

## POPULATION GENETICS OF TWO BIVALVE SPECIES (*PROTOTHACA STAMINEA* AND *MACOMA BALTHICA*) IN PUGET SOUND, WASHINGTON

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**ABSTRACT** Allozyme polymorphisms from individuals of *Protothaca staminea* and *Macoma balthica* were examined electrophoretically and scored at five loci. Both species were sampled at three sites located in different hydrologically defined basins of Puget Sound, Washington. Highly significant differences in allele frequencies among the three *P. staminea* populations were found at all five loci. Significant differences in allele frequencies were detected consistently at only one locus among the *M. balthica* populations. Genetic distances between the three *P. staminea* populations, determined using both Cavalli-Sforza and Edwards (1967) chord distance and Nei's (1972) genetic distance measures, revealed the South Sound population as the genetic outlier. This pattern is consistent with the hydrology of the Puget Sound basins and the mixing that occurs at the sills between basins. Two to four of the allozyme loci demonstrated heterozygote deficiencies in *P. staminea*, depending on population. Only one locus exhibited a heterozygote deficiency in each of the three *M. balthica* populations. Potential contributing factors to the heterozygote deficiencies include a temporal Wahlund effect, selection, and null alleles. When data were corrected for the presence of a putative null allele, conclusions about population differentiation did not change.

**KEY WORDS:** population, genetics, allozymes, bivalves, *Protothaca staminea*, Puget Sound, *Macoma balthica*

### INTRODUCTION

Early genetic studies of marine populations found little evidence for genetic differentiation over large geographic distances. It was generally believed that open aquatic environments permit extensive dispersal of planktonic larvae, resulting in little genetic heterogeneity over wide spatial scales (e.g., Buroker et al. 1979, Crisp 1978, Gooch et al. 1972). This notion was soon challenged, however, by several studies presenting compelling evidence for population structure even along open coastlines (Scheltema 1975, Burton 1983). Increasingly, studies now find that any number of factors can contribute to population differentiation in apparently open systems. Populations may be defined not only by their reproductive mode (Hellberg 1996), but by hydrological forcing (Reeb & Avise 1990), chemical gradients (KoeHN et al. 1976, Ma et al. 2000), or changes in source populations (Kordos & Burton 1993). Population subdivision is evident even among the bivalves, whose long-lived planktonic larvae might otherwise be equated with high dispersal potential (e.g., Mariani et al. 2002). Other examples of genetic differentiation in marine populations over both small and large spatial scales are reviewed in Shaklee and Bentzen (1998). Collectively, these studies demonstrate that reproductive and dispersal strategies are not the only determinants of genetic differentiation among marine populations.

In this study, we examined the potential for hydrological forcing to promote differentiation of broadcast spawners with planktonic larvae in a small estuarine system. Puget Sound, Washington, is a fjord-like estuary composed of five contiguous basins with constrictions and sills that strongly influence the tidally-driven currents. The basins fall into two categories: well-mixed with rapidly circulating water masses (Admiralty Inlet, Main basin, and Southern basin), or stratified with slow-moving water masses (Hood Canal and Whidbey basin; Fig. 1). Ebbesmeyer et al. (1988) proposed that as much as 50% of the water in each basin

is recirculated back into the basin of origin because of intense mixing at the sills. This recirculation includes the upper layer of the water column (10–30 m deep) where planktonic larvae of marine invertebrates are commonly found, possibly leading to partial restriction of larvae to their basin of origin. Such a barrier to dispersal could create genetically differentiated subpopulations among basins.

Few population genetic studies of marine invertebrates have been conducted in Puget Sound despite the presence of many managed commercial and recreational fisheries. Grant and Utter (1988) examined allele frequencies from two polymorphic loci in the intertidal gastropod *Nucella [Thais] lamellosa* at several sites within Puget Sound, adjacent waters and along the open coasts of Oregon and Washington. They found evidence for population subdivision at various geographic scales, however the differences were attributed primarily to the nonplanktonic life history of this species. A more limited study in Puget Sound involving a species with a planktonic larval stage, the bivalve *Saxidomus giganteus*, found a geographic cline in populations in one of the two allozyme loci examined (Johnson & Utter 1973). Unfortunately, this study did not investigate any differences that might be attributed to separation by the hydrologically defined basins.

