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MICROSATELLITE AND ALLOZYME ANALYSES REVEAL FEW GENETIC DIFFERENCES AMONG SPATIALLY DISTINCT AGGREGATIONS OF GEODUCK CLAMS (*PANOPEA ABRUPTA*, CONRAD 1849)

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ABSTRACT The genetic population structure of geoduck clams (*Panopea abrupta*) in inland waters of Washington may affect fishery management and aquacultural practices involving this species. To investigate genetic differentiation in geoduck clams, samples were collected from 16 Washington State sites located in the five Puget Sound sub basins, southern Georgia Strait, and the Strait of Juan de Fuca. A collection from Clarence Strait in SE Alaska was included as an outgroup. Individuals were genotyped at 11 allozyme and 7 microsatellite loci. There was little differentiation overall, but the Freshwater Bay collection in the Strait of Juan de Fuca was differentiated from others at both microsatellite and allozyme loci. For both marker classes, there was no evidence of significant correlation between genetic and geographic distance measures. In contrast to the microsatellite loci, the allozyme loci were in Hardy-Weinberg Equilibrium (HWE). Deviations from HWE expectations at microsatellite loci were interpreted as being primarily due to primer-site sequence variation rather than population level processes such as inbreeding.

KEY WORDS: *Panopea abrupta,* geoduck, population genetics, larval dispersal

INTRODUCTION

Understanding the extent of gene flow provides insight into the demographic dynamics among natural populations. Generally, gene flow is correlated with dispersal ability in many organisms (Bohonak 1999), including many marine fishes and shellfishes (reviewed in Shaklee & Bentzen 1998). In sedentary marine bivalves, dispersal and gene flow occur only during the pelagic larval phase. Gene flow and larval dispersal may be correlated with spatial distribution in marine mollusks (Johnson et al. 2001); many investigators have failed to falsify the null hypothesis of panmixia at broad geographic scales in a variety of broadcast spawning marine species with pelagic larvae (e.g., *Crassostrea virginica* [McDonald et al. 1996]; *Littorina striata*, [De Wolf et al. 2000]; *Mytilus galloprovincialis* [Skalamera et al. 1999]). Nevertheless, some studies have demonstrated genetic structuring in a variety of marine invertebrate species with pelagic larvae (e.g., the American oyster, *Crassostrea virginica* [Reeb & Avise 1990]; *Ostrea edulis* [Launey et al. 2002]; the sea urchins *Strongylocentrotus purpuratus* [Edmands et al. 1996] and *S. franciscanus* [Moberg & Burton 2000]; and black abalone *Haliotis cracherodii* [Hamm & Burton 2000]). Examples of genetic differentiation on smaller geographic scales include the limpet *Siphonaria jeanae* (Johnson & Black 1984), the oyster *Crassostrea virginica* (Buroker 1983, [King et al. 1994]), cockles *Cerastoderma glaucum* (Mariani et al. 2002), and *Mytilus edulis* (Ridgway 2001). Most population genetic studies of marine invertebrates have focused on populations that are distributed along open coasts or island populations with discontinuous distributions separated by deep oceanic water. However, Parsons (1996) demonstrated significant genetic subdivision (9-locus F_{ST} = 0.16) over an area of only 75 km^2 in the intertidal gastropod, *Austrocochlea constricta*. Limited water movement in the area studied may have caused localized recruitment, resulting in the detected population differentiation (Parsons 1996).

The complex hydrology and bathymetry of Puget Sound in Washington State suggests the potential for restricted dispersal and population subdivision of marine invertebrates in the region. Puget Sound is a fjord-like estuarine system that is highly subdivided by numerous peninsulas, narrow passes, islands, and underwater sills (Fig. 1). It is comprised of five sub-basins oriented approximately along a north-south axis. At the north end is Admiralty Inlet, separated from the Strait of Juan de Fuca by a shallow sill. Admiralty Inlet connects to three long, narrow basins: from west to east these are Hood Canal, Main Basin, and Whidbey Basin. Southern Basin is connected at the south end of the Main Basin. Potentially important for larval dispersal, each basin is separated from its neighbor by a shallow sill, except for Whidbey Basin's constricted connection to the Main Basin (Ebbesmeyer et al. 1988). The Strait of Juan de Fuca, the sole connection of Puget Sound to the Pacific Ocean, is itself bounded by an inner and an outer sill, as well as the bathymetrically and hydrologically complex Georgia Strait to the north (Herlinveaux & Tully 1961). Collectively, these features restrict the flow of surface waters, leading to the possibility that the dispersal of pelagic larvae may be restricted, and hence that genetic differentiation of marine invertebrates might occur on relatively small geographic scales. On the other hand, Puget Sound's freshwater inputs and their typical estuarine surface outflow increase the propensity of passive surface particles to disperse in a seaward direction, and could be sufficient to genetically homogenize molluscan populations colonized by pelagic larvae drifting seaward from populations in inner inlets. In a study of marine bivalve population genetics in Puget Sound, Parker et al. (2003) recently found significant differences in allele frequencies among collections of the endemic *Protothecа staminea*, but no differences among collections of *Macoma balthica*.

One of the most fecund species with pelagic larvae in Puget Sound, Washington, the bivalve *Panopea abrupta*, or geoduck clam, is found there in high densities and is among the largest, longest-lived (> 100 y), and deepest burrowing clams known. It occurs from the low intertidal zone to a depth of 100 m, buried up

