozygosity (HW_{exp}) were calculated for each locus at each sampling location using BIOSYS-1 (Swofford and Selander 1981). A and HW_{exp} were compared between fragmented and unfragmented sites using ANOVA blocked by locus. A and HW_{exp} for genetically differentiated and undifferentiated streams were compared in the same way when within-stream genetic differentiation was moderate or greater ($\theta > 0.05$, see below). Deviations from HW equilibrium were examined by cal-

TABLE 2. Locus name, enzyme system (including peptidase substrate), International Enzyme Commission (E.C.) number, running buffer, and number of alleles scored for each locus. Buffer systems are those indicated by Richardson et al. (1986). Blanks indicate that the respective locus was not resolved successfully for the species.

				No. of alleles		
Locus	Enzyme	E.C. number	Buffer	*Rhithrogena loyolaea*	*Allogamus auricollis*	*Baetis alpinus*
Gda	Guanine deaminase	3.5.4.3	I	7		
Mpi	Mannose-phosphate isomerase	5.3.1.8	A	4	4	9
Pep-A	Peptidase (valine-leucine)	3.4.11 or 13	I		4	
Pep-B	Peptidase (leucine-glycine-glycine)	3.4.11 or 13	I			7
Pep-C-1	Peptidase (leucine-alanine)	3.4.11 or 13	I		4	5
Pep-C-2	Peptidase (leucine-alanine)	3.4.11 or 13	I		4	
Pep-D	Peptidase (phenylalanine-proline)	3.4.13.9	I	5		7
Pgi	Phosphoglucose isomerase	5.3.1.9	I	8	5	8
Pgm	Phosphoglucomutase	2.7.5.1	I	4	4	8

culating the inbreeding coefficient (*f*) and testing for significance using GENEPOP version 3.1d (M. Raymond and F. Rousset, Université de Montpellier II, Montpellier, France). Significant (Bonferroni-corrected) positive values of *f* indicate heterozygote deficiency and significant negative values indicate heterozygote excess. Linkage disequilibrium also was assessed using GENEPOP.

Genetic differentiation of populations was determined by estimating θ, a measure of the relative fixation of alternate alleles in different subpopulations (Weir and Cockerham 1984). Values of θ, 95% confidence intervals, and significant difference from 0 were examined using FSTAT version 2.9.1 (J. Goudet, Université de Lausanne, Switzerland). The option that compares genotype frequencies rather than allele frequencies was used because of significant deviation from HW equilibrium (see Results). When θ was significant, the degree of genetic differentiation was assessed using the ranges specified by Hartl and Clark (1997), where $\theta < 0.05$ indicates little differentiation, 0.05 to 0.15 indicates moderate differentiation, 0.15 to 0.25 indicates great differentiation, and >0.25 indicates very great differentiation. Levels of genetic differentiation were assessed at 3 levels of spatial hierarchy: 1) among the 3 major drainages (Rhine, Inn, and Ticino headwater populations), 2) among streams within each major drainage, and 3) within streams. At the within-stream scale, 7 streams contained potential dispersal barriers and 4 streams did not. When populations were genetically differentiated within streams (e.g., *B. alpinus* data from Monaghan et al. 2001), we calculated θ among streams and among drainages once for all populations and once using only those populations from streams that were not genetically differentiated. Thus, it was possible to examine whether small-scale differentiation affected large-scale patterns.

Results

Genetic diversity in fragmented and unfragmented streams

Allele frequencies and locus *n*-sizes are reported in Appendices 1 to 3. Mean number of alleles per locus, *A*, was not significantly different between fragmented and unfragmented sample locations for either *R. loyolaea* ($F_{1,52} = 0.18$, $p = 0.68$) or *A. auricollis* ($F_{1,33} = 0.20$, $p = 0.66$), as was the case for *B. alpinus* ($F_{1,135} = 1.03$, $p = 0.31$) reported previously (Fig. 2A). The same was true for mean HW_{exp} (Fig. 2B; statistics not reported). For *B. alpinus*, neither *A* nor HW_{exp} was significantly different between sites in genetically differentiated and undifferentiated streams ($F_{1,136} = 2.28$, $p = 0.13$). The inbreeding coefficient, *f*, was significantly >0 for *R. loyolaea* in 4 of 60 instances (~7%; Table 3). For *A. auricollis*, a significant *f* was observed in 3 of 36 instances (~8%; Table 4). These values were in contrast to the large number of significant deviations for *B. alpinus* (28%; Monaghan et al. 2001). Of the 375, 110, and 90 pairwise compar-