Our objective in this study was to test whether the bivalves *Protothaca staminea* (Conrad) and *Macoma balthica* (L.) exhibit evidence of genetic differentiation in Puget Sound consistent with its unique hydrology. Both species broadcast spawn between April and September with planktonic larvae that feed for weeks prior to settlement. Given the long planktonic larval phase of the two species and the small length scale of Puget Sound (on the order of 130 km), one might expect genetic homogeneity in the absence of any physical barriers to dispersal. Differentiation of the populations might suggest that recirculation of water masses at the sills contributes to partial isolation of populations in Puget Sound. To determine whether the hydrology of Puget Sound is the principle mechanism for any observed differentiation we chose two species that share similar reproductive and dispersal strategies yet have

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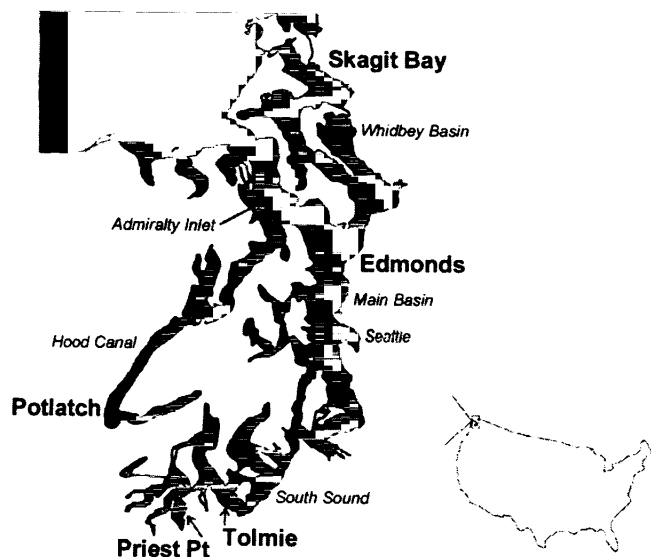


Figure 1. Sampling sites for *Protothaca staminea* (Edmonds, Potlatch, Priest Pt.) and *Macoma balthica* (Skagit, Potlatch, Tolmie) in Puget Sound, Washington.

sected from each *P. staminea*. The tissues from each clam were then combined in a single test tube. Because of the small size of the *Macoma* clams, they were stored whole (minus shell) in individual test tubes. All samples were stored at  $-80^{\circ}\text{C}$  for subsequent electrophoretic analysis.

### Electrophoresis

Following the methods of LeClair and Phelps (1994), tissue samples were homogenized in TC-1 gel buffer (Shaw & Prasad 1970) and centrifuged at 1,000 g for 5 min. Supernatants were absorbed with filter-paper wicks (Schleicher & Schuell no. 470) and used for starch gel electrophoresis. Details of the electrophoretic method are described in Aebersold et al. (1987) and Harris & Hopkinson (1976). Gels were run in a refrigerator at  $8^{\circ}\text{C}$ . Enzyme and gene nomenclature follow the guidelines of Shaklee et al. (1990). Both species were assayed for allozyme polymorphisms on four different buffer systems: CAME 6.8 (LeClair & Phelps 1994, modified from Clayton & Tretiak 1972); LiOH-RW (Ridgeway et al. 1970), TRIS-GLY (Holmes & Masters 1970); and TC-4 (buffer "a" of Schaal & Anderson 1974). The following enzyme/buffer combinations were tested: aspartate aminotransferase (AAT), isocitrate dehydrogenase (IDHP), malate dehydrogenase (MDH), malic enzyme (MEP), phosphogluconate dehydrogenase (PGDH), and phosphoglycerate kinase (PGK) on CAME 6.8; esterase-D (ESTD), formaldehyde dehydrogenase (FDHG), nucleoside-triphosphate pyrophosphatase (NTP), octopine dehydrogenase (OPDH), and strombine dehydrogenase (STDH) on LiOH-RW; alanine aminotransferase (ALAT), arginine kinase (ARGK), ESTD, glucose-6-phosphate isomerase (GPI), lactate dehydrogenase (LDH), mannose-6-phosphate dehydrogenase (MPI), cytosol nonspecific dipeptidase (PEPA), tripeptide aminopeptidase (PEPB), peptidase-S (PEPS), phosphoglucomutase (PGM), STDH, and triose-phosphate isomerase (TPI) on TRIS-GLY; adenosine deaminase (ADA), aconitate hydratase (AH), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), PEPA, and proline dipeptidase (PEPD) on TC-4.