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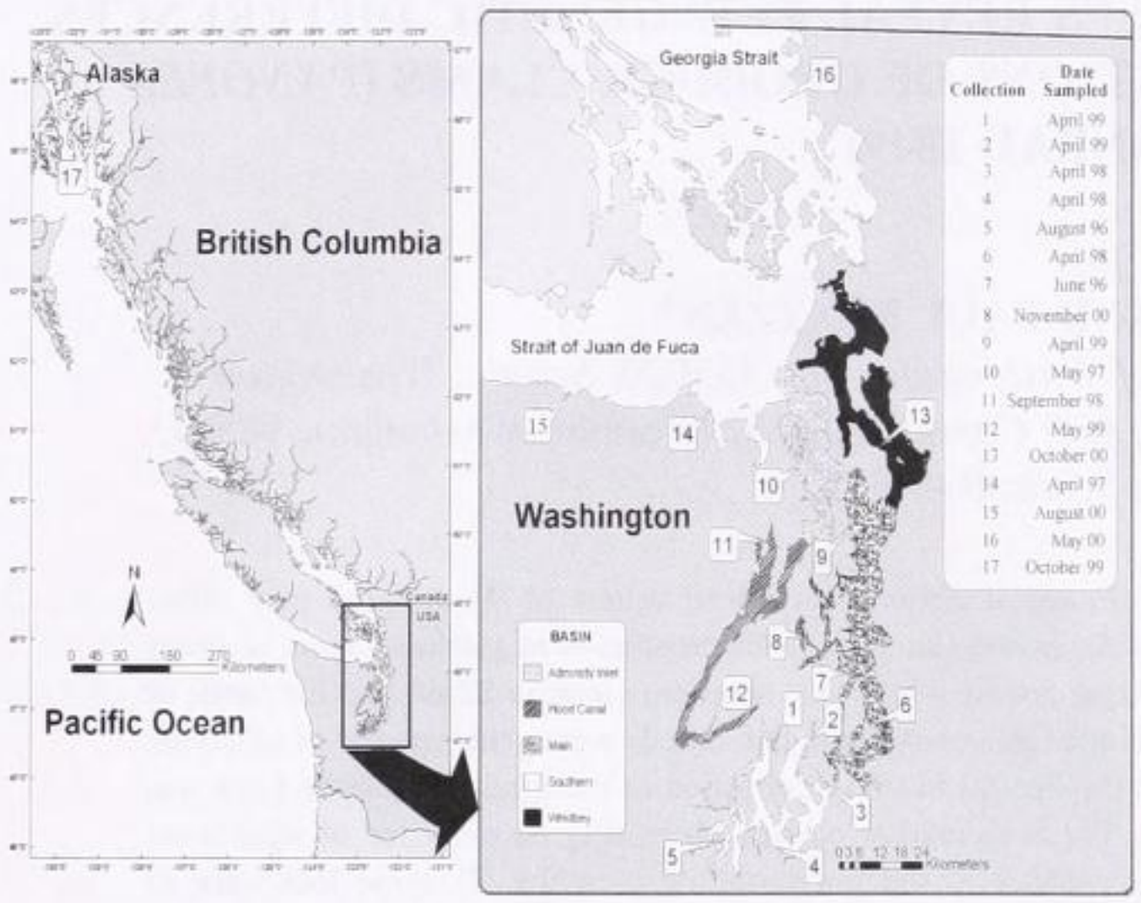

Figure 1. Puget Sound hydrographic basins, and sample dates and geographic locations for 17 geoduck clam (*Panopea abrupta*) collections from Washington and Southeast Alaska.

to 1 m in loose stable substrates in marine and estuarine environments from Alaska to Mexico (Goodwin & Pease 1989). Geoducks are dioecious synchronized broadcast spawners, with a pelagic larval phase that lasts from 3–6 weeks (Goodwin 1976). Experimental evidence indicates mean fecundities of 40 million eggs per year (Beattie 1992). A species may use the strategy of high fecundity to overcome the spatial and temporal odds of gamete union (Hedgecock 1994) and/or larval survival, or, secondarily, to maximize dispersal (Johnson et al. 2001).

Geoducks are the target of a lucrative international commercial fishery, and recently have become a commercially cultivated species. Assessing potential impacts of hatchery outplants on their wild counterparts will depend, in part, on the characterization of naturally occurring genetic stock structure. To date there has been no available information on the genetic stock structure of *P. abrupta*, or any of its congeners, and with the high fecundity and longevity of *P. abrupta* potentially maximizing dispersal and genetic homogeneity, large-scale panmixia has been the assumption. Here, we evaluate the null hypothesis of genetic homogeneity in the Strait of Juan de Fuca—Georgia Strait—Puget Sound complex. We examine population differentiation among collections from sites in the complex using 11 allozyme and seven microsatellite loci. As alternative hypotheses, we also consider genetic differentiation due to isolation by distance, as well as stochastic genetic differentiation relative to geographic distance.

A secondary objective is to compare the outcome of parallel analyses using two classes of genetic marker, allozymes and microsatellites. Both are codominant, however allelic variability is usually much higher in microsatellites, providing potentially higher power to detect differences (Goudet et al. 1996, Grant et al. 2000; but see Allendorf & Seeb 2000 and DeWoody & Avise 2000). Because discordant results have been achieved with the two marker classes in a number of studies of genetic variation (e.g., Lemaire et al. 2000, de Innocentiis et al. 2001 but see Allendorf & Seeb 2000), we investigate the relative strengths of these two marker classes for detection of genetic differentiation in a broadcast spawning marine bivalve.

METHODS

Sample Collection

Using SCUBA, divers collected geoducks ($n = 1645$) from a total of 17 sites distributed among the five sub-basins of Puget Sound, the Strait of Juan de Fuca, Georgia Strait, and near Ketchikan, Alaska (Fig. 1). Where possible, sites were chosen based on maximum watercourse distances, least amount of surface water outflow, maximum potential wind-mediated retention, and geographic coverage. Tissue samples were dissected from live animals within 24 hours of harvest. Animals that were not dissected immediately after harvest were held live in flow-through troughs

TABLE 1.

Pairwise watercourse distances (upper triangle) and Fst values (allozyme/microsatellite) for 17 collections of *Panopea abrupta* from Puget Sound, Washington and S.E. Alaska. Bold type indicates significant pairwise tests of genotypic (7 microsatellite loci) and genic (11 allozyme loci) differentiation; underlining indicates significant pairwise tests of genotypic differentiation for all 18 loci combined. Indicated significance is at the table-wide Bonferroni *P*-value of 0.00028 (0.05/178).

	1	2	3	4	5	6	7	8
1		50	19	29	7	93	99	130
2	/0.0017		44	34	35	56	70	92
3	–0.0012/	/		27	35	37	54	74
4	–0.0026/	/	–0.0005/		19	52	68	88
5	0.0049/	/	0.0020/	0.0034/		83	92	120
6	0.0004/0.0007	/0.0016	0.0008/	0.0019/	0.0024/		17	37
7	0.0012/	/	0.0023/	–0.0007/	0.0001/	0.0013/		20
8	0.0071/–0.0007	**/0.0007**	0.0049/	0.0078/	0.0021/	0.0009/0.0000	0.0050/	
9	0.0000/	/	0.0018/	0.0014/	0.0054/	0.0039/	–0.0032/	0.0128/
10	0.0039/	/	0.0037/	0.0053/	–0.0011/	0.0002/	0.0012/	–0.0008/
11	0.0024/–0.0003	/0.0007	0.0007/	0.0027/	0.0003/	0.0021/0.0011	0.0035/	–0.0001/–0.0004
12	0.0007/0.0010	**/0.0016**	0.0006/	0.0027/	0.0014/	0.0007/0.0014	–0.0008/	0.0028/0.0000
13	0.0053/0.0003	**/0.0028**	0.0040/	0.0049/	0.0008/	–0.0013/–0.0004	0.0013/	–0.0015/–0.0004
14	0.0014/	/	0.0035/	0.0016/	0.0042/	0.0026/	0.0001/	0.0027/
15	0.0081/0.0022	**/0.0033**	**0.0097**/	**0.0068**/	0.0024/	0.0020/**0.0031**	0.0025/	0.0068/**0.0028**
16	0.0003/0.0019	/0.0005	0.0019/	0.0039/	0.0058/	–0.0012/0.0007	0.0072/	–0.0002/–0.0005
17	/0.0016	**/0.0027**	/	/	/	/0.0003	/	/0.0004

supplied with natural seawater. From each specimen, samples were taken for allozyme, microsatellite, or both analyses: approximately 1 cm^3 samples of siphon and ctenidia tissues were collected and cryogenically preserved at −80 °C, and foot tissues were divided into 0.5 cm pieces, and stored in 95% ethanol at room temperature (DNA) until laboratory analyses. Sampling the SE Alaska specimens for allozyme analyses was not possible because tissues for microsatellite analyses were obtained opportunistically via a commercial harvest.