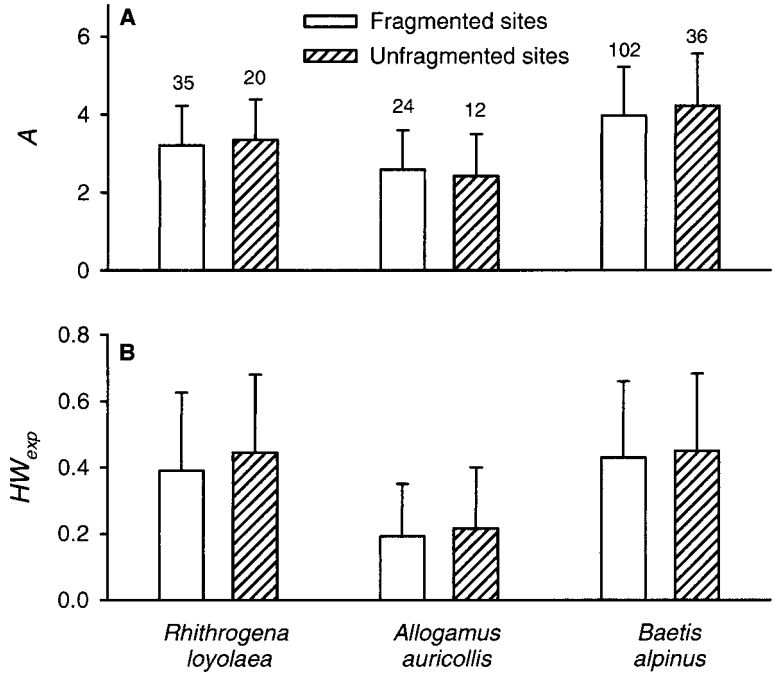

FIG. 2. Genetic diversity measured as (A) mean number of alleles per locus (*A*) and (B) mean expected Hardy–Weinberg heterozygosities (HW_{exp}) of each species in fragmented and unfragmented streams. Error bars indicate +1 SD. Sample sizes (no. populations × no. loci) for both variables are indicated above the error bars in panel A.

TABLE 3. Inbreeding coefficient (*f*) for each locus in each population of *Rhithrogena loyolaea*. * = significant difference from 0 following Bonferroni correction for the number of tests. – = locus was monomorphic. Locus names as in Table 2.

Location	*Gda*	*Mpi*	*Pep-D*	*Pgi*	*Pgm*
Rhine					
Schwellisee-1	0.538	0.473	0.580*	0.366	0.133
Schwellisee-2	−0.062	−0.041	0.062	0.017	−0.154
Upper Jöri-1	−0.047	–	0.375	0.205	0.019
Upper Jöri-2	0.280	0.653	0.183	−0.094	−0.093
Lower Jöri-2	−0.036	−0.036	0.745*	0.079	0.105
Arosa-1	−0.079	0.500	−0.141	−0.048	0.183
Arosa-2	−0.048	0.000	0.000	0.028	0.102
Inn					
Upper Julierpass-1	−0.015	−0.024	0.713	0.252	0.469
Upper Julierpass-2	0.319	−0.076	0.650*	0.081	0.317
Minor-2	0.118	0.063	0.056	0.005	0.079
Ticino					
Bianco-1	−0.096	0.000	0.618	0.259	0.073
Bianco-2	−0.051	0.999*	−0.045	0.077	−0.179

TABLE 4. Inbreeding coefficient (*f*) for each locus in each population of *Allogamus auricollis*. * = significant difference from 0 following Bonferroni correction for the number of tests. Locus names as in Table 2.

Location	*Mpi*	*Pep-A*	*Pep-C-1*	*Pep-C-2*	*Pgi*	*Pgm*
Livigno-1	−0.255	0.228	0.174	−0.036	−0.020	−0.010
Livigno-2	0.223	−0.011	0.000	−0.171	−0.057	−0.067
Marmorera-1	1.000*	0.000	1.000*	−0.209	−0.204	−0.048
Marmorera-2	1.000*	0.000	−0.032	0.050	0.000	−0.076
Ticino River-1	0.084	0.481	−0.067	−0.005	−0.057	−0.166
Ticino River-2	−0.096	0.000	0.000	0.098	−0.020	−0.204

isons used to test for linkage disequilibrium of *B. alpinus*, *R. loyolaea*, and *A. auricollis*, respectively, 18, 4, and 4 were significant ($p < 0.05$); such results are expected through chance alone.

Genetic differentiation, θ, at multiple spatial scales

Values of θ for *R. loyolaea* were significant within and among drainages (Table 5). θ among major drainages of the Alps was much more pronounced (θ = 0.080) than among streams within any drainage (θ = 0.026–0.032; Table 5). Within streams, θ was significant across Upper Jöri Lake but indicated little differentiation (θ = 0.030; Fig. 3). No genetic differentiation of *R. loyolaea* was observed within any of the other streams, including those where relatively large differentiation occurred for *B. alpinus* (Fig. 3). θ for *A. auricollis* was significant among major drainages but indicated little differentiation (Table 5). θ for *A. auricollis* was significant across the reservoir Livigno, but also low (θ = 0.023); θ was not significant across Marmorera or along the unfragmented Ticino River (Fig. 3). The lack of even moderate differentiation at the reservoir sites was similar to that observed for *B. alpinus* (Fig. 3).

Values of θ for *B. alpinus* were significant within and among drainages when all streams were included in the analysis and when only genetically undifferentiated streams were included (Table 5). Considering all streams together, differentiation within drainages (θ = 0.064–0.089) was much higher than among drainages (θ = 0.010). Considering only undifferentiated streams (i.e., with values of θ < 0.05,

TABLE 5. Estimates of genetic differentiation (θ) jackknifed across loci within and among each of the 3 major drainages (Rhine, Inn, and Ticino rivers). For *Baetis alpinus*, θ values are presented for all streams and separately for only the undifferentiated streams. Only 1 stream (Moesa) in the Ticino drainage was undifferentiated, so an among-stream θ could not be calculated. *Rhithrogena loyolaea* was not genetically differentiated in any streams and so only a single analysis was performed. *Allogamus auricollis* was sampled from a single stream in each major drainage so only an among-stream analysis was performed. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 1$. – = no analysis was performed.

