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Genetic diversity vs geographic distribution of five congeneric caddisflies

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Abstract

We compared the genetic structure and diversity of five *Cheumatopsyche* (Trichoptera: Hydropsychidae) species from Upper Three Runs Creek in South Carolina to analyze the relationship between genetic variability and potential gene flow (i.e., geographic distribution) in a group of lotic macroinvertebrates. Among these species is an endemic to the stream (*Cheumatopsyche richardsoni*), a southeastern U.S.A. endemic (*C. edista*), and three widely distributed species (*C. pasella*, *C. pettiti*, and *C. pinaca*). Using cellulose acetate plates, we reliably scored 19 presumptive allozyme loci for each species. *C. richardsoni* and *C. pettiti* were the least genetically variable taxa, *C. pasella* and *C. pinaca* the most, and *C. edista* fell in between. Therefore, unless this *C. pettiti* population is not representative of the species in general, the genetic diversity of *C. richardsoni* and *C. edista* fall within the range exhibited by other congeners. In turn, each species' genetic diversity is evidently not correlated to its relative geographic distribution. Four species (all but *C. pettiti*) had moderate to high proportions of polymorphic loci in Hardy-Weinberg disequilibria, the cause of which is likely one of three factors: (1) each species experiences disruptive selection, (2) we inadvertently sampled adults from more than one genetically distinct stream population, or (3) each species is divided into partially reproductively isolated subpopulations within the stream.

Introduction

A species' long term persistence at least partly depends upon its inherent level of genetic variability to respond to new selection pressures presented by changing environmental conditions (Schonewald-Cox, 1983). In his review of the genetic diversity of natural populations, Nevo (1978) concluded that narrowly distributed invertebrate species are less genetically diverse than widely distributed invertebrate species. This suggests that in general, narrowly distributed species might be less likely to exist as long as widely distributed species. Although lotic macroinvertebrates should also theoretically follow this genetic diversity vs geographic distribution trend, they could have very different population genetic structures than terrestrial and marine species due to their necessarily patchy distributions. This patchiness results not only from the physical dispersion of streams and drainages across the landscape, but also from the spatial and temporal heterogeneity inherent to all streams (Palmer et al., 1997).

Upper Three Runs Creek (UTR) in Aiken County, South Carolina has an extraordinary assemblage of aquatic insects. Among the 650+ inhabitant species are three species of caddisflies (Trichoptera) which are known from nowhere else (hereafter called endemic, although see Materials and methods) (Floyd et al., 1993). One of these species, *Cheumatopsyche richardsoni* Gordon (Hydropsychidae), is one of five congeners that is relatively prevalent in the stream. The other cheumatopsychids include a southeastern U.S.A. endemic (*Cheumatopsyche edista* Gordon) and three ubiquitously distributed species (*Cheumatopsy-*

che pasella Ross, *Cheumatopsyche pettiti* (Banks), and *Cheumatopsyche pinaca* Ross). All five species occur together in UTR, and while presumably partitioning the habitat both spatially and temporally, they theoretically experience nearly identical environmental conditions. Therefore, this assemblage provides a unique opportunity to compare the genetic diversity of a local and regional endemic to that of three non-endemic congeners, with the confounding effect of environmental variability standardized.

Following Nevo's (1978) conclusions, we hypothesized that *C. richardsoni*, with no gene flow from outside populations, would be the least genetically diverse cheumatopsychid within UTR; *C. edista*, with potential gene flow from populations within a limited geographic range, would be slightly more variable; and *C. pasella*, *C. pettiti*, and *C. pinaca*, with potential gene flow from populations across a broad geographic range, would be the most variable.

Materials and methods

Site description

UTR is a fifth-order blackwater stream that flows through the Savannah River National Environmental Research Park, Savannah River Site, on the Atlantic Upper Coastal Plain. It drains about 492 km^2 and has a length of approximately 39 km. We sampled two sites on the downstream portion of UTR for this study. Site 1 was approximately 25 km downstream from the headwater and site 2 was approximately 11 km downstream of site 1.

The stream bottom consists mainly of shifting sand with numerous logs and debris dams. At site 1, the stream is confined to a single channel with very little canopy shading. At site 2, the stream becomes braided through multiple channels with extensive canopy shading in the summer. The riparian forest community at site 1 is dominated by red maple (*Acer rubrum* L.), american holly (*Ilex opaca* Aiton), sweet gum (*Liquidambar styraciflua* L.), sweet bay (*Magnolia virginiana* L.), and black gum (*Nyssa sylvatica* Marshall), while site 2 is dominated by red maple, red ash (*Fraxinus pennsylvanica* Marshall), tupelo gum (*Nyssa aquatica* L.), and bald cypress (*Taxodium distichum* (L.) Richard) (Whipple et al., 1981).