Microsatellite Data Collection

Microsatellite genotypes were collected from specimens from sites 1, 2, 6, 8, 11, 12, 13, 15, 16, and 17 (Fig. 1). DNA was extracted from 10 mg of foot tissue using DNEasy 96 Tissue Kits (Qiagen) following the manufacturer's protocol, and used to amplify seven loci. Four of these loci, Pab3, Pab4, Pab5, and Pab6 (Genbank accession numbers AF213657–AF213660) were described by Vadopalas and Bentzen (2000). For Pab5, the primer TAATTTCAGGTGGCCGATTT was used as an alternate forward primer. Pab7 (CA repeat, 137–199 bp, primers TTTTCAACTGGATTGCGTGA and GAACCAATCAATAGAAGCTCCA), Pab8 (compound tetranucleotide repeat, 200–634 bp, primers TCAATGAGATAAAAATGTCGCTAAC and AACAATACCTGCACCCAATCT), and Pab9 (GATA repeat, 202–300 bp, primers CGTAAATGTTTATGCCTGCAA and GATCACAACTCTCTTTTTCTTC) reported here for the first time, were isolated as described in Vadopalas and Bentzen (2000) (Genbank accession numbers AF541256–AF541258). One of each primer pair was labeled on the 5′ end with NED (Pab4, Pab8), FAM (Pab5, Pab6, Pab7), or HEX (Pab1, Pab9) fluorescent dyes to enable detection on the MegaBACE 1000 genotyping platform. Reactions were carried out in 384 well microtitre plates in 10 μL volumes containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01 μM BSA, 0.1% Triton-X100, 0.2 mM each dNTP, 0.5 μM each primer, 0.5 U *Taq* DNA polymerase, and 25–100 ng genomic DNA. Cycling conditions were as follows: 95 °C (3 min); five cycles 95 °C (30 s), 52 °C (30 s) and 72 °C (30 s); 25 cycles 90 °C (15 s), 52 °C (15 s), 72 °C (30 s); 72 °C (40 min) final extension. Loci were amplified individually and then pooled in sets of four according to nonoverlapping allelic ranges and differing fluorescent end labels. The two electrophoresis multiplexes consisted of Pab3-Pab5-Pab8 and Pab4-Pab6-Pab7-Pab9, and were desalted using hydrated Sephadex G-75 (medium) in Millipore Multiscreen HV 0.45 μm filter plates. Some PCR products were treated with Procipitate (CPG, Inc. New Jersey) following the manufacturer's protocol prior to the desalting step to prolong MegaBACE capillary life. Prepared samples were further diluted 2–5 times, and 2.05 μL added to 2.75 μL MegaBACE loading buffer (Amersham), plus 0.2 μL 900 bp internal size standard (25 bp rungs, Amersham). Pooled samples were denatured in a 96-well heat block at 95 °C for 55 sec and snap-cooled in a 96-well cold block for 1.5 min before electrophoresis through a 96 well MegaBACE 1000 capillary system. Allele sizes were estimated by visual inspection of raw electrophoretic traces using Genotyper 1.0 software (Amersham) and subsequently binned by grouping to the nearest full repeat.

Allozyme Data Collection

Preliminary allozyme screening included over 30 enzymes and a wide array of tissue-buffer combinations. Based on those results, an 11-enzyme, 2-tissue (siphon muscle and ctenidium) screening protocol was developed and used for all subsequent assays. The following enzyme/buffer combinations were used: alanine aminotransferase (ALAT), arginine kinase (ARGK), cytosol nonspecific dipeptidase (PEPA), mannose-6-phosphate isomerase (MPI), and superoxide dismutase (SOD) on TRIS-GLY ph 8.5 (Holmes & Masters 1970); aspartate aminotransferase (AAT-1), glucose-6-phosphate isomerase (GPI), glyceraldehyde-3-phosphate dehyrogenase (GAPDH), and phosphogluconate dehydrogenase (PGDH) on CAME pH 6.8 (Clayton & Tretiak 1972); isocitrate dehydrogenase (IDHP), malate dehydrogenase 9 (MDH), and the *SOD** 155 allele on CAME pH 5.9. The histochemical staining recipes of Harris and Hopkinson (1976) were used to visualize all allozymes except SOD, which was stained according to Fevolden (1989).

For each locus, variant alleles are described in terms of the mobility of the encoded allozymes, relative to the mobility of the

TABLE 1.

continued

	9	10	11	12	13	14	15	16	17
1	145	155	217	249	158	199	244	258	1270
2	109	130	192	224	132	180	219	233	1245
3	91	101	174	206	114	155	200	215	1227
4	105	115	188	220	129	170	215	229	1241
5	138	148	210	242	151	192	237	251	1273
6	54	64	137	169	77	118	163	178	1190
7	50	60	135	167	75	116	161	176	1173
8	57	67	140	172	80	121	166	181	1149
9		15	73	105	34	54	99	113	1126
10	0.0062/		63	95	44	44	89	103	1116
11	0.0066/	0.0008/		65	107	107	152	166	1178
12	0.0010/	−0.0003/	0.0044/−0.0003		139	139	184	198	1210
13	0.0057/	−0.0003/	0.0004/0.0002	0.0028/0.0006		88	133	147	1159
14	0.0048/	0.0021/	0.0008/	0.0026/	0.0022/		45	69	1171
15	0.0069/	0.0021/	0.0055/**0.0009**	**0.0080**/0.0023	**0.0022**/0.0021	0.0088/		114	1126
16	0.0094/	0.0012/	0.0012/0.0002	0.0025/0.0004	0.0021/0.0015	0.0012/	**0.0102**/0.0018		1012
17	/	/	/0.000	/0.0007	/0.0000	/	/−0.0007	/0.0004	

TABLE 2.

Allelic variability in 15 populations of *Panopea abrupta* at 11 allozyme loci. Shown for each population and locus: number of alleles (#), expected heterozygosity (H_e), observed heterozygosity (H_o), *p*-value for test of conformance to HWE (p), within-population variability (F_{is}), and number of individuals genotyped (n). Means are given in the last column.