Of the 25 enzymes assayed, activity of six (AAT, ESTD, GPI, IDHP, PGDH, PGM) were well resolved and indicated encoding by polymorphic loci (more than one allelic form detected). These enzymes were subsequently screened in all clams except AAT, which was screened only in *P. staminea*, and IDHP, which was screened only in *M. balthica*. Allelic variants are designated by their electrophoretic mobility relative to the most frequent variant encountered during the initial screening. Variants preceded by a minus sign indicate cathodal migration.

### Data Analysis

The population genetics software GENEPOP version 1.2 (Raymond & Rousset 1995a) was used to run analyses of population differentiation and heterozygote deficiency or excess relative to Hardy-Weinberg equilibrium. For testing population differentiation, both "genetic" and "genotypic" tests were run. The genic test is used to determine whether allelic distributions are identical across populations. Contingency tables for each locus were tested using the  $R \times C$  Fisher test to arrive at an unbiased estimate of the  $P$  value (Raymond & Rousset 1995b). The genotypic test is used to determine whether genotypic distributions are identical across populations. Although less powerful, the genotypic test is more appropriate when alleles within individuals are not independent, which may occur when there is nonrandom mating (Goudet et al.

disparate adult characteristics. *Protothaca staminea* occurs from the Aleutian Islands of Alaska to Baja California; is a suspension feeder preferring coarse sand to gravel substrate; attains a maximum valve length of around 7 cm; and is preyed upon primarily by starfish, moonsnails, and octopuses. *Macoma balthica*, conversely, is circumboreally distributed; may switch between surface-deposit and suspension feeding; prefers muddy substrate; may inhabit brackish waters; in Puget Sound, rarely exceeds 2 cm in length; and is preyed upon primarily by flounder, crabs and sea birds. By examining two species with similar reproductive and dispersal strategies but with different adult characteristics, we hoped to assess the influence hydrology may have on population distributions of different species in this estuary.

We examined allozyme polymorphisms at five presumptive gene loci in each of the two species of intertidal bivalve clams: *Protothaca staminea* and *Macoma balthica*. Genotype and allele frequencies from each species were then compared among three of the hydrologically defined basins of Puget Sound, Washington.

## METHODS

### Field Sampling

*Protothaca staminea* were collected at Potlatch (Hood Canal Basin;  $n = 94$ ), Priest Point (Southern Basin;  $n = 114$ ), and Edmonds (Main Basin;  $n = 114$ ) between March 1 and Sept. 7, 1998. *Macoma balthica* were collected from Potlatch ( $n = 113$ ), Tolmie Park at Big Slough (Southern Basin;  $n = 116$ ), and Skagit Bay (Whidbey Basin;  $n = 132$ ) between March 2, 1998 and June 29, 1999 (Fig. 1). All samples were obtained during low low tide along 100- to 500-m transects running parallel to the shore. Care was taken to sample individuals from the full extent of their range in the intertidal zone as well as across size classes. The length of the right valve of *P. staminea* specimens sampled ranged from 9 mm to 57 mm and for *M. balthica* from 4.5 mm to 17 mm. *M. balthica* specimens included the white, pale pink, and dark pink color morphologies. The clams were transferred live in ambient seawater to the laboratory. Immediately upon arrival, foot muscle, ctenidium, digestive gland, mantle, and adductor muscle were dis-

