NOTE

Analysis of an Apparent Genetic Cline in the Stonefly *Pteronarcys scotti* (Plecoptera: Pteronarcyidae)[1]

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Pteronarcys stonefly larvae (Plecoptera: Pteronarcyidae) inhabit lotic systems and are generally considered allochthonous shredders (Merritt and Cummins, 1996, An Introduction to the Aquatic Insects of North America, Kendall/Hunt, Dubuque, IA), primarily consuming leaf material that falls into streams. However, *Pteronarcys* spp. in the Little Tennessee River (LTR) drainage basin in southern North Carolina shift their diet through the stream continuum, with individuals in the relatively small and heavily canopied upstream reaches consuming mainly leaf material and individuals in the relatively large and open downstream reaches consuming mainly algae (Plague et al., 1998, Am. Midl. Nat. 139: 224-234). Commensurate with this diet shift, the *Pteronarcys* species composition also shifts through the continuum, with one species inhabiting the upstream reaches (fourth- through sixth-order sites), a second inhabiting the downstream reaches (sixth- and seventh-order sites), and a third inhabiting the mid-reach where the other two co-occur (sixth-order site) (Plague et al., 1998, Am. Midl. Nat. 139: 224-234). Furthermore, the *phosphoglucomutase* (*Pgm*) allele frequencies in the upstream species, *Pteronarcys scotti* Ricker, also shift through the continuum, thereby forming an apparent genetic cline: the most common allele (Pgm^3) has a frequency of 0.91 at the fourth-order collecting site, 0.80 at a fifth-order site (2.2 km downstream), 0.65 at a second fifth-order site (3.4 km farther downstream), and 0.50 at a sixth-order site (6.0 km farther downstream) (Plague et al., 1998, Am. Midl. Nat. 139: 224-234). Although these allele frequencies are quite disparate through the LTR continuum, they are not significantly different from one another ($P = 0.315$, see below for statistical analysis description), largely because the sample sizes were relatively small (N = 11, 10, 10, and 3 individuals at each sampling site, respectively).

Genetic clines are formed by one of two proximate mechanisms: natural selection

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and secondary contact between allopatrically differentiated populations (Futuyma, 1998, Evolutionary Biology, Sinauer Associates, Sunderland, MA). Natural selection forms a cline when a locus experiences differential selection pressure over some environmental gradient (e.g., Berry and Kreitman, 1993, Genetics 134: 869-893; Dahlhoff and Rank, 2000, Proc. Natl. Acad. Sci. USA 97:10056-10061). Such a population is panmictic across the gradient, so differentially selected loci have variable allele frequencies while selectively neutral loci have homogeneous allele frequencies over the gradient. Alternately, clines can form when two allopatric populations come into secondary contact with one another (e.g., Hare and Avise, 1996, Evolution 69: 2305-2315; Kirby et al., 1997, Biol. J. Linn. Soc. 62: 299-312). Under this scenario, the two populations were historically reproductively isolated, so all genetically differentiated loci will form clines in the zone of secondary contact.

Our objectives were to: (1) confirm the *Pgm* cline in *P. scotti* in the LTR with larger sample sizes than in Plague et al. (1998, Am. Midl. Nat. 139: 224-234), and (2) assess the putative proximate mechanism controlling the cline by analyzing the allele frequencies at other polymorphic loci.

We collected 30 *P. scotti* larvae from each of three sites along the LTR continuum on 26-27 May 2000, and again on 8-9 December 2000. The collecting sites, one on the fourth-order Lower Ball Creek (Site 4) and two on the fifth-order Coweeta Creek (sites 5a and 5b, upstream and downstream, respectively), comprise 5.6 km of the LTR continuum, and correspond to the three upper-most sites in Plague et al. (1998, Am. Midl. Nat. 139: 224-234; see this for site descriptions). Unlike Plague et al. (1998, Am. Midl. Nat. 139: 224-234), we were unable to locate any *P. scotti* larvae at the sixth-order LTR site.