Collection		Locus											
		ALAT	MPI	PEPA	PGDH	SOD	ARGK	GPI	AATI	GAPDH	MDH	IDHP	Means
1	#	3	8	4	4	4	4	4	5	2	2	3	3.909
	H_e	0.138	0.755	0.484	0.031	0.477	0.568	0.589	0.258	0.041	0.481	0.497	0.392
	H_o	0.135	0.750	0.406	0.032	0.448	0.635	0.635	0.250	0.042	0.438	0.406	0.380
	p	0.201	0.775	0.041	1.000	0.838	0.063	0.787	0.115	1.000	0.399	0.073	0.180
	F_{is}	0.017	0.006	0.160	−0.005	0.062	−0.119	−0.080	0.032	−0.016	0.090	0.182	0.016
	n	103	103	103	102	103	103	103	103	103	103	103	
3	#	4	7	3	5	4	4	5	5	2	2	4	4.091
	H_e	0.121	0.746	0.451	0.046	0.464	0.594	0.547	0.176	0.044	0.491	0.516	0.381
	H_o	0.127	0.773	0.509	0.047	0.482	0.573	0.527	0.173	0.046	0.518	0.518	0.390
	p	1.000	0.000	0.384	1.000	0.909	0.826	0.759	0.599	1.000	0.697	0.373	0.279
	F_{is}	−0.051	−0.031	−0.125	−0.010	−0.033	0.040	0.041	0.021	−0.019	−0.051	0.000	−0.019
	n	110	110	110	107	110	110	110	110	110	110	110	
4	#	4	8	4	7	5	7	3	6	2	3	5	4.909
	H_e	0.198	0.754	0.455	0.074	0.474	0.592	0.570	0.204	0.017	0.501	0.500	0.394
	H_o	0.208	0.658	0.483	0.076	0.417	0.617	0.525	0.175	0.017	0.542	0.467	0.380
	p	0.221	0.038	0.518	1.000	0.534	0.159	0.487	0.421	1.000	0.003	0.664	0.085
	F_{is}	−0.050	0.131	−0.058	−0.019	0.126	−0.038	0.084	0.145	−0.004	−0.076	0.070	0.040
	n	120	120	120	119	120	120	120	120	120	120	120	
5	#	4	8	3	5	4	5	4	4	2	3	4	4.182
	H_e	0.198	0.757	0.362	0.069	0.426	0.588	0.582	0.177	0.068	0.495	0.501	0.384
	H_o	0.202	0.675	0.395	0.070	0.377	0.553	0.711	0.175	0.070	0.483	0.500	0.383
	p	1.000	0.085	0.604	1.000	0.509	0.201	0.025	0.674	1.000	0.912	1.000	0.652
	F_{is}	−0.019	0.108	−0.089	−0.020	0.114	0.059	−0.220	0.011	−0.032	0.025	0.002	0.003
	n	114	114	114	114	114	114	114	114	114	114	114	
6	#	3	8	3	6	3	4	5	3	2	3	4	4.000
	H_e	0.199	0.712	0.443	0.076	0.506	0.607	0.590	0.247	0.032	0.490	0.514	0.401
	H_o	0.185	0.706	0.380	0.077	0.554	0.663	0.576	0.261	0.033	0.435	0.446	0.392
	p	0.290	0.679	0.140	1.000	0.637	0.294	0.036	1.000	1.000	0.230	0.212	0.380
	F_{is}	0.070	0.008	0.142	−0.019	−0.095	−0.092	0.023	−0.057	−0.011	0.113	0.133	0.023
	n	92	92	92	91	92	92	92	92	92	92	92	
7	#	4	6	3	4	4	5	4	4	2	3	3	3.818
	H_e	0.252	0.741	0.443	0.053	0.493	0.627	0.588	0.182	0.069	0.510	0.471	0.403
	H_o	0.268	0.661	0.429	0.054	0.464	0.661	0.607	0.196	0.071	0.536	0.339	0.390
	p	0.270	0.116	0.222	1.000	0.867	0.147	0.182	1.000	1.000	0.886	0.051	0.364
	F_{is}	−0.065	0.108	0.032	−0.009	0.058	−0.054	−0.032	−0.081	−0.028	−0.051	0.280	0.032
	n	56	56	56	56	56	56	56	56	56	56	56	
8	#	3	8	4	2	5	6	4	5	3	2	3	4.091
	H_e	0.180	0.709	0.361	0.020	0.427	0.632	0.595	0.165	0.076	0.459	0.508	0.376
	H_o	0.176	0.647	0.314	0.020	0.422	0.549	0.598	0.157	0.078	0.412	0.451	0.348
	p	0.644	0.216	0.313	1.000	0.481	0.020	0.939	0.534	1.000	0.385	0.276	0.495
	F_{is}	0.021	0.087	0.013	−0.005	0.012	0.131	−0.005	0.051	−0.028	0.103	0.112	0.074
	n	102	102	102	99	102	102	102	102	102	102	102	
9	#	2	7	3	4	3	3	4	2	2	3	4	3.364
	H_e	0.130	0.757	0.510	0.064	0.455	0.570	0.568	0.226	0.058	0.506	0.462	0.391
	H_o	0.140	0.640	0.460	0.065	0.520	0.640	0.540	0.220	0.060	0.600	0.360	0.386
	p	1.000	0.010	0.047	1.000	0.681	0.450	0.770	1.000	1.000	0.252	0.060	0.223
	F_{is}	−0.065	0.165	0.107	−0.011	−0.132	−0.114	0.060	0.038	−0.021	−0.176	0.230	0.024
	n	50	50	50	46	50	50	50	50	50	50	50	
10	#	5	8	4	5	4	4	3	4	3	4	4	4.364
	H_e	0.244	0.717	0.393	0.033	0.419	0.598	0.580	0.203	0.057	0.479	0.501	0.384
	H_o	0.208	0.658	0.392	0.033	0.392	0.558	0.542	0.192	0.058	0.467	0.483	0.362
	p	0.076	0.219	0.834	1.000	0.599	0.276	0.094	0.549	1.000	0.001	0.909	0.085
	F_{is}	0.150	0.086	0.007	−0.006	0.069	0.070	0.070	0.060	−0.022	0.031	0.038	0.061
	n	120	120	120	120	120	120	120	120	120	120	120	

continued on next page

TABLE 2.