Table 1. Distributional records of the *Cheumatopsyche* species analyzed in this study.

Species	Distribution	Reference
C. edista	Alabama, Georgia, and South Carolina	Floyd et al. (1993)
C. pasella	Continental United States except for southwestern states	Gordon (1974)
C. pettiti	Continental United States and Canada	Gordon (1974)
C. pinaca	Eastern United States	Gordon (1974)
C. richardsoni	Upper Three Runs Creek, Aiken County, South Carolina	Floyd et al. (1993)

Study group

Members of the genus *Cheumatopsyche* are small, net-spinning caddisflies that are distributed on every continent except South America (Wiggins, 1996). Six *Cheumatopsyche* species have been collected from UTR: *C. edista*, *C. gyra* Ross, *C. pasella*, *C. pettiti*, *C. pinaca*, and *C. richardsoni* (Morse et al., 1980; Floyd et al., 1993), although no *C. gyra* were collected in this study. The distributional records of the five species collected are outlined in Table 1. We stress that although *C. richardsoni* is considered endemic to UTR and *C. edista* endemic to the southeastern U.S.A., both statements are true only so far as they have never been collected outside these respective areas.

Collections

We collected adult cheumatopsychids between 15 July and 4 September 1994. Individuals were attracted to an ultraviolet fluorescent light (40 watt, Sylvania® F40/BLB) placed on the stream's edge, where they were aspirated. The caddisflies were transported back to the laboratory on ice and stored at -80 °C in individual 1.5-ml microcentrifuge tubes. We removed the apex of each individual's abdomen for species identification by genitalia characters (Gordon, 1974) while the rest of the body was stored at -80 °C until electrophoretic analysis. All voucher abdomens are stored in the University of Georgia Natural History Museum.

Electrophoretic analysis

Specimens were ground with a glass rod, females in 20 μl and males in 16 μl (females are larger) of

crushing buffer (10 ml diH_2O, 1 mg NADP, 10 μl β-mercaptoethanol) in their respective microcentrifuge tubes. Samples were centrifuged at 10 000 rpm for 90 s. We performed allozyme electrophoresis on cellulose acetate plates (Titan III, Helena Laboratories, Beaumont, TX) using the techniques of Hebert & Beaton (1993). We screened a total of 35 enzymes using female *C. richardsoni*. Sixteen of these were able to be reliably scored, representing 19 presumptive loci (Table 2). All stain recipes were taken from Hebert & Beaton (1993) except ALD which was taken from Richardson et al. (1986). We analyzed between 16 and 63 individuals from each species. Alleles and loci (when more than one was present) were numbered in order of increasing electrophoretic mobility (e.g., *Idh-1* is slower than *Idh-2*). We confirmed allele designations by running presumed homologues side by side on the same gel (i.e., line-up gels, sensu Richardson et al., 1986).

Data analysis

We compared genetic diversity among the five *Cheumatopsyche* species using H_{exp} (the unbiased estimate of expected heterozygosity, i.e., the expected mean proportion of heterozygous loci per individual) (Nei, 1978), the mean number of alleles per locus, and the proportion of polymorphic loci. We calculated significance among H_{exp} values and mean number of alleles per locus using Wilcoxon's signed ranks two tailed test (SYSTAT, Inc. 1992). A locus was considered polymorphic if the frequency of the most common allele was ≤ 0.95. The proportion of polymorphic loci is a single measurement for each population and therefore cannot be compared statistically among populations. Our intention in presenting these data is to simply show a trend, either supporting or not supporting the other two diversity measures.

Allele frequencies at all loci were calculated for each species. Concordance between observed genotype frequencies and those expected under Hardy-Weinberg equilibrium were measured using the fixation index (F_{IS}) (Wright, 1978). We evaluated the null hypothesis that $F_{IS} = 0$ (i.e., the locus meets Hardy-Weinberg expectations) using the χ^2 test of Li & Horvitz (1953). All calculations, apart from testing the significance of F_{IS}, H_{exp}, and mean number of alleles per locus, were performed by BIOSYS-1 (Version 1.6, Swofford & Selander, 1981).