1996). For this test, an unbiased estimate of the  $P$  value is achieved by using the  $G$ -based test (Goudet et al. 1996) on contingency tables for each locus. Tests for both heterozygote deficiency and excess are concerned with the same  $H_0$ , random union of gametes. For both tests, the unbiased  $P$  value was estimated using the score test ( $U$  test; Rousset & Raymond 1995). Because of the presence of rare alleles, defined as having frequencies  $<0.005$  (Hartl & Clark 1997), the exact tests used by GENEPOP are more appropriate than the commonly used  $\chi^2$  test because the results will not be biased by rare alleles (Guo & Thompson 1992). Expected heterozygosities ( $H_E$ ), fixation indices ( $F_{IS}$ ) and the extent of population divergence ( $F_{ST}$ ) were also calculated for each locus in each population using GENEPOP. The  $F$ -statistics used by GENEPOP follow Weir & Cockerham (1984). GENEPOP was also used to test for genotypic linkage disequilibrium. The program BIOSYS-1 (Swofford & Selander 1981) was used to determine Cavalli-Sforza and Edwards (1967) chord distances and Nei's (1972) genetic distances. Finally, when individuals without a banding pattern are observed, yet are not conclusively null homozygotes, the frequency of a putative null allele can be estimated using  $(H_E - H_O)/(H_E + H_O)$ , where  $H_E$  and  $H_O$  refer to the expected and observed heterozygosities, respectively (Brookfield 1996). Using this algorithm allele frequencies for the populations of both species were corrected for the presence of a null allele.

## RESULTS

Stains for GPI and PGM were most successful on the Tris-Gly buffer system; PGDH, AAT, and IDHP on CAME 6.8; ESTD on LiOH-RW. In each species, two private alleles (alleles only detected in one population) were found: *GPI\*17* (*P. staminea*, Potlatch), *AAT\*1500* (*P. staminea*, Edmonds), *IDHP\*150* (*M. balthica*, Skagit), *PGDH\*114* (*M. balthica*, Skagit). Four rare alleles occurred in *P. staminea* populations and eight in *M. balthica* populations (Table 1).

Expected heterozygosities ( $H_E$ ) and fixation indices ( $F_{IS}$ ) varied widely in both species depending on the locus (Table 1). Notably,  $F_{IS}$  values for the *P. staminea* population at Edmonds were consistently higher than values for the population at Potlatch or, with most loci, at Priest Point suggesting strong heterozygote deficiencies in this population. The tests for Hardy-Weinberg equilibrium revealed significant heterozygote deficiencies ( $P < 0.05$ ) in up to four of the five loci in the *P. staminea* populations (Table 2). Only at the *ESTD\** locus was a significant heterozygote deficiency detected in the *M. balthica* populations ( $P < 0.001$ ; Table 2). In neither species was a heterozygote excess detected.

For both species, locus pairs were also tested for genotypic linkage disequilibrium within each population. A significant linkage disequilibrium suggests the genotypes at different loci are not independent. Linked loci may be an indication of inbreeding. After applying a sequential Bonferroni correction (Ury, 1976), only one population (*P. staminea*, Edmonds) had loci with significant linkage disequilibrium. The two locus pairs demonstrating a significant disequilibrium were: *GPI\** and *AAT\** ( $P < 0.001$ ) and *AAT\** and *ESTD-2\** ( $P < 0.005$ ).

With both the genic and genotypic tests, we found strong evidence for population differentiation among all three *P. staminea* populations at all loci ( $P < 0.001$ ; Table 3). Both chord and Nei's distances indicated that the populations from Edmonds and Potlatch are more closely related than either is to the Priest Point

population (Table 4). When distances were determined locus by locus, four of five loci were in agreement with this pattern.  $F_{ST}$  values for the *P. staminea* populations ranged from 0.07 (*PGM\**) to 0.13 (*ESTD\**).

In the *M. balthica* populations, both the genic and genotypic tests demonstrated differentiation at one of the five loci (*PGDH\** Table 3). The genic test revealed an additional differentiation at the *ESTD\** locus (Table 3).  $F_{ST}$  values for *M. balthica* ranged from  $-0.002$  (*PGM\**) to 0.009 (*ESTD\**). Because of the lack of differentiation among *M. balthica* populations at most loci, distance measures were not significant (data not shown).