continued

Collection		Locus											
		ALAT	MPI	PEPA	PGDH	SOD	ARGK	GPI	AATI	GAPDH	MDH	IDHP	Means
11	#	3	9	4	4	5	6	4	5	3	2	2	4.273
	H_e	0.155	0.764	0.413	0.031	0.445	0.589	0.627	0.175	0.071	0.469	0.500	0.385
	H_o	0.125	0.802	0.375	0.031	0.521	0.500	0.677	0.188	0.073	0.448	0.448	0.381
	p	0.122	0.079	0.214	1.000	0.170	0.128	0.486	1.000	0.107	0.668	0.411	0.159
	F_{is}	0.195	−0.049	0.092	−0.005	−0.169	0.151	−0.080	−0.070	−0.028	0.044	0.105	0.010
	n	99	99	99	99	99	99	99	99	99	99	99	
12	#	3	8	4	4	5	6	3	4	2	2	5	4.182
	H_e	0.137	0.716	0.437	0.043	0.440	0.618	0.539	0.247	0.010	0.478	0.507	0.379
	H_o	0.146	0.590	0.396	0.043	0.385	0.600	0.531	0.271	0.010	0.510	0.531	0.365
	p	1.000	0.015	0.122	1.000	0.038	0.547	0.975	0.247	1.000	0.528	0.806	0.204
	F_{is}	−0.068	0.177	0.094	−0.010	0.124	0.029	0.015	−0.097	0.000	−0.067	−0.047	0.038
	n	100	99	100	97	100	99	100	100	100	100	100	
13	#	3	6	3	5	4	6	4	3	3	3	4	4.000
	H_e	0.213	0.733	0.358	0.072	0.477	0.597	0.613	0.189	0.041	0.501	0.510	0.391
	H_o	0.240	0.625	0.406	0.074	0.500	0.677	0.677	0.208	0.042	0.458	0.448	0.396
	p	0.651	0.069	0.428	1.000	0.948	0.022	0.279	1.000	1.000	0.413	0.258	0.423
	F_{is}	−0.125	0.148	−0.136	−0.019	−0.048	−0.134	−0.105	−0.100	−0.012	0.085	0.121	−0.005
	n	117	117	117	117	117	117	117	117	117	117	117	
14	#	4	8	4	6	4	4	5	4	2	5	4	4.545
	H_e	0.247	0.753	0.423	0.041	0.472	0.608	0.592	0.232	0.013	0.469	0.493	0.395
	H_o	0.210	0.676	0.442	0.041	0.435	0.655	0.601	0.237	0.014	0.426	0.493	0.385
	p	0.044	0.012	0.494	1.000	0.043	0.260	0.136	1.000	1.000	0.435	0.351	0.058
	F_{is}	0.153	0.103	−0.045	−0.009	0.078	−0.077	−0.017	−0.019	−0.003	0.092	0.000	0.026
	n	148	148	147	146	147	148	148	148	148	148	148	
15	#	5	8	3	3	3	5	4	5	2	2	4	4.000
	H_e	0.200	0.741	0.402	0.042	0.477	0.628	0.572	0.191	0.031	0.479	0.499	0.387
	H_o	0.198	0.649	0.354	0.042	0.542	0.667	0.663	0.156	0.031	0.427	0.432	0.378
	p	0.701	0.078	0.393	1.000	0.187	0.096	0.209	0.069	1.000	0.390	0.199	0.136
	F_{is}	0.010	0.124	0.119	−0.012	−0.136	−0.061	−0.160	0.181	−0.011	0.108	0.135	0.018
	n	105	103	105	104	105	105	104	105	105	105	103	
16	#	4	8	4	3	3	5	5	3	3	3	4	4.091
	H_e	0.211	0.738	0.456	0.022	0.446	0.592	0.583	0.232	0.052	0.449	0.518	0.391
	H_o	0.158	0.684	0.474	0.022	0.358	0.547	0.484	0.211	0.053	0.411	0.442	0.349
	p	0.038	0.539	0.764	1.000	0.090	0.734	0.049	0.172	1.000	0.652	0.110	0.157
	F_{is}	0.252	0.073	−0.040	−0.003	0.197	0.075	0.169	0.094	−0.017	0.086	0.147	0.104
	n	98	98	98	93	98	98	98	98	98	98	98	
	total #	7	9	5	11	7	8	6	8	4	6	9	7.27
	p	0.1879	<0.0001	0.1719	1.0000	0.3496	0.0051	0.0849	0.7546	1.0000	0.0907	0.1801	<0.0001

most frequently encountered allele during the preliminary survey. Allozyme genotypes were collected from the same sites noted earlier, except for sites 2 and 17. Additional collections analyzed for allozyme variation only included three samples from the Southern Basin, (sites 3, 4, and 5), two samples from the Main Basin (7 and 9), and one sample from Admiralty Inlet (10).

Data Analyses

Genepop version 3.3 population genetics software of Raymond and Rousset (1995) was used to test for conformation to Hardy-Weinberg Equilibrium (HWE) at each locus in each collection using the Markov chain exact test method (Guo & Thompson 1992) for loci with five or more alleles, and the Louis and Dempster (1987) enumeration method for loci with less than five alleles (allozyme loci *PEPA**, *GAPDH**, and *MDH**). Genepop was also used to estimate gametic linkage disequilibrium between loci via the algorithm described by Cockerham and Weir (1979). For analyses of population differentiation, the genotypic log-likelihood (g) based exact test (Goudet et al. 1996), as implemented in Genepop, was used for all loci. To increase power in groups of loci in which assumptions of HWE were not violated, the genic test (Raymond & Rousset 1995) was used as implemented in Genepop. Weir and Cockerham's (1984) unbiased estimators of single and multilocus F-statistics were computed using the program FSTAT version 2.9.3.2 (Goudet 1995), and F_{ST} values were jackknifed over loci, and bootstrapped over individuals to obtain 95% confidence limits. The programs described in Beaumont and Nichols (1996) were used to evaluate whether F_{ST} values might indicate spatial selection at individual loci. Subsets of the allozyme and microsatellite datasets were used to perform a hierarchical analyses of molecular variance (AMOVA, Excoffier et al. 1992) based on the infinite allele model, to partition the genetic variance into among basins, among collections within basins, and within collections. The significance of the fixation indices among and

TABLE 3.

Allelic variability in 10 populations of *Panopea abrupta* at 7 microsatellite loci. Shown for each population and locus: number of alleles (#), expected heterozgosity (H_e), observed heterozygosity (H_o), p-value for test of conformance to HWE (p), within-population variability (F_{is}), and number of individuals genotyped (n). Means are given in the last column.