Results

Of the 19 presumptive loci examined, 13 were polymorphic in at least one species (*Aldh*, *Ald*, *Est-2*, *Fum*, *Gpi*, *α-Gpdh*, *Hk*, *Idh-1*, *Me*, *Mpi*, *Pep-D*, *Pgm*, *6Pgdh*), two were monomorphic in all species by the 0.95 criterion (*Ark-2*, *Idh-2*), and four were completely monomorphic in all species (*Acon*, *Ark-1*, *Mdh-1*, *Mdh-2*) (Table 3). *Est-2* did not stain for *C. richardsoni*, possibly because the locus is fixed for a null allele (i.e., an allele that shows no banding pattern when stained) or the locus has been eliminated from its genome. Because each *Cheumatopsyche* species is apparently panmictic between the two sampling sites (Plague, unpublished data), we pooled individuals from both sites and considered each taxon a single population.

The three genetic diversity measures all displayed roughly the same trends among species, with *C. pasella* and *C. pinaca* consistently the most diverse, *C. richardsoni* and *C. pettiti* the least, and *C. edista* in between (Figure 1). H_{exp} for these five species ranged from 0.036–0.151 (Figure 1). *Cheumatopsyche pasella* exhibited significantly higher H_{exp} than *C. richardsoni*, *C. edista*, and *C. pettiti* ($p < 0.05$), while *C. pinaca* was significantly higher than *C. richardsoni* and *C. pettiti*. The mean number of alleles per locus ranged from 1.37–1.95 (Figure 1). *Cheumatopsyche pasella* had significantly more alleles per locus than *C. richardsoni* and *C. pettiti* ($p < 0.05$), and *C. pinaca* had significantly more than *C. pettiti*. The proportion of polymorphic loci in each species ranged from 0.105–0.421 (Figure 1).

All polymorphic loci for *C. pettiti* were in Hardy-Weinberg equilibrium ($p > 0.05$) (Table 4). However, every other species exhibited significant genotypic frequency deviations from Hardy-Weinberg equilibrium at at least one of the following loci: *Aldh*, *Ald*, *Est-2*, *Fum*, *Me*, *Mpi*, *Pep-D*, *Pgm*, and *6Pgdh* (Table 4). Six of these loci (*Aldh*, *Ald*, *Fum*, *Me*, *Pgm*, and *6Pgdh*) were in disequilibrium for all species in which they were polymorphic. The F_{IS} values for all significantly different loci were positive, indicating that in each case the cause of divergence was a heterozygote deficiency.

Discussion

The genetic variability of the endemic species, *C. richardsoni* and *C. edista*, falls within the range