To determine whether heterozygote deficiencies had any effect on the population differentiation tests, the allele frequencies were recalculated to account for the potential presence of a null allele. An indication of null alleles is a null homozygote demonstrating no banding pattern. In the *P. staminea* samples, absence of enzymatic activity occurred with only one individual from Priest Pt. when stained for GPI and two individuals from Edmonds when stained for ESTD and PGM. In the *M. balthica* samples, absence of enzymatic activity occurred in three individuals from Skagit Bay (all using the stain for IDHP, one additionally did not stain for ESTD) and four individuals from Potlatch (all using the stain for ESTD, one additionally did not stain for PGDH). Because this absence of activity could also have been caused by tissue degradation, staining inconsistencies, or tissue samples that are too small (for *M. balthica*), we could not conclusively assign these individuals as null homozygotes. It is possible to estimate the frequency of a putative null allele based on the heterozygote deficiency in a population. Following Brookfield (1996), allele frequencies were corrected in each population to account for the presence of a null allele and the genic and genotypic tests re-run. The level of population differentiation observed did not decline for either species. On the contrary, both the chord and Nei's genetic distances increased slightly with the addition of the null allele (between 1 and 30% increase, data not shown).

## DISCUSSION

### *Evidence for Distinct Populations of P. staminea But Not M. balthica*

Both *Protothaca staminea* and *Macoma balthica* are free-spawning bivalves, with feeding larvae that spend about 3–4 wk in the plankton. These larvae are the dispersal propagules, largely at the mercy of local horizontal currents. Given the similar reproductive and dispersal strategies of *P. staminea* and *M. balthica*, one might expect consistency in the level of population differentiation of these species when exposed to the same estuarine currents. The population structure of these two species, however, is very different in the complex estuarine system of Puget Sound, Washington. Populations of *P. staminea* were found to be highly differentiated at all loci surveyed, whereas the *M. balthica* populations were significantly different at only one locus using both the genic and genotypic tests. While it is possible that allozymes are not variable enough to detect differences between the populations of *M. balthica*, it is likely that species-specific selective pressures also play a role in structuring these populations.

*Protothaca staminea* and *Macoma balthica* occupy very different ecological niches. It is possible that these two species experience different selective pressures in Puget Sound from the physical environment or from local predators, including humans (van der Veer et al. 1998, Ejdung & Elmgren 1998, Chew & Ma 1987). *P.*

TABLE 1.

Allele frequencies at loci for *Protothaca staminea* and *Macoma balthica* individuals from three locations in Puget Sound, WA

Locus, allele	<i>Protothaca staminea</i>			Locus, allele	<i>Macoma balthica</i>		
	Potlatch	Edmonds	Priest Pt.		Potlatch	Skagit	Tolmie
<i>GPI</i>							
-17	0.011	0.000	0.000	-22	0.004*	0.004*	0.000
14	0.074	0.039	0.138	8	0.009	0.019	0.022
36	0.420	0.237	0.170	38	0.434	0.481	0.457
58	0.351	0.202	0.589	66	0.128	0.092	0.116
77	0.112	0.167	0.085	100	0.376	0.385	0.353
100	0.032	0.285	0.013	130	0.049	0.019	0.052
127	0.000	0.070	0.004*	(N)	(113)	(130)	(116)
(N)	(94)	(114)	(112)	$H_E$	0.654	0.614	0.652
$H_E$	0.685	0.791	0.600	$F_{IS}$	-0.014	-0.027	-0.097
$F_{IS}$	0.177	0.358	-0.012				
<i>PGM</i>							
66	0.101	0.108	0.090	38	0.018	0.008	0.030
85	0.261	0.171	0.232	62	0.159	0.129	0.156
100	0.314	0.230	0.602	86	0.053	0.057	0.065
112	0.245	0.387	0.076	100	0.611	0.621	0.593
132	0.080	0.104	0.000	124	0.124	0.159	0.113
(N)	(94)	(111)	(105)	154	0.035	0.027	0.043
$H_E$	0.761	0.750	0.574	(N)	(113)	(132)	(115)
$F_{IS}$	0.148	0.267	0.238	$H_E$	0.585	0.570	0.609
				$F_{IS}$	0.107	0.097	0.000
<i>AAT</i>							
-1500	0.000	0.004*	0.000	70	0.004*	0.000	0.004*
-700	0.293	0.180	0.009	82	0.062	0.036	0.039
-100	0.670	0.798	0.947	94	0.013	0.008	0.000
500	0.021	0.004*	0.044	100	0.903	0.933	0.953
900	0.016	0.013	0.000	124	0.018	0.020	0.004*
(N)	(94)	(114)	(114)	150	0.000	0.004*	0.000
$H_E$	0.467	0.332	0.101	(N)	(113)	(126)	(116)
$F_{IS}$	0.112	0.207	-0.043	$H_E$	0.182	0.129	0.091
				$F_{IS}$	0.124	0.017	0.152
<i>PGDH</i>							
-1100	0.048	0.024	0.253	62	0.004*	0.004*	0.000
-600	0.425	0.524	0.552	74	0.022	0.068	0.030
-100	0.495	0.423	0.155	82	0.157	0.209	0.129
500	0.011	0.014	0.041	90	0.511	0.392	0.500
1000	0.022	0.014	0.000	100	0.305	0.316	0.341
(N)	(93)	(104)	(97)	114	0.000	0.011	0.000
$H_E$	0.575	0.548	0.599	(N)	(111)	(131)	(116)
$F_{IS}$	0.178	0.299	0.333	$H_E$	0.625	0.700	0.619
				$F_{IS}$	0.063	0.052	0.025
<i>ESTD-2</i>							
75	0.000	0.045	0.004*	90	0.081	0.116	0.078
84	0.202	0.134	0.425	100	0.720	0.633	0.759
92	0.069	0.290	0.294	106	0.199	0.251	0.164
100	0.723	0.513	0.276	(N)	(105)	(129)	(116)
111	0.005	0.018	0.000	$H_E$	0.443	0.526	0.393
(N)	(94)	(112)	(114)	$F_{IS}$	0.448	0.485	0.519
$H_E$	0.433	0.635	0.659				
$F_{IS}$	0.166	0.270	0.002				