	All streams	95% CI	Undifferentiated streams	95% CI
Baetis alpinus				
Rhine	0.089***	0.022	0.052***	0.022
Inn	0.069***	0.044	0.049***	0.024
Ticino	0.064***	0.040	–	–
Among	0.010***	0.005	0.015***	0.006
Rhithrogena loyolaea				
Rhine	0.032***	0.021	–	–
Inn	0.026**	0.005	–	–
Ticino	0.026**	0.010	–	–
Among	0.080***	0.005	–	–
Allogamus auricollis				
Among	0.042*	0.011	–	–

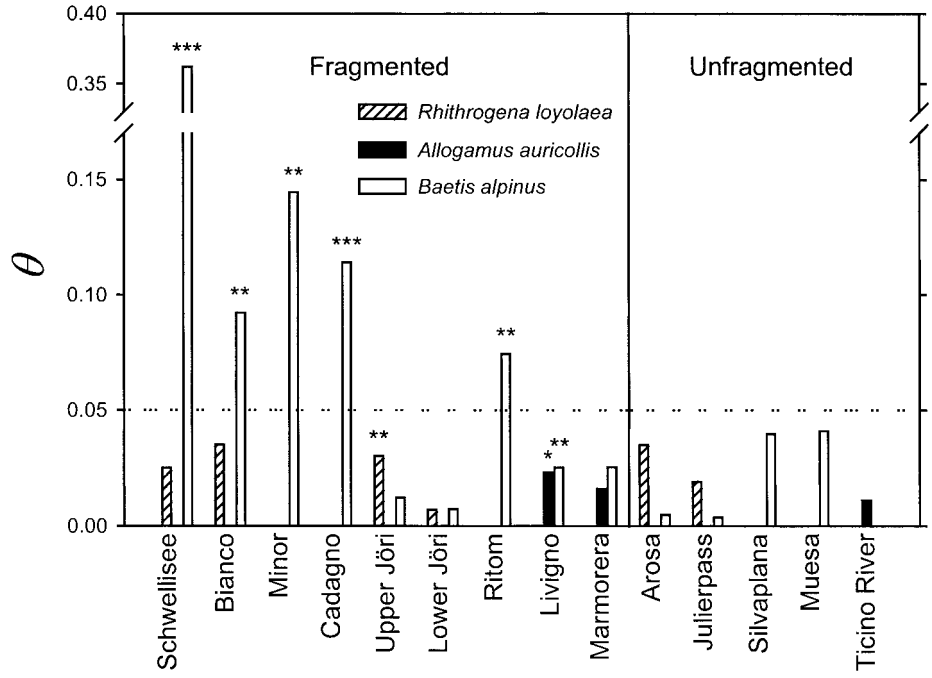

FIG. 3. Genetic differentiation (θ) within fragmented (lake or reservoir) and unfragmented streams for each species. Asterisks indicate significant differences from 0 (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$). The dotted line represents levels above which moderate differentiation occurs (Hartl and Clark 1997).

Fig. 3), among-stream θ values were lower than those computed using all streams together. Nonetheless, θ remained at levels indicative of moderate differentiation. The pattern of greater differentiation within drainages than among drainages was opposite to the pattern observed for *R. loyolaea*.

Multiscale patterns of θ are presented graphically for simultaneous comparison of species and spatial scales (Fig. 4). *Rhithrogena loyolaea* genetic population structure was most pronounced at the largest spatial scale, indicating populations were structured primarily among drainages. *Allogamus auricollis* exhibited very little structure at the scales of the present study, with only a slight increase in θ moving up 2 steps in the spatial hierarchy (Fig. 4). Considering *B. alpinus* populations undifferentiated within streams (Fig. 4), genetic population structure appeared most pronounced among streams, with lower values of θ at both smaller and larger spatial scales. Considering populations with high levels of within-stream differentiation, genetic structure of *B. alpinus* often was most pronounced within streams, with subsequent reduction in θ moving to larger (among streams) and larger (among drainages) scales (Fig. 4).

Discussion

Genetic diversity in fragmented streams

Results for all 3 species suggest that genetic diversity was not reduced by the fragmentation of lotic habitat by lentic water bodies. We observed no difference between fragmented and unfragmented populations using 2 informative estimates of genetic diversity (A and HW_{exp}). In addition, we observed no difference in genetic diversity when populations of *B. alpinus* from genetically differentiated streams ($\theta > 0.05$) were compared with undifferentiated streams. These results contrast with expectations from empirical and theoretical work in fragmented populations, which have often found that genetic diversity is reduced (Lacy 1987, van Dongen et al. 1998, Morden and Loeffler 1999). The maintenance of genetic diversity implies population sizes may be large enough in fragments

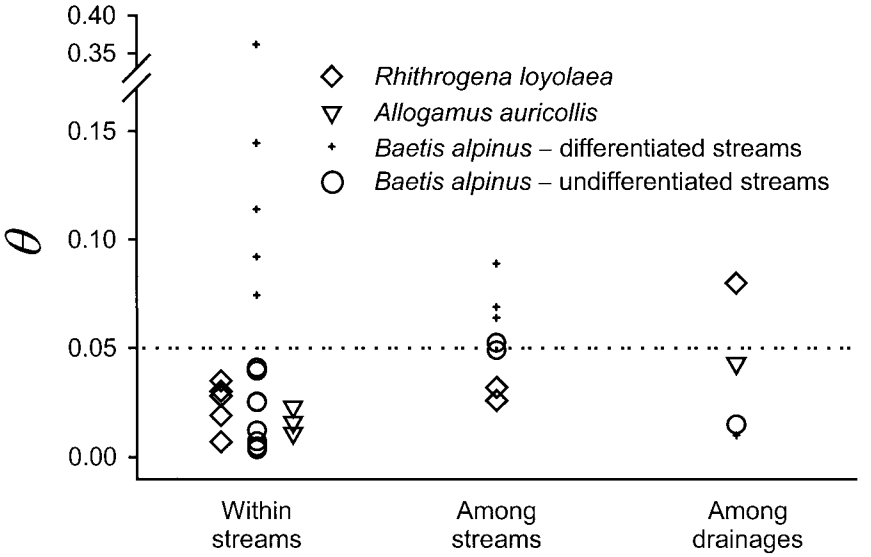

FIG. 4. Multilocus genetic differentiation (θ) of each species at 3 hierarchical spatial scales. Within-stream values are the same as presented in Fig. 3 and are offset along the x-axis for clarity of presentation.

so that the loss of alleles by genetic drift is minimal, or gene flow among subpopulations sustains genetic diversity (Slatkin 1985).