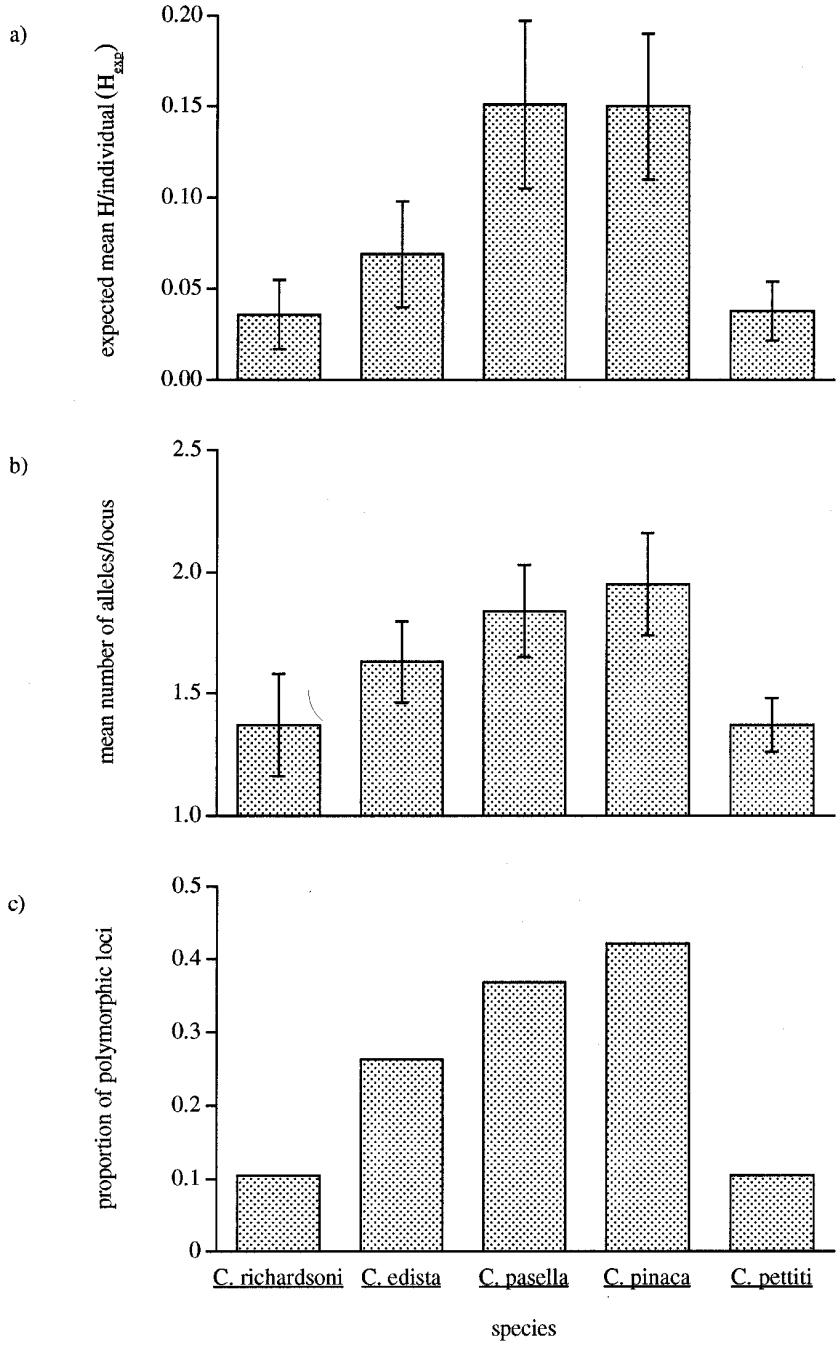

Figure 1. Genetic diversity measures for each *Cheumatopsyche* species occurring in the lower reaches of UTR: (a) expected mean proportion of heterozygous loci per individual (H_{exp}) ($\pm$ 1 S.E.), (b) mean number of alleles per locus ($\pm$ 1 S.E.), and (c) proportion of polymorphic loci (0.95 criterion).

Table 2. Enzyme systems assayed for *Cheumatopsyche* spp., with buffer and stain recipe references.

E.C. no.	Name	Symbol	# of scorable loci	Reference for buffer system[a]
4.2.1.3	Aconitase	ACON	1	A
1.2.1.5	Aldehyde dehydrogenase	ALDH	1	B[b]
4.1.2.13	Aldolase	ALD	1	A
2.7.3.3	Arginine kinase	ARK	2	J[c]
3.1.1.1	Esterase	EST	1	C
4.2.1.2	Fumarase	FUM	1	A
5.3.1.9	Glucose-phosphate isomerase	GPI	1	A
1.1.1.8	Glycerol-3-phospate dehydrogenase	α-GPDH	1	C
2.7.1.1	Hexokinase	HK	1	D
1.1.1.42	Isocitrate dehydrogenase	IDH	2	A
1.1.1.37	Malate dehydrogenase	MDH	2	B
1.1.1.40	Malic enzyme	ME	1	B
5.3.1.8	Mannose-6-phosphate isomerase	MPI	1	A
3.4.13.9	Peptidase D (phe-pro)	PEP-D	1	C
5.4.2.2	Phosphoglucomutase	PGM	1	B
1.1.1.44	6-Phosphogluconate dehydrogenase	6PGDH	1	D

[a] See Richardson et al. (1986) for recipes; continuous buffer system unless otherwise noted.
[b] Soak in D buffer.
[c] Soak in A buffer.

exhibited by their non-endemic congeners. If the measurements for the UTR *C. pettiti* population are representative of the species in general, then we must reject the original hypothesis; specifically, endemic cheumatopsychids are not necessarily less diverse than non-endemic cheumatopsychids. Therefore, *C. richardsoni* and *C. edista* should be no less able to respond to changing environmental conditions than other widely distributed relatives. Although most invertebrates exhibit a positive correlation between geographic distribution and genetic variability (Nevo, 1978), some insect species do not follow this trend (e.g., Steiner, 1979a, b; González et al., 1983). Whether 'non-correlation' is the general rule for lotic macroinvertebrates (the cause of which may be related to their patchy distributions, which could potentially foretell other fundamental differences between terrestrial and aquatic insect populations) or is unique to these cheumatopsychids is uncertain and requires similar analyses on other groups.