		Locus							
Collection		Pab3	Pab4	Pab5	Pab6	Pab7	Pab8	Pab9	Mean
1	#	31	40	19	31	16	60	20	31
	H_e	0.95	0.97	0.89	0.93	0.91	0.98	0.93	0.93
	H_o	0.48	0.33	0.53	0.91	0.73	0.51	0.80	0.61
	p	<.0001	<.0001	<.0001	0.253	0.000	0.001	0.011	<0.0001
	F_{is}	0.491	0.655	0.406	0.018	0.198	0.486	0.137	0.345
	n	95	90	95	92	91	95	90	
2	#	26	33	21	28	19	49	19	27.86
	H_e	0.94	0.96	0.90	0.93	0.91	0.98	0.92	0.93
	H_o	0.50	0.43	0.50	0.89	0.57	0.52	0.74	0.59
	p	<.0001	<.0001	<.0001	0.563	<.0001	<.0001	<0.001	<.0001
	F_{is}	0.472	0.547	0.447	0.048	0.081	0.467	0.197	0.365
	n	96	92	96	96	96	96	96	
6	#	26	31	18	29	15	59	18	28
	H_e	0.96	0.97	0.91	0.92	0.88	0.99	0.92	0.94
	H_o	0.52	0.28	0.42	0.85	0.59	0.41	0.75	0.55
	p	<.0001	<.0001	<.0001	0.052	<.0001	<.0001	<.0001	<.0001
	F_{is}	0.454	0.713	0.536	0.080	0.332	0.580	0.191	0.417
	n	92	83	92	92	87	87	91	
8	#	31	28	21	28	20	56	18	28.857
	H_e	0.96	0.97	0.90	0.93	0.92	0.98	0.92	0.94
	H_o	0.53	0.33	0.63	0.88	0.82	0.44	0.83	0.64
	p	<.0001	<.0001	<.0001	0.241	<.0001	0.055	0.026	<.0001
	F_{is}	0.444	0.664	0.304	0.049	0.113	0.551	0.019	0.323
	n	96	89	96	95	92	91	95	
11	#	28	32	25	31	17	52	19	29.14
	H_e	0.95	0.96	0.92	0.94	0.91	0.98	0.93	0.94
	H_o	0.49	0.43	0.44	0.90	0.53	0.44	0.79	0.57
	p	<.0001	<.0001	<.0001	0.107	<.0001	<.0001	0.074	<.0001
	F_{is}	0.484	0.551	0.525	0.044	0.417	0.552	0.145	0.390
	n	96	81	96	96	96	96	96	
12	#	28	31	19	26	18	52	17	27.29
	H_e	0.96	0.96	0.91	0.93	0.91	0.98	0.92	0.94
	H_o	0.45	0.31	0.45	0.87	0.60	0.45	0.75	0.55
	p	<.0001	<.0001	<.0001	0.163	<.0001	<.0001	0.004	<.0001
	F_{is}	0.533	0.673	0.506	0.067	0.339	0.546	0.182	0.410
	n	94	89	94	91	96	92	92	93
13	#	30	32	19	29	15	53	18	28
	H_e	0.96	0.96	0.90	0.93	0.90	0.98	0.92	0.93
	H_o	0.26	0.33	0.49	0.95	0.61	0.43	0.79	0.55
	p	<.0001	<.0001	<.0001	0.696	<.0001	<.0001	<.0001	<.0001
	F_{is}	0.728	0.657	0.459	−0.024	0.321	0.566	0.141	0.411
	n	96	79	96	96	96	94	96	
15	#	28	35	21	24	19	67	18	30.29
	H_e	0.95	0.96	0.93	0.93	0.91	0.99	0.93	0.94
	H_o	0.44	0.31	0.57	0.89	0.84	0.39	0.81	0.61
	p	<.0001	<.0001	<.0001	0.284	0.010	0.002	0.047	<.0001
	F_{is}	0.535	0.675	0.385	0.038	0.081	0.601	0.134	0.355
	n	95	83	95	93	94	94	93	
16	n	31	37	19	34	17	52	21	30.14
	H_e	0.95	0.97	0.92	0.94	0.91	0.98	0.93	0.94
	H_o	0.71	0.35	0.63	0.87	0.72	0.81	0.81	0.7
	p	<.0001	<.0001	<.0001	0.017	<.0001	<.0001	0.0213	<.0001
	F_{is}	0.252	0.642	0.321	0.07	0.208	0.168	0.124	0.257
	n	94	89	96	92	96	96	96	
17	#	31	34	19	29	17	58	21	29.86
	H_e	0.95	0.96	0.92	0.93	0.91	0.98	0.93	0.94
	H_o	0.53	0.34	0.48	0.87	0.64	0.44	0.84	0.59
	p	<.0001	<.0001	<.0001	0.2219	<.0001	<.0001	0.2845	<.0001
	F_{is}	0.443	0.647	0.482	0.061	0.305	0.554	0.098	0.373
	n	94	88	96	93	96	91	94	
total #		40	50	29	50	25	96	31	
P		<.0001	<.0001	<.0001	0.078	<.0001	<.0001	<.0001	

within basins and among samples was tested by nonparametric genotypic permutation tests as implemented in Arlequin (Schneider et al. 2000). Two distance matrices, one using linear watercourse distances (Table 1), the other with distances weighted by average subbasin retention time (Ebbesmeyer et al. 1988) between each pair of samples, were tested for correlation with genetic distance matrices via Mantel tests (Mantel 1967) using rank correlations (Raymond & Rousset 1995). A significance level of 0.05 was used throughout, and to avoid Type I error the Bonferroni correction (Rice 1989) was applied for table-wide comparisons.

RESULTS

Within-Population Genetic Diversity

As expected, levels of genetic diversity exhibited by the microsatellites were greater than those observed at allozyme loci (Tables 2 and 3). All of the microsatellites, but only 11 of 30 allozyme loci assayed were polymorphic. The mean number of alleles per allozyme locus per sample was 3.3 to 4.5 (overall mean 4.1), compared with 27.3 to 31.0 (overall mean 29.9) for the microsatellite loci. Expected heterozygosities were 0.01 to 0.76 (mean 0.39) for allozyme loci and 0.88 to 0.99 (mean 0.94) for microsatellites. No significant linkage disequilibrium was detected between pairs of allozyme or microsatellite loci, or between allozyme and microsatellite locus pairs. There were no significant differences among collections in mean observed heterozygosities for either marker set. Over microsatellite loci, the site 1 collection had the greatest mean number of alleles, but this was not significant (ANOVA, $P = 0.98$) due to the high variance over loci.

The allozyme and microsatellite loci differed markedly in respect to their relative conformity to Hardy Weinberg Equilibrium (HWE). Deviations from HWE expectations were generally small for allozymes, with only a single locus out of HWE over all populations after Bonferroni correction ($\alpha = 0.05/11 = 0.0045$). Two allozyme loci were out of HWE in individual collections after Bonferroni correction: *MPI** in the site 3 collection due to heterozygote deficiency and *MDH** in the site 10 collection due to heterozygote deficiency and the site 4 collection due to heterozygote excess. There were 2 additional marginally significant ($P < 0.05$) F_{IS} values involving heterozygote excesses: *ARGK** in the site 13 collection and *GPI** in the site 5 collection. Departures from HWE over all allozyme loci were not significant in any collection after Bonferroni correction ($\alpha = 0.05/15 = 0.0033$, Table 2).

In contrast, the microsatellite loci, with few exceptions, exhibited significantly fewer heterozygotes than expected (in most cases, $P < 0.001$; $\alpha = 0.05/10 = 0.005$, Table 3). The most notable exception was Pab6, which exhibited no significant departures from HWE in any population ($P = 0.0780$, Table 3); however, departures from HWE were also not significant after Bonferroni correction for Pab9 in collections from sites 1, 8, 11, 15, 16, and 17, and, for Pab7 in the site 15 collection and Pab8 in the site 8 collection.