Gene flow estimates at multiple spatial scales

Multilocus estimates of θ were distinctly different among species and varied within species depending on the spatial scale considered. We observed little or no genetic differentiation in *R. loyolaea* except among the major drainages of the study, suggesting that at least a moderate level of dispersal occurs within and among streams. The multiscale pattern of θ (Fig. 4) presumably typifies species with a relatively large-scale population structure, an equilibrium between genetic drift and gene flow, and a decreased relatedness with increasing geographic distance (Slatkin 1993). One conclusion is that failure to observe even moderate genetic differentiation across any lakes, including those where *B. alpinus* was differentiated, results from gene flow among habitat fragments. Thomas (1975) observed *R. loyolaea* flying upstream and gaining twice as much elevation as *B. alpinus*, suggesting they are stronger flyers and capable of traveling considerable distances as adults. Flecker and Allan (1988) observed a congener, *R. hageni*, to fly randomly, including away from the stream, suggesting *Rhithrogena* may be capable of crossing areas of unsuitable habitat and may not be confined to following the drainage pattern.

The low level of differentiation in *B. alpinus* among the 3 major drainages was surprising, based on our previous conclusion that gene flow was limited over lentic water bodies ~300 to 1000 m across (Monaghan et al. 2001). The homogeneity among major drainages also seems contradictory to among-stream θ values in the present study. These values suggest limited dispersal of *B. alpinus* among different valleys. Small-scale differentiation and large-scale homogeneity is evidence that a species has not yet reached equilibrium between genetic drift and gene flow (Hellberg 1994). We suggest 2 possible mechanisms responsible for the genetic population structure of *B. alpinus*. Either, or both, could account for the lack of equilibrium between gene flow and genetic drift (as indicated by different estimates of θ at different spatial scales), and for the lack of HW equilibrium in populations. One mechanism concerns heterozygote deficiency as evidence for recurring changes in small-scale population structure in the midst of large-scale equilibrium. The other mechanism concerns consistent small-scale patterns of θ as evidence for limited present-day dispersal in the midst of large-scale patterns that continue to reflect historical patterns of gene flow.

Recurring small-scale changes in genetic structure.—We observed a large number of heterozygote deficiencies for *B. alpinus* and frequency appeared unrelated to habitat fragmentation, longitudinal position in the stream, or geographical location in the study. Heterozygote deficiency may result from nonrandom mating, the presence of null alleles, misscoring of gels, or the presence of multiple species. We observed no null homozygotes in an analysis of >1000 individuals and, although misscoring of gels can never be ruled out, it was an unlikely source of error because of consistent HW equilibrium observed at the same loci for the 2 other species. It is unlikely that multiple species of *Baetis* were analyzed at any sampling site because of good larval taxonomic descriptions (see Sartori and Landolt 1999), the lack of linkage disequilibrium, and a preliminary examination of DNA fragment-length polymorphism for the same individuals (MTM, unpublished data).

Heterozygote deficiency has been observed in several studies of aquatic insects, often at levels similar to what we observed for *B. alpinus* (28% of possible instances). Schmidt et al. (1995) reported 25% for *Baetis* sp., Wishart and Hughes (2001) reported 30% for *Elporia barnardi* (Blephariceridae), and Hughes et al. (1998) reported 23% for *Tasiagma ciliata* (Trichoptera). Schmidt et al. (1995) proposed and Bunn and Hughes (1997) extended an explanatory mechanism, suggesting that reduced direct-count heterozygosity results from larval populations at any given location being the result of only a few ovipositing females. If *B. alpinus* populations are the result of a small number of matings, then allele frequencies at any given sample site could fluctuate randomly from one generation to the next. If genetic homogeneity at large scales results from contemporary wide-ranging dispersal ability, then ovipositing females constitute a small but random sample of females drawn from a very large gene pool, allowing genetic differentiation at local scales to arise by chance. Such a mechanism suggests equilibrium has been reached at the scale of the Alps but that random sampling of alleles (bottlenecking) occurring each generation results in changes in allele frequencies too rapid for equilibrium to be reached within streams.

One limitation of this mechanism is that it seems unable to account for the consistent pattern of differentiation among streams for *B. alpinus*, regardless of whether we considered all streams or only undifferentiated streams. In addition, we observed a lack of HW equilibrium at all but 2 sampling sites for *B. alpinus*, but genetic differentiation was consistently observed only in fragmented streams and only in those streams where fragmentation was comparatively old (Monaghan et al. 2001).

Present-day and historical gene flow.—A 2nd possible mechanism is that small-scale population differentiation reflects present-day levels of gene flow and large-scale homogeneity reflects historical processes and a slow rate of approach to equilibrium between genetic drift and gene flow for *B. alpinus*. Major glacial advances (occurring twice in the last 200,000 y) forced populations downward in river drainages and likely mixed headwater populations below major confluences. During and after glacial retreat, populations dispersed into headwaters and slowly began to diverge genetically because of the tight coupling of downstream drift and upstream flight (Lavandier 1982). Populations have genetically diverged within streams in drainages fragmented by lakes formed during and soon after glacial retreat (Monaghan et al. 2001). The overall distribution of alleles among the headwaters of the Alps remains similar to its historical configuration, however, because of the slow rate of approach to equilibrium between gene flow and genetic drift at this largest scale.