N = the number of clams scored in each collection. Frequencies in bold indicate private alleles. Asterisks (\*) indicate rare alleles (frequencies <0.005).  $H_E$  = expected heterozygosities;  $F_{IS}$  = fixation index for individuals within each population.

*staminea* has a larger adult size and often occupies much more sandy substrates than *M. balthica*. Sanchez-Salazar et al. (1987a, 1987b) demonstrated the influence both tidal elevation and shore crabs can have on the population structure of the bivalve, *Ceras-*

*toderma edule*. The recreational harvest of *P. staminea* in Puget Sound may also contribute to selective pressures in this species. In addition, harvesting of *P. staminea* may reduce its effective population size ( $N_e$ ), contributing to differentiation of populations

TABLE 2.

Probability values for the test of heterozygote deficiency relative to Hardy-Weinberg expectations at each locus for each population

Locus	<i>Protothaca staminea</i>			Locus	<i>Macoma balthica</i>		
	Potlach	Edmonds	Priest Pt.		Potlach	Skagit	Tolmie
<i>GPI</i>	0.096	<0.001*	0.347	<i>GPI</i>	0.333	0.835	0.942
<i>PGM</i>	0.084	<0.001*	0.035*	<i>PGM</i>	0.100	0.403	0.173
<i>PGDH</i>	<0.001*	0.057	<0.001*	<i>PGDH</i>	0.123	0.097	0.474
<i>AAT</i>	0.019*	0.030*	1.000	<i>IDHP</i>	0.087	0.447	0.149
<i>ESTD-2</i>	0.008*	<0.001*	0.383	<i>ESTD</i>	<0.001*	<0.001*	<0.001*

Asterisks (\*) indicate significant heterozygote deficiencies ( $P < 0.05$ ).

through genetic drift. *M. balthica* is too small to attract recreational or commercial interest and may therefore also have a much larger  $N_e$ . Additionally, neither selective pressures nor genetic drift may be strong enough to drive population differentiation of *M. balthica* if there are sufficient migrants to homogenize the populations (Hartl & Clark 1997).