However, because *C. pettiti* is genetically depauperate relative to the other non-endemic taxa, the possibility exists that the UTR *C. pettiti* population is also depauperate relative to the species in general. Although we have no *a priori* reason to suspect that this is the case, we must nonetheless consider the possibility. Exceptionally low genetic diversity can be caused by population level phenomena (e.g., a founder effect or a population bottleneck) (Hartl, 1988) or sampling error (in this case, having analyzed only 16 individuals). If either of these is responsible for *C. pettiti's* low variability, then this population is not appropriate to include in our analysis because it is not indicative of the species as a whole. By not including the population, we would conclude that cheumatopsychids exhibit a positive correlation between geographic distribution and genetic variability, which supports our original hypothesis. However, further analysis of other *C. pettiti* populations is necessary before this population is discarded from the comparison.

Heterozygote deficiencies in natural populations can be caused by several factors, including the presence of null alleles, selection against heterozygotes, inbreeding, assortative mating, and the presence of multiple genetically distinct groups within each species. However, only two of these factors, selection and presence of multiple genetically distinct groups within each species, seem to potentially be the cause of the heterozygote deficiency in these cheumatopsy-

Table 3. Sample sizes and allele frequencies at each of 15 presumptive loci for each *Cheumatopsyche* species in the lower reaches of UTR. Frequencies are not shown for *Acon*, *Ark-1*, *Mdh-1*, or *Mdh-2* because they were completely monomorphic in all species.

Locus	Species				
	C. richardsoni	*C. edista*	*C. pasella*	*C. pinaca*	*C. pettiti*
Aldh					
(N)	62	37	36	26	16
1		0.932	0.264		
2		0.068	0.736	0.846	
3				0.115	
4	1.000				
5				0.038	1.000
Ald					
(N)	63	32	35	26	16
1		0.984	0.829	0.038	0.969
2	1.000	0.016	0.171	0.962	0.031
Ark-2					
(N)	63	38	37	26	16
1	1.000	1.000	0.014		0.031
2			0.986	1.000	0.969
Est-2					
(N)	63	36	37	24	16
1		0.014	0.730	0.875	0.156
2				0.021	
3		0.931	0.203	0.083	0.844
4		0.056	0.068	0.021	
Fum					
(N)	63	38	37	26	16
1	0.992	1.000		0.788	
2	0.008				
3			1.000	0.192	0.969
4				0.019	0.031
Gpi					
(N)	63	38	37	26	16
1				0.231	
2	1.000	0.987	1.000	0.769	1.000
3		0.013			
α-Gpdh					
(N)	63	38	37	26	16
1			0.014		
2	0.651	1.000	0.986	1.000	1.000
3	0.333				
4	0.016				
Hk					
(N)	61	38	33	25	16
1			0.015	0.100	
2	1.000	1.000	0.985	0.900	1.000
Idh-1					
(N)	62	38	37	26	16
1		0.961		0.096	0.031
2	1.000	0.039	1.000	0.904	0.969

Table 3. Continued.

Locus	Species				
	C. richardsoni	*C. edista*	*C. pasella*	*C. pinaca*	*C. pettiti*
Idh-2					
(N)	62	38	37	26	16
1	0.008			0.038	
2	0.992	1.000	0.986	0.962	1.000
3			0.014		
Me					
(N)	63	34	36	24	16
1		0.132		0.021	
2	1.000	0.868	0.819	0.979	1.000
3			0.181		
Mpi					
(N)	58	35	36	22	15
1				0.114	
2	0.069		0.097	0.568	1.000
3	0.931	0.014	0.847	0.318	
4		0.014	0.056		
5		0.971			
Pep-D					
(N)	62	32	36	26	15
1	1.000				
2					0.067
3			0.389		
4		0.469	0.611	1.000	0.933
5		0.531			
Pgm					
(N)	63	38	35	26	16
1				0.019	
2	0.008	0.026	0.043		
3	0.968	0.934	0.586	0.962	0.969
4	0.016	0.039	0.300	0.019	
5	0.008		0.071		0.031
6Pgdh					
(N)	55	28	34	25	15
1			0.015	0.300	
2	1.000	1.000	0.985	0.700	1.000

chids. When heterozygotes have a lower fitness than homozygotes at a locus, one of the alleles will drift to fixation unless the allele frequencies are at equilibrium (Hartl, 1988). It is improbable that heterozygotes at nine of 19 assayed loci are being selected against, all with allele frequencies at equilibrium. On the other hand, balancing selection could cause a heterozygote deficiency in adults while maintaining genetic variability within the population. For example, general

Table 4. The fixation index (F_{IS}) of each *Cheumatopsyche* population (= species) in the lower reaches of UTR at each of 13 polymorphic loci ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p, <, 0.001$).