Although neither mechanism can be explicitly ruled out with our data set, we can propose testable hypotheses based on each. Recurring bottlenecks should mean that allele frequencies at any given site change randomly from one generation to the next in a manner similar to a metapopulation (Piertney and Carvalho 1995). If the lack of HW equilibrium results from populations being founded by only a few ovipositing females, then these populations should contain relatively few mtDNA haplotypes. Such a test would require comparison with another species whose populations are in HW equilibrium, presumably because populations are founded by many more females. On the other hand, equilibrium between gene flow and genetic drift should be achieved more rapidly with faster evolving molecular markers (e.g., mtDNA; Brown et al. 1982), thus allowing one to distinguish between historical and present-day gene flow.

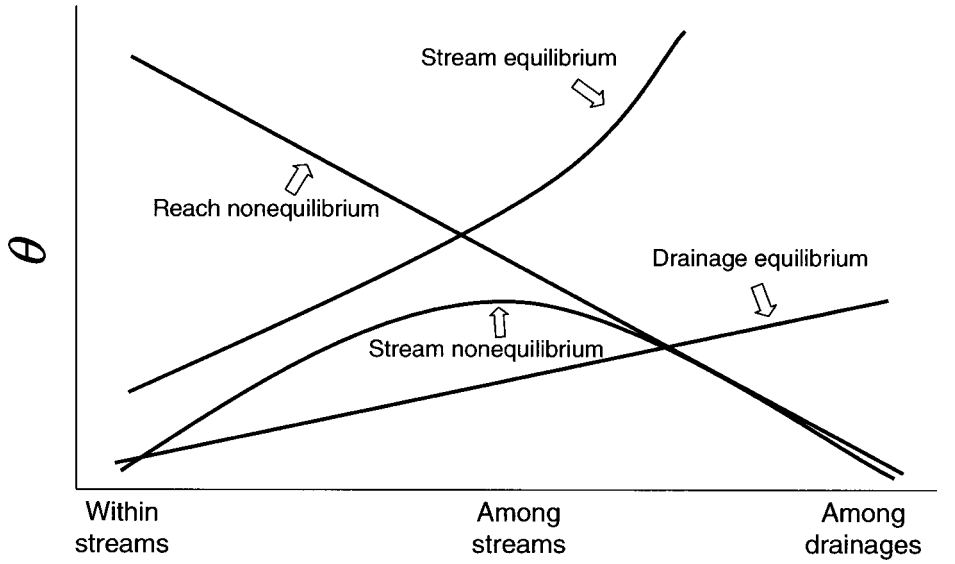

FIG. 5. Relationships between genetic differentiation (θ) and spatial scale for stream benthic macroinvertebrates as a function of the scales of dispersal and the mechanisms responsible for the patterns.

Allogamus auricollis and lack of genetic differentiation over reservoirs

As with *B. alpinus*, we observed little or no genetic differentiation of *A. auricollis* over the reservoirs Livigno and Marmorera. There also was little or no subpopulation structure even among major drainages. Limnephilidae typically are strong flyers (Svensson 1974) and dispersal among major drainages suggests that *A. auricollis* is able to cross reservoirs. However, the conclusion that gene flow continues over reservoirs can only be tentative because of uncertainty as to how rapidly genetic markers can detect recent fragmentation. Sweeney et al. (1986) observed no genetic differentiation between populations of mayflies (*Ephemerella subvaria* and *Eurylophella verisimilis*) above and below reservoirs of the Delaware River, USA, and Stiven and Kreiser (1994) observed no differentiation of stream-dwelling gastropod (*Goniobasis proxima*) populations separated by a reservoir. Thus, to our knowledge, researchers have never observed genetic isolation of benthic invertebrates, using allozymes, across reservoirs up to 10 km long.

Dispersal modes and genetic population structure of stream insects

Using our data and other studies of stream insect population genetic structure, we present a synthesis of observed relationships between genetic population structure and dispersal modes. We examined studies that investigated at least 2 spatial scales and we consider those taxa that have a wide enough geographical distribution such that θ among drainages can be calculated. Figure 5 depicts 4 different relationships between θ and spatial scale for stream macroinvertebrates taken from the published literature. Note that the x-axis depicts increasing spatial scales used in our study rather than linear distance as would occur in a strict isolation-by-distance (IBD) model (Slatkin 1993). The names of the 4 different curves are defined by the spatial scale at which dispersal becomes limited and by the presence or absence of gene flow–genetic drift equilibrium. In general, increasing θ from left to right in Fig. 5 should indicate species in equilibrium between genetic drift and gene flow. The rate of increase in θ depends on whether or not species disperse readily among streams and drainages. A decrease from left to right or a hump-shaped distribution should indicate species that are not in equilibrium. This pattern may be the result of local dynamics that recurringly alter allele frequencies, or may be the result of historical levels of gene flow confounding present-day population genetic signatures.

Progressively increasing subpopulation dif-

ferentiation (*drainage equilibrium,* Fig. 5) indicates a relatively widespread species with gene flow occurring within and among streams (i.e., gene flow becomes limited only among drainages) and large-scale equilibrium between genetic drift and gene flow (Slatkin 1993). Such species are likely to exhibit an IBD pattern, and include *R. loyolaea* in our study and the caddisfly *Helicopsyche borealis* studied by Jackson and Resh (1992). We note, however, that strong relationships of IBD could result even in the absence of gene flow at smaller spatial scales if recent population differentiation (for example by habitat fragmentation) is not yet manifest in population genetic signatures (e.g., Barber 1999).