Exchange of individuals between populations may be facilitated by larval behavior. The planktonic larvae of many estuarine invertebrates do not behave as strictly passive particles, instead exhibiting selective transport in horizontal currents mediated by vertical migration (Morgan 1995). Although the most extensive research has focused on crustaceans (e.g., Sandifer 1975, Cronin 1982, Forward et al. 1995), a few studies have confirmed selective larval transport among bivalves (Wood & Hargis 1971, Manuel et al. 1997). It is possible that *P. staminea* and *M. balthica* larvae exhibit divergent swimming behaviors that could affect their transport out of their respective estuarine basins of origin in Puget Sound. Unfortunately, there have not been any studies investigating vertical migration behavior of *P. staminea* larvae. Work by Roegner (2000) suggests that the larvae of *M. balthica* are passively distributed. However, there is evidence for selective post-metamorphic drifting of *M. balthica* juveniles (Beukema & de Vlas 1989). Byssal threads attached to these post-larvae provide drag and lift allowing transport on horizontal flow. A recent study of *Macoma* spp. post-larval distributions in the York River estuary of the Chesapeake Bay strongly suggests that this life-history stage exerts a behavioral control over position in the water column (Garrison & Morgan 1999). Because byssal thread-drifting has not been demonstrated in *P. staminea*, one possibility is that *M. balthica* populations in Puget Sound are less differentiated due to selective thread-drifting of the post-metamorphic juveniles.

TABLE 3.

Probability values for the genic and genotypic tests for population differentiation of three *Protothaca staminea* and three *Macoma balthica* populations

Locus	<i>Protothaca staminea</i>		Locus	<i>Macoma balthica</i>	
	Genic test	Genotypic test		Genic test	Genotypic test
<i>GPI</i>	<0.001*	<0.001*	<i>GPI</i>	0.467	0.524
<i>PGM</i>	<0.001*	<0.001*	<i>PGM</i>	0.589	0.681
<i>AAT</i>	<0.001*	<0.001*	<i>IDHP</i>	0.273	0.320
<i>PGDH</i>	<0.001*	<0.001*	<i>PGDH</i>	0.011*	0.007*
<i>ESTD-2</i>	<0.001*	<0.001*	<i>ESTD</i>	0.043*	0.140

Asterisks (\*) indicate significant differences ( $P < 0.05$ ).

### *P. staminea* Populations May Be Constrained by Puget Sound Hydrology

Because we found substantial differentiation among *Protothaca staminea* populations, we hypothesize that gene flow between these populations may indeed be restricted. The chord distances as well as Nei's genetic distances suggest that the populations of *P. staminea* in Hood Canal and the Main Basin are more similar to each other than either is to the South Sound population (Table 4). Hydrology of the Puget Sound estuary supports the hypothesis of South Sound isolation. Cokelet et al. (1991) determined that as much as 52% of the water entering Admiralty Inlet from Puget Sound is recycled back through mixing at the sill (Fig. 1). This refluxing coupled with their proximity suggests a large potential for exchange between Hood Canal and the Main Basin. Cokelet et al. (1991) also estimated that the longest residence times in Puget Sound are for waters originating in the southernmost reaches of the Sound. Populations from the South Sound and Main Basin might therefore be restricted in their ability to exchange individuals. In fact, there are two minor sills and one major sill (at Tacoma Narrows) between the Priest Point population in South Sound and the Edmonds population in the Main Basin. Recently, the slow flushing times of South Sound have been implicated in the die-off of a number of benthic species, perhaps due to pollutant retention (Ebbesmeyer et al., 1998). It remains to be seen whether the refluxing of South Sound waters is directly preventing dispersal of *P. staminea* larvae. There is, however, a correlation between the observed genetic pattern and the expected circulation pattern of Puget Sound.

### Deviations from Hardy-Weinberg Equilibrium

Heterozygote deficiencies are commonly found with a variety of molecular methods, especially in marine bivalve populations (Raymond et al. 1997, Gaffney 1994, Zouros & Foltz 1984, Singh

TABLE 4.

Genetic distance measures for the three *Protothaca staminea* populations (Edm = Edmonds; Pot = Potlatch; PPt = Priest Pt.)

	<i>Protothaca staminea</i> , all loci		
	Edm-Pot	Edm-PPt	Pot-PPt
CSE	0.1997	0.3141	0.2858
NEI	0.0723	0.1947	0.2085

CSE = Cavalli-Sforza and Edwards (1967) chord distance; NEI = Nei's distance from Nei (1972).

& Green 1984). Often, heterozygote deficiencies are indicative of reproductive isolation resulting in inbreeding. Additional causes ascribed to heterozygote deficiencies are wide ranging but may include aneuploidy, molecular imprinting, genotype-dependent spawning, selection, population mixing, null alleles, scoring bias, and tissue degradation. Aneuploidy, molecular imprinting, and genotype-dependent spawning have not been reported for either of these species and there is little evidence to support these phenomena in bivalves. Heterozygote deficiencies resulting from spatial population mixing do not seem likely either, given the large sampling area (100- to 500-m transects), high abundances, and long pelagic phases of these two species in Puget Sound.