Locus	Species				
	C. richardsoni	*C. edista*	*C. pasella*	*C. pinaca*	*C. pettiti*
Aldh	–	0.786***	0.786***	0.714***	–
Ald	–	–	0.598***	–	–
Est-2	–	−0.062	0.359**	0.264	−0.185
Fum	–	–	–	0.662***	–
Gpi	–	–	–	0.350	–
α-Gpdh	0.249	–	–	–	–
Hk	–	–	–	−0.111	–
Idh-1	–	–	–	0.336	–
Me	–	0.872***	0.718***	–	–
Mpi	0.463***	–	0.485***	0.273	–
Pep-D	–	1.000***	1.000***	–	−0.071
Pgm	–	0.368**	0.337**	–	–
6Pgdh	–	–	–	0.905***	–

heterozygosity could be detrimental for larvae but beneficial for adults. In this case, heterozygotes will be rare (because they are eliminated as larvae) but the alleles will not drift to fixation because of the advantage heterozygosity confers on adults (e.g., Singh & Green, 1984). This possibility can be tested by analyzing larval genotypes and doing comparative fitness studies of larvae and adults.

Although analyzing two species as one can give the impression of a heterozygote deficiency (e.g., Snyder & Linton, 1984; Jackson & Resh, 1992), population gene frequencies and individual genotypes do not indicate the obvious presence of more than five *Cheumatopsyche* species (i.e., no cryptic species). However, even partial reproductive isolation within a species can result in subpopulations with different allele frequencies at a locus. Pooling these subpopulations will result in an apparent heterozygote deficiency at that locus (i.e., a Wahlund effect). While nothing is known about the aerial dispersal capabilities of any species analyzed in this study, most caddisflies apparently do not fly far from their larval stream (Svensson, 1974; Jackson & Resh, 1989; Sode & Wiberg-Larsen, 1993), including *Cheumatopsyche campyla* and *Cheumatopsyche speciosa* (Kovats et al., 1996). Jackson & Resh (1992) concluded that limited inter-stream dispersal of *Helicopsyche borealis* has led to significant genetic differentiation between streams. If this is similarly true for the *Cheumatopsyche* species in this study, our U.V. light may have attracted individuals from other stream subpopulations that normally would not migrate to UTR. However, Morse & Culin (1992) found an inverse relationship between the number of caddisflies attracted to a light trap and the trap's distance from the stream from which the insects emerged. Although this suggests that inter-stream dispersal is minimal, even a few migrants can potentially cause discordant Hardy-Weinberg allele frequencies. At any rate, this possibility can be tested by emergence trapping adults from several streams, including UTR, and analyzing the allele frequencies of each stream population separately. We would like to note that immigration does not explain the heterozygote deficiencies in *C. richardsoni*, however, as none have ever been collected outside of UTR.

Wahlund effects could also theoretically result from subpopulation structure within the stream rather than between streams. Because net-spinning caddisfly larvae filter suspended organic matter from the water column, and because there are numerous flow microhabitats within a stream (Hart et al., 1996), some co-occurring net-spinners have been found to interspecifically partition food resources within a single stream reach (e.g., Williams & Hynes, 1973; Malas & Wallace, 1977; Wallace et al., 1977). For example, *Parapsyche cardis* occurs primarily in high velocity flow areas, constructs large mesh nets, and consumes large food items while *Dolophilodes distinctus* occurs in low flow areas, constructs small mesh nets, and consumes small food items (Malas & Wallace, 1977). Similarly, microhabitat partitioning could theoretically occur intraspecifically, which could lead to reproductive isolation and potentially sympatric speciation. If this is the cause of these observed heterozygote deficiencies, and if it is restricted to UTR, then this could provide support for possible *in situ* speciation events in progress within the stream. This in turn might suggest that at least part of the stream's endemic fauna originated from intra-stream speciation. However, this possibility would be difficult to test because the analyses would have to be conducted on larvae collected from different presumptive microhabitats, yet no diagnostic characters are currently known which identify any of these cheumatopsychid species' larvae.

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