Among-Population Genetic Diversity

Estimated values of F_{ST} were small for both marker classes ($F_{ST} \leq 0.01$, Table 1). Across all samples assayed, the global F_{ST} values for allozymes and microsatellites was small yet significant after bootstrapping ($F_{ST} = 0.002$, $P < 0.001$; $F_{ST} = 0.001$, $P = 0.004$, respectively). Across the eight samples assayed with both marker types, the global F_{ST} value was 0.002, also significant after bootstrapping ($P < 0.001$). The allozyme locus *GPI** and the microsatellite locus Pab4 had the greatest influence on F_{ST}, with values of 0.0018 and 0.0005, respectively, after jackknifing (Table 4). Significant genetic differentiation was detected among collections using allozymes ($P = 0.006$), microsatellites ($P < 0.0001$), and both marker classes ($P < 0.0001$) via global genic (allozyme) and genotypic (microsatellites and combined) tests (as implemented in Genepop). There was no evidence for selection at any locus: F_{ST} values for all loci plotted against heterozygosity were within the range of the expected distribution for neutral markers as determined by the Beaumont and Nichols (1996) method.

The hierarchical AMOVAs performed for both allozyme and microsatellite datasets yielded similar results, with 99.71% (allozymes) and 99.75% (microsatellites) of the variation occurring within collections. Variation among basins was nonsignificant for both marker classes (Table 5). Variation among collections was significant for allozymes ($P = <0.0001$) but not for microsatellites ($P = 0.19062$), and F_{ST} values were significant for both allozymes ($P = < 0.0001$) and microsatellites ($P = 0.041$).

Among the eight populations analyzed with both allozymes and microsatellites, the site 15 collection (Freshwater Bay) was the most genetically divergent; it differed significantly from all common collections except sites 1 (Case Inlet) and 13 (Langley) in pairwise exact genotypic tests with at least one of the two classes of marker (Table 1). Only one of the significant differences, involving site 12 (South Hood Canal) was detected with both allozymes and microsatellites. Significant divergence in genotypic frequencies were observed in four comparisons using both marker sets, but not detected with either marker set separately after Bonferroni corrections. Three of these comparisons involved site 8 (Dyes Inlet), which differed from sites 11, 12, and 16. The fourth comparison revealed differences between sites 11 and 13, again only with both sets of markers combined.

Three other significant differences among common collections (sites 6, 8, and 11) were detected with microsatellites alone. Significant differences between site 15 and sites 3, 4, and 14 for allozymes, and site 2 for microsatellites were also observed. Among populations analyzed only with microsatellites, site 2 stood out as relatively distinct; it differed significantly from sites 8, 12, 13, 15, and 17.

For collections analyzed with both allozymes and microsatellites, pairwise F_{ST} values calculated with allozyme and microsatellite loci separately were correlated (Mantel test, $R^2 = 0.47$, $P = 0.032$), but were typically higher for allozymes (Fig. 2) Without the allozyme locus *GPI**, however, the mean $F_{ST} = 0.0017$, and the T test was no longer significant. There was no correlation between expected heterozygosity and F_{ST} at individual loci for either allozymes or microsatellites. Mantel tests revealed no significant correlations between F_{ST} and either linear or adjusted linear watercourse distance (distance matrix A and B, $P >$ observed $= 0.10$, 0.57 respectively; Fig. 2), not even including the site 17 (Alaska) outgroup (Fig. 3).

DISCUSSION

The results presented are among the first studies of marine bivalve population genetics involving both microsatellite and allozyme loci on the same sample set. In general, geoduck aggregation localities appear genetically homogenous within the region studied, although both marker classes were concordant in the detection of genetic differentiation of the Freshwater Bay (site 15) collection from others. The possible biologic significance of these findings is discussed later.

TABLE 4.

F-statistics and Jacknifed F-statistics for 11 allozyme and 7 microsatellite loci at 15 and 10 collections of *Panopea abrupta*, respectively.

F statistics				Jackknifed			
Allozyme Locus	**F_{IS}**	**F_{IT}**	**F_{ST}**	**Excluded locus**	**F_{IS}**	**F_{IT}**	**F_{ST}**
ALAT	0.0429	0.0439	0.0011	ALAT	0.0288	0.0313	0.0025
MPI	0.0836	0.0871	0.0038	MPI	0.0182	0.0203	0.0021
PEPA	0.0226	0.0270	0.0045	PEPA	0.0302	0.0323	0.0022
PGDH	–0.0129	–0.0134	–0.0004	PGDH	0.0299	0.0323	0.0025
SOD	0.0165	0.0160	–0.0005	SOD	0.0310	0.0337	0.0028
ARGK	0.0031	0.0024	–0.0007	ARGK	0.0338	0.0366	0.0029
GPI	–0.0184	–0.0117	0.0066	GPI	0.0370	0.0387	0.0018
AAT1	0.0110	0.0113	0.0003	AAT1	0.0304	0.0329	0.0025
GAPDH	–0.0218	–0.0207	0.0010	GAPDH	0.0300	0.0324	0.0024
MDH	0.0199	0.0211	0.0012	MDH	0.0307	0.0332	0.0026
IDHP	0.0748	0.0776	0.0030	IDHP	0.0234	0.0257	0.0023
Total	0.0295	0.0318	0.0024	mean	0.0300	0.0324	0.0025
Microsatellite Locus							
Pab3	0.4841	0.4844	0.0005	Pab3	0.3440	0.3446	0.0010
Pab4	0.6422	0.6432	0.0029	Pab4	0.3165	0.3168	0.0005
Pab5	0.4369	0.4375	0.0010	Pab5	0.3526	0.3532	0.0009
Pab6	0.0449	0.0450	0.0001	Pab6	0.4169	0.4175	0.0010
Pab7	0.2683	0.2686	0.0005	Pab7	0.3797	0.3803	0.0010
Pab8	0.5058	0.5065	0.0013	Pab8	0.3395	0.3400	0.0008
Pab9	0.1446	0.1444	–0.0002	Pab9	0.4003	0.4010	0.0011
total	0.3643	0.3649	0.0009	mean	0.3649	0.3655	0.0009

Within-population Genetic Diversity

In marked contrast to the allozyme loci, most of the microsatellite loci were out of HWE. For allozyme loci, deficiencies of heterozygotes relative to the expectations of HWE have been frequently reported for invertebrates, and bivalves in particular (Zouros & Foltz 1984, Raymond et al. 1997). These deficiencies have variously been characterized as resulting from null alleles (Foltz 1986), a Wahlund effect (David et al. 1997), inbreeding and genotype–dependent spawning (Rios et al. 1996) or selection (Ridgway 2001). Because the assumption of discrete generations, inherent to the Hardy-Weinberg principle, was violated by this study, that the allozyme loci overall were in HWE may indicate a degree of genetic stability across generations. Because little evi-

TABLE 5.