Stream equilibrium (Fig. 5) includes species that experience gene flow primarily within streams or among reaches of streams, thereby having a more rapid increase in θ as one moves to larger spatial scales. A clear example of such a species is the waterstrider *Aquarius remigis* investigated by Preziosi and Fairbairn (1992). They observed orders of magnitude increase in θ from within to among streams. The atyid shrimp *Paratya australiensis* also exhibited an order of magnitude increase in θ from within to among streams (Hughes et al. 1995, Bunn and Hughes 1997). *Paratya australiensis* does not fly and *A. remigis* dispersal by flight is very rare (Preziosi and Fairbairn 1992). We suggest that most taxa with limited dispersal abilities would fall into this category. Taxa with very high in-stream dispersal ability but limited dispersal among streams should display a similar pattern, but with the curve shifted downward.

Several taxa exhibit patterns of reduced differentiation at progressively larger spatial scales (*reach nonequilibrium,* Fig. 5). These patterns indicate large-scale structure is relatively homogeneous but that small-scale (within-stream) structuring exists, thus implying that small-scale structuring forces are more evolutionarily recent events (Hellberg 1994). The populations of *B. alpinus* that were genetically differentiated within streams in our study exhibited such a pattern, as did the caddisfly *T. ciliata* (Hughes et al. 1998). Species that undergo the process of repeated bottlenecks (e.g., oviposition by only a few females) should match this distribution because such a process constitutes small-scale substructuring of each generation.

Last, some taxa may structure genetically at intermediate spatial scales, with highest θ found among streams. Such a curve (*stream nonequilibrium*) was the case for *B. alpinus* in unfragmented streams in the present study. This pattern may be predominant in species that are widespread biogeographically and have colonized areas in evolutionarily recent times from large source populations (e.g., mountain ranges following Pleistocene glaciation), but that maintain relatively low levels of gene flow among streams. Of note, the stonefly *Yoroperla brevis* examined by Hughes et al. (1999) may exhibit a pattern of stream equilibrium (shifted downward as described earlier) or stream nonequilibrium, depending on its genetic structure at the largest spatial scale.

In conclusion, we observed no reduction in genetic diversity in fragmented streams for any of the 3 species. Populations in fragments may remain large enough that no loss of alleles occurs via genetic drift, or levels of gene flow among fragments may remain high enough to counteract the loss of alleles. For *R. loyolaea*, the consistent pattern of increasing genetic differentiation with increasing spatial scale suggests populations were in equilibrium between genetic drift and gene flow. We conclude *R. loyolaea* disperses readily both within and among streams, but less so among major drainages. *Allogamus auricollis* did not exhibit genetic differentiation at any scale, suggesting that dispersal occurs throughout the geographical range of the study. In contrast, homogeneity at large spatial scales and differentiation at small spatial scales suggest a lack of equilibrium for *B. alpinus*. Consistent differentiation of *B. alpinus* between older stream fragments (~10,000 y) indicates dispersal is limited among fragments and that large-scale structure reflects historical levels of gene flow. Pronounced heterozygote deficiencies suggest structure at small spatial scales reflects genetic bottlenecks during recruitment. Last, limited dispersal among fragments and demographic processes likely affect small-scale patterns, and historical processes likely affect large-scale patterns. The simultaneous study of multiple spatial scales can help us to determine the relative importance of each process.

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APPENDIX 1. Allele frequencies of the 5 polymorphic loci examined for populations of *Rhithrogena loyolaea* for upstream (1) and downstream (2) sampling sites. Alleles were scored by their relative mobility, with A being slowest. Locus names as in Table 2.

		Schwellisee		Upper Jöri		Lower Jöri	Arosa		Upper Julierpass		Minor	Bianco	
Locus	Allele	1	2	1	2	2	1	2	1	2	2	1	2
Gda	*n*	*25*	*18*	*16*	*18*	*30*	*25*	*12*	*22*	*25*	*20*	*19*	*24*
	A				0.06								
	B											0.05	
	C			0.06	0.06		0.06	0.04	0.05	0.08		0.08	0.02
	D	0.74	0.92	0.91	0.83	0.93	0.88	0.88	0.80	0.80	0.88	0.84	0.92
	E	0.02		0.03	0.03	0.03		0.04	0.11	0.02			0.06
	F	0.08	0.08		0.03	0.03	0.06	0.04	0.05	0.10	0.13	0.03	
	G	0.16											
Mpi	*n*	*25*	*18*	*19*	*18*	*30*	*25*	*9*	*22*	*17*	*20*	*22*	*22*
	A						0.02						
	B	0.08	0.06		0.08	0.05	0.04			0.03	0.08	0.02	0.09
	C	0.92	0.92	1.00	0.92	0.95	0.76	0.94	0.96	0.77	0.90	0.98	0.91
	D		0.03				0.18	0.06	0.05	0.21	0.03		
Pep-D	*n*	*25*	*18*	*17*	*18*	*30*	*25*	*12*	*22*	*25*	*20*	*22*	*24*
	A					0.02			0.02				
	B	0.14		0.15		0.25	0.02			0.24		0.07	0.06
	C	0.60	0.92	0.62	0.81	0.65	0.72	1.00	0.82	0.60	0.93	0.80	0.94
	D	0.26	0.08	0.24	0.14	0.08	0.26		0.16	0.16	0.08	0.14	
	E				0.06								
Pgi	*n*	*25*	*17*	*19*	*18*	*30*	*25*	*12*	*23*	*24*	*19*	*20*	*23*
	A		0.03								0.03		
	B		0.06	0.03	0.03	0.02			0.07	0.08	0.05	0.13	0.22
	C	0.26	0.15	0.32	0.39	0.37	0.38	0.38	0.41	0.27	0.13	0.25	0.28
	D										0.16		
	E	0.52	0.15	0.18	0.28	0.30	0.30	0.38	0.22	0.29	0.03	0.25	0.33
	F		0.03						0.02				
	G	0.22	0.56	0.47	0.28	0.32	0.20	0.25	0.17	0.35	0.58	0.38	0.17
	H		0.03		0.03		0.12		0.11		0.03		
Pgm	*n*	*25*	*18*	*19*	*18*	*30*	*25*	*12*	*22*	*20*	*20*	*22*	*24*
	A					0.03	0.16			0.03		0.02	
	B	0.40	0.33	0.53	0.31	0.35	0.20	0.13	0.30	0.23	0.30	0.34	0.17
	C	0.18	0.17	0.03	0.06	0.08	0.24	0.25	0.32	0.15	0.23	0.64	0.83
	D	0.42	0.50	0.45	0.64	0.53	0.40	0.63	0.39	0.60	0.48		