However, it is possible that we encountered temporal population mixing since we likely sampled over several generations by sampling over a wide range of sizes. It has been hypothesized that the chance reproductive success of free-spawners may lead to large variances in the genetic composition of each successive generation due to random drift (Hedgcock 1994). The result is a small number of individuals contributing disproportionately to the next generation. Sampling across these generations may lead to temporal population mixing, also known as a temporal Wahlund effect. To maintain differences between year-classes, selection and/or assortative mating may also be occurring (Hartl & Clark 1997). Similar to Ruzzante et al. (1996), we investigated the effect of pooled age-classes on Hardy-Weinberg equilibrium by dividing the *P. staminea* individuals into large- and small-size classes and re-testing for heterozygote deficiencies. For all populations, the number of loci with heterozygote deficiencies decreased in both size classes except one (small class, Potlatch) compared with populations that had both size classes pooled (data not shown). This suggests that pooling the size classes may have contributed to the observed heterozygote deficiencies.

Selection may also act to reduce the number of heterozygotes in a population. An ongoing debate in bivalve genetics is the apparent paradox between observations of hybrid vigor and heterozygote deficiencies. Individuals in both the laboratory and natural field populations demonstrate strong correlations between heterozygosity and fitness-related traits, e.g., size, growth rate, and reproductive capacity (Hedgcock et al. 1996, Zouros 1987). Yet field populations of many bivalve species are heterozygote deficient. One possible explanation is genotype-dependent larval mortality (Singh & Green 1984, Zouros & Foltz, 1984). Investigating the timing of the heterozygote deficit, Fairbrother and Beaumont (1993) found heterozygote deficiencies in a cohort of newly settled mussel (*Mytilus edulis*) spat, concluding that the loss of heterozygotes must have occurred during the larval stage or early settlement. Singh (1982) suggested that selection might act against the more heterozygous, faster-growing larvae because of their increased food requirements during the critical period of larval development. If plankton abundances are not high during this period,

these larvae face a greater mortality. This phenomenon has yet to be investigated in either *P. staminea* or *M. balthica*.

Finally, the presence of null alleles may also contribute to the observed deficiencies. It is possible that either true null alleles or artifacts, such as insufficient tissue or staining inconsistencies, caused the deficiency in the one locus (*ESTD*) across all *Macoma balthica* populations. However, heterozygote deficiencies occurred in most loci and in all populations of *Protothaca staminea*, suggesting null alleles are not sufficient to explain the observed deficiencies in this species. For these populations, selection and inbreeding due to partial reproductive isolation could explain the deficiencies we observed. In addition, it is possible that we encountered a temporal Wahlund effect in the *P. staminea* populations. Importantly, when all other allele frequencies were corrected for the presence of a null allele and the analytical tests re-run, the population differentiation conclusions did not change.

## CONCLUSIONS

Many factors may contribute to population differentiation of marine invertebrates in Puget Sound. To prevent genetic homogeneity over such a small geographic scale, however, selective forces must be strong, gene flow must be restricted, and/or temporal variance of the populations must be extreme. Environmental fluctuations can be dramatic in the estuarine ecosystem. Extremes of salinity and temperature can be found over small spatial scales. In such a heterogeneous environment, selection may take the form of both physical and biological constraints. They may act in concert to vary pressures on adult clams or the recruiting larvae. Populations may vary from generation to generation simply due to pulsed recruitment or sweepstakes sampling from the previous generation. Factors that might limit gene flow between populations in this estuary include large-scale reflux via mixing at sills, larval behavior, or small-scale circulation patterns such as nearshore eddies. We have demonstrated a correlation between the population differentiation of *P. staminea* and the circulation pattern of Puget Sound warranting further study of the effects of Puget Sound hydrology on larval dispersal. The hydrology of Puget Sound, however, does not ensure differentiation in every species. In stark contrast to *P. staminea*, we have shown that *M. balthica* populations reveal little differentiation among the same basins. The amount of differentiation between sites is highly species dependent, and therefore population dynamics should not be generalized based on reproductive characters alone.

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