The pro- and mesothorax of each individual was homogenized in 40 to 60 µL of crushing buffer (1 mg NADP, 10 µL 2-mercaptoethanol, 10 mL diH_20), depending on the size of the individual. Allozyme electrophoresis was performed on cellulose acetate plates as described in Hebert and Beaton (1993, Methodologies for Allozyme Analysis Using Cellulose Acetate Electrophoresis, Helena Laboratories, Beaumont, TX). In the Plague et al. (1998, Am. Midl. Nat. 139: 224-234) study, only five taxonomically diagnostic allozyme loci for North American *Pteroncarcys* spp. (Wright and White, 1992, Biochem. Syst. Ecol. 20: 515-521) were screened. Therefore, we assayed 36 enzymes for their potential suitability. Subsequently, 15 loci were screened for polymorphism using 36 individuals, 12 from each site. Of these, three loci were polymorphic: *isocitrate dehydrogenase-1* (*Idh-1;* EC 1.1.1.42), *isocitrate dehydrogenase-2* (*Idh-2*), and *malic enzyme* (*Me;* EC 1.1.1.40). *Idh-2* was electrophoresed in 0.05M Tris-maleate buffer, pH 7.8 (soaked in 0.1M Tris-citrate buffer, pH 8.2) for 75 min. *Pgm* was electrophoresed in 0.015M Tris-EDTA-borate-$MgCl_2$, pH = 7.8 for 45 min. *Idh-1* and *Me* could not be reliably resolved for all individuals and were not analyzed further. The genotypes of all *P. scotti* larvae (N = 180) were then assessed at *Idh-2* and *Pgm.*

We calculated allele frequencies for both loci at each collecting site and date. Deviations from Hardy-Weinberg equilibrium expected genotype frequencies were measured for each locus using an exact test of significance, as calculated by the BIOSYS-1 computer program (Swofford and Selander, 1981, J. Hered. 72: 281-283). We compared allele frequencies between dates and among sites using a Monte Carlo technique (1000 iterations) to generate a χ^2 distribution of expected values, using the observed allele frequencies for each locus (Roff and Bentzen, 1989, Mol. Biol. Evol. 6: 539-545).

Table 1. Allele frequencies of two polymorphic loci for *Pteronarcys scotti* in the Little Tennessee River drainage. N = 60 at each sampling site and locus

		Sample site		
Locus	Allele	4	5a	5b
Idh-2				
	1	0.008		
	2	0.392	0.358	0.325
	3	0.600	0.642	0.675
Pgm				
	1			0.008
	2	0.125	0.183	0.125
	3	0.858	0.808	0.850
	4	0.017	0.008	0.017

The allele frequencies at both polymorphic loci (*Pgm* and *Idh-2*) are not significantly different between collection dates at any site ($P > 0.05$). Therefore, we combined the *P. scotti* larvae collected in May and December to calculate allele frequencies at each sampling site. The genotype frequencies at both loci are in Hardy-Weinberg equilibrium at each site ($P > 0.25$), and the allele frequencies at both loci are similar at all sites (*Idh-2,* $P = 0.553$; *Pgm,* $P = 0.692$) (Table 1). Therefore, we did not confirm the presumed *Pgm* cline observed by Plague et al. (1998, Am. Midl. Nat. 139: 224-234).

Because the allele frequencies of two polymorphic loci are concordantly homogeneous through the LTR continuum, *P. scotti* is apparently panmictic in this drainage. Furthermore, when all stoneflies are pooled into a single sample for analysis (N = 180), both loci are consistent with Hardy-Weinberg expectations for a randomly mating population ($P > 0.30$). Although several phenomena could explain why there was a presumptive *Pgm* cline in 1995 (Plague et al., 1998, Am. Midl. Nat. 139: 224-234) but not in 2000 (e.g., temporally different selection pressures, elimination of admixture between genetically distinct populations), the most plausible explanation for the apparent 1995 cline is sampling error due to the relatively small sample sizes in the Plague et al. (1998, Am. Midl. Nat. 139: 224-234) study.

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