APPENDIX 2. Allele frequencies of the 6 polymorphic loci examined for populations of *Allogamus auricollis* for upstream (1) and downstream (2) sampling sites. Alleles were scored by their relative mobility, with A being slowest. Locus names as in Table 2.

		Livigno		Marmorera		Ticino River	
Locus	Allele	1	2	1	2	1	2
Mpi	*n*	*24*	*24*	*18*	*12*	*15*	*17*
	A	0.04	0.15	0.11		0.10	
	B	0.75	0.60	0.89	0.92	0.67	1.00
	C	0.21	0.19		0.08	0.23	
	D		0.06				
Pep-A	*n*	*26*	*25*	*30*	*17*	*25*	*27*
	A	0.02	0.02			0.06	
	B	0.87	0.96	1.00	1.00	0.92	1.00
	C	0.08	0.02			0.02	
	D	0.04					
Pep-C-1	*n*	*26*	*24*	*28*	*17*	*25*	*27*
	A	0.02					
	B	0.85	0.98	0.96	0.94	0.92	1.00
	C	0.10	0.02	0.04	0.06	0.08	
	D	0.04					
Pep-C-2	*n*	*26*	*25*	*27*	*17*	*25*	*21*
	A	0.02				0.02	
	B	0.92	0.84	0.59	0.77	0.78	0.81
	C	0.02					
	D	0.04	0.16	0.41	0.24	0.20	0.19
Pgi	*n*	*26*	*25*	*30*	*17*	*25*	*26*
	A		0.02				
	B			0.03		0.02	
	C	0.96	0.90	0.95	1.00	0.90	0.96
	D		0.06			0.06	0.04
	E	0.04	0.02	0.02		0.02	
Pgm	*n*	*26*	*25*	*30*	*17*	*25*	*27*
	A			0.07		0.10	0.09
	B	0.02		0.10	0.09	0.10	0.20
	C	0.96	0.92	0.80	0.88	0.78	0.70
	D	0.02	0.08	0.03	0.03	0.02	

APPENDIX 3. Allele frequencies of the 6 polymorphic loci examined for populations of *Baetis alpinus* in upstream (1) and downstream (2) study sites. Alleles were scored by their relative mobility, with A being slowest. These data also were used for the analysis by Monaghan et al. (2001). Locus names as in Table 2.

		Schwellisee		Upper Jöri		Lower Jöri	Marmorera		Arosa		Minor		Livigno		Upper Julierpass		Lower Julierpass	Bianco		Cadagno		Ritom	Muesa	
Locus	Allele	1	2	1	2	2	1	2	1	2	1	2	1	2	1	2	2	1	2	1	2	2	1	2
Mpi	*n*	*15*	*25*	*21*	*5*	*22*	*16*	*14*	*26*	*26*	*15*	*10*	*24*	*19*	*27*	*16*	*24*	*20*	*17*	*15*	*20*	*19*	*25*	*25*
	A		0.02						0.02	0.02	0.03	0.20												
	B	0.07			0.10				0.08	0.06	0.07			0.16	0.02	0.03	0.04	0.10	0.09		0.05	0.05	0.08	0.02
	C	0.13	0.14	0.14	0.10	0.09	0.22	0.29	0.21	0.27	0.17	0.60	0.06	0.13	0.11	0.25	0.23	0.25	0.38	0.13	0.10	0.24	0.12	0.20
	D									0.02				0.16										
	E	0.77	0.82	0.81	0.80	0.86	0.66	0.21	0.67	0.60	0.67	0.20	0.46	0.18	0.80	0.63	0.44	0.53	0.38	0.80	0.33	0.45	0.70	0.64
	F							0.07						0.05			0.04							
	G	0.03	0.02	0.05		0.05	0.13	0.29	0.02	0.04	0.07		0.40	0.24	0.07	0.09	0.25	0.13	0.15	0.07	0.40	0.18	0.10	0.14
	H							0.14					0.04	0.08							0.13	0.08		
	I												0.04											
Pep-B	*n*	*25*	*27*	*25*	*25*	*25*	*24*	*24*	*27*	*26*	*26*	*18*	*25*	*25*	*28*	*24*	*26*	*23*	*25*	*25*	*24*	*24*	*25*	*25*
	B	0.04		0.06	0.06	0.02			0.04	0.02			0.04		0.02	0.08	0.08		0.04	0.10	0.17	0.13	0.02	0.02
	C	0.54	0.06	0.26	0.10	0.14	0.19	0.10	0.32	0.27	0.10	0.14	0.08	0.02	0.14	0.23	0.14	0.11	0.04	0.22	0.52	0.27	0.16	0.26
	D												0.02											
	E	0.40	0.48	0.60	0.76	0.68	0.69	0.63	0.50	0.46	0.50	0.47	0.74	0.76	0.75	0.65	0.58	0.61	0.78	0.44	0.31	0.54	0.58	0.32
	G	0.02	0.39	0.04	0.08	0.14	0.06	0.25	0.11	0.25	0.37	0.33	0.12	0.22	0.09	0.04	0.21	0.28	0.14	0.16		0.06	0.24	0.40
	H		0.07	0.04		0.02	0.06	0.02	0.04		0.04	0.06								0.02				
	I																			0.06				
Pep-C	*n*	*25*	*27*	*25*	*25*	*25*	*25*	*25*	*27*	*26*	*26*	*18*	*26*	*25*	*28*	*26*	*26*	*24*	*26*	*25*	*25*	*25*	*26*	*25*
	A								0.02	0.02														
	B											0.03	0.02											
	C			0.02	0.02	0.04		0.08	0.02									0.02	0.02	0.08		0.04		
	E	1.00	0.98	0.98	0.96	0.96	1.00	0.92	0.96	0.98	1.00	0.97	0.98	1.00	1.00	1.00	1.00	0.98	0.98	0.92	1.00	0.90	1.00	1.00
	G		0.02		0.02																	0.06		
Pep-D	*n*	*25*	*26*	*23*	*23*	*23*	*20*	*21*	*22*	*26*	*16*	*16*	*26*	*25*	*26*	*25*	*22*	*22*	*25*	*25*	*24*	*25*	*24*	*25*
	A						0.05																	
	B	0.20					0.10	0.02	0.21	0.12			0.02	0.04		0.08	0.05			0.08		0.02		0.06
	C	0.08		0.20	0.28	0.07	0.05	0.10	0.05	0.02			0.04		0.08	0.18	0.05	0.02	0.06	0.14	0.38	0.12	0.04	0.20

APPENDIX 3. Continued.

Locus	Allele	Schwellisee		Upper Jöri		Lower Jöri	Marmorera		Arosa		Minor		Livigno		Upper Julierpass		Lower Julierpass	Bianco		Cadagno		Ritom	Muesa	
		1	2	1	2	2	1	2	1	2	1	2	1	2	1	2	2	1	2	1	2	2	1	2
	E	0.68	0.02	0.78	0.54	0.70	0.75	0.83	0.61	0.42	0.56	0.50	0.89	0.90	0.65	0.64	0.52	0.39	0.66	0.64	0.52	0.70	0.73	0.70
	F							0.05					0.04	0.04	0.04										
	G	0.04	0.79	0.02	0.13	0.22	0.05		0.05	0.33	0.31	0.28	0.02	0.02	0.08	0.10	0.25	0.41	0.24	0.08	0.10	0.16	0.23	0.04
	H		0.19		0.04	0.02			0.09	0.12	0.13	0.22			0.15		0.14	0.18	0.04	0.06				
Pgi	*n*	*25*	*26*	*21*	*25*	*25*	*24*	*25*	*27*	*25*	*26*	*18*	*25*	*25*	*28*	*26*	*26*	*24*	*25*	*25*	*23*	*25*	*26*	*25*
	A								0.02			0.03			0.02									
	B						0.02			0.06	0.06	0.03			0.02						0.04			0.02
	C		0.06			0.04	0.08	0.02	0.07	0.04	0.04		0.06	0.02		0.02		0.02			0.04	0.06	0.04	0.12
	D								0.04															
	E	0.68	0.94	0.81	0.80	0.88	0.79	0.80	0.76	0.80	0.89	0.94	0.82	0.78	0.73	0.87	0.94	0.92	0.82	0.82	0.72	0.74	0.90	0.66
	F	0.04													0.02								0.02	
	G	0.28		0.19	0.20	0.08	0.10	0.18	0.11	0.10	0.02		0.12	0.20	0.21	0.12	0.06	0.06	0.16	0.18	0.20	0.20	0.04	0.20
	H																		0.02					
Pgm	*n*	*25*	*27*	*21*	*25*	*16*	*24*	*25*	*27*	*26*	*26*	*18*	*26*	*23*	*28*	*26*	*26*	*24*	*26*	*22*	*25*	*25*	*26*	*25*
	A											0.03							0.12	0.07			0.08	0.02
	B			0.07	0.12			0.04	0.02	0.04	0.02	0.08	0.04	0.07			0.02	0.38	0.14	0.11			0.12	0.04
	C	0.08	0.17	0.26	0.36	0.31	0.13	0.22	0.28	0.35	0.06	0.14	0.17	0.30	0.07	0.19	0.12	0.31	0.08	0.18	0.08		0.17	0.12
	D	0.68	0.83				0.29	0.38	0.35	0.39	0.31	0.64	0.33	0.17	0.59	0.50	0.37	0.15	0.37	0.43	0.70	0.46	0.29	0.36
	E	0.24		0.55	0.42	0.47	0.54	0.34	0.30	0.23	0.62	0.08	0.42	0.35	0.32	0.31	0.46	0.17	0.31	0.21	0.22	0.54	0.25	0.46
	F						0.04	0.02	0.06				0.04	0.09	0.02		0.04						0.08	
	G			0.12	0.10	0.22						0.03											0.02	
	I													0.02										