Hierarchical analysis of molecular variance (AMOVA: Weir, B.S. and Cockerham, C.C. 1984; Excoffier, L., Smouse, P., and Quattro, J. 1992. Weir, B.S., 1996.) using A) microsatellite data for collections of *P. abrupta* grouped into 4 basins: Straits (sites 14, 15), Main (sites 6, 8), Hood Canal (sites 11, 12) and Southern (sites 1, 2), and B) allozyme data for collections of *Panopea abrupta* grouped into 4 basins: Straits (sites 14–16), Main (sites 6–9), Hood Canal (sites 11, 12) and Southern (sites 1, 3–5).

A

Source of variation	DF	Variance components	Percent variation	Fixation indices	P
Among basins	3	0.00086 Va	0.03	F_{CT} 0.00028	0.19257
Among collections within basins	4	0.00683 Vb	0.22	F_{SC} 0.00219	0.19062
Within collections	1520	3.10694 Vc	99.75	F_{ST} 0.00247	0.04106
Total	1527	3.11463			

B

Source of variation	DF	Variance components	Percent variation	Fixation indices	P
Among basins	3	–0.00133 Va	–0.06	F_{CT} –0.00062	0.79765
Among collections within basins	9	0.00762 Vb	0.36	F_{SC} 0.000355	<0.0001
Within collections	2581	2.13812 Vc	99.71	F_{ST} 0.00293	<0.0001
Total	2593	2.14440			

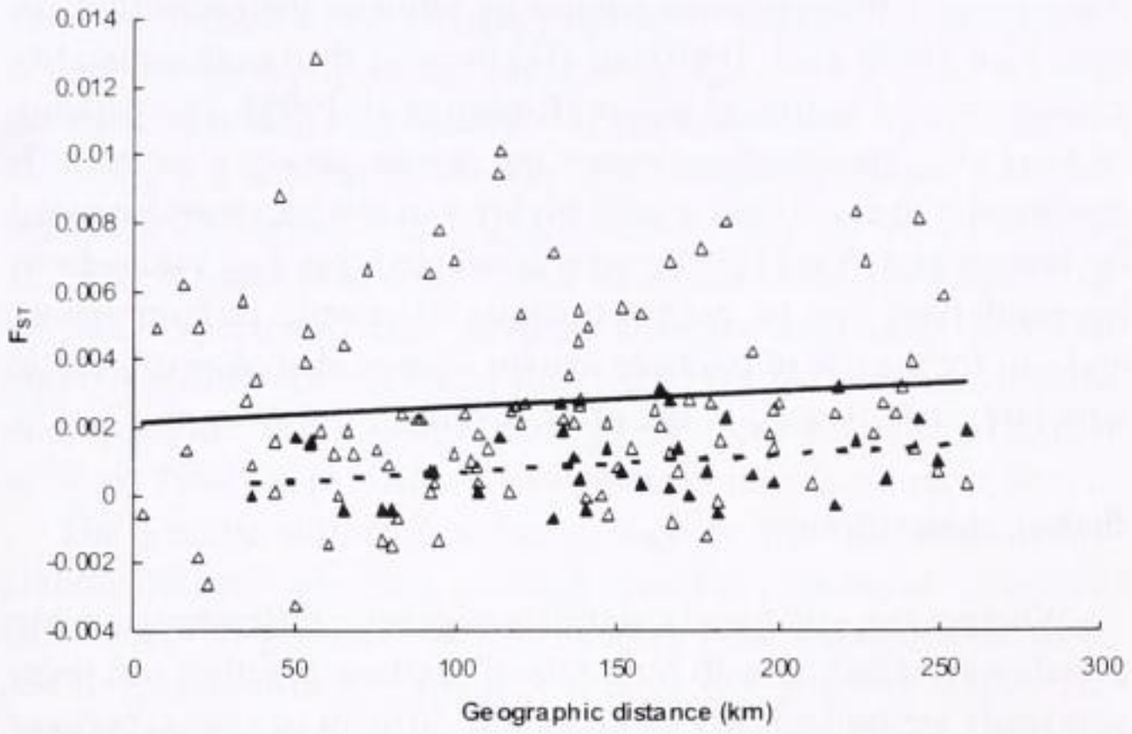

Figure 2. *Panopea abrupta* sample site pairwise genetic and geographic distances: Genetic distance, indicated as F_{ST}, over all allozyme (□) and microsatellite (Δ) loci for each population pair, as a function of watercourse distance (km) between sites. Indicated trendlines for allozyme (solid line) and microsatellite (dashed line) loci are nonsignificant.

dence of overall heterozygote deficiency is apparent in the allozyme data, our data suggest that population level processes are unlikely to be the cause of the heterozygote deficiencies that we observed with microsatellites.

Aside from population level processes, potential causes of the observed heterozygote deficiencies in microsatellites are size homoplasy or mis-scoring of alleles, upper allele dropout, and nonamplifying alleles. Mis-scoring or inadvertent binning of neighboring alleles is akin to size homoplasy, and undoubtedly occurred to some extent in this dataset. Indeed, at a single locus, as many as seven different microsatellite alleles with identical mobilities were detected via sequencing in the gastropod *Bulinus truncatus* (Viard et al. 1998). Given the high variability of the microsatellite loci used in this study, and the high resolution of the genotyping platform, alleles with very close mobilities are unlikely to have occurred (or to have been inadvertently binned) with a high enough frequency to produce the strong heterozygote deficiencies observed. Upper allele dropout was minimized by both the optimization of PCR conditions and the exploitation of the high sensitivity of the genotyping platform (see Materials and Methods): two alleles could differ in fluorescence intensity by more than 50,000 relative fluorescence units (RFUs), and still be accurately scored via internal lane standards. Even though observed heterozygosities were slightly lower on the less sensitive ABI 373 slab gel platform whose software allowed only a lower fluorescence intensity differential between alleles (Vadopalas, unpublished data), upper allele dropout is unlikely to account for the full magnitude of the deficit of heterozygotes. We explored the possibility of mispriming as a cause of our heterozygote deficiencies during the development of the loci (Vadopalas & Bentzen 2000) by designing primers for alternate flanking sequence. Although the number of alleles present in some individuals changed either from homozygote to heterozygote or vice versa, there was no net change in observed heterozygosities (unpublished data). We were unable to find suitably conserved flanking sequence to alleviate the occurrence of putative mispriming. Thus a likely explanation for the observed heterozygote deficit in our microsatellite data is primer site sequence variation resulting in null alleles. Hedgecock et al. (2004) gave a conservative estimate of one single nucleotide polymorphism (SNP) every 82 base pairs in the Pacific oyster *Crassostrea gigas*; this figure may be considerably higher in geoduck clams. We used the Brookfield (1996) method to calculate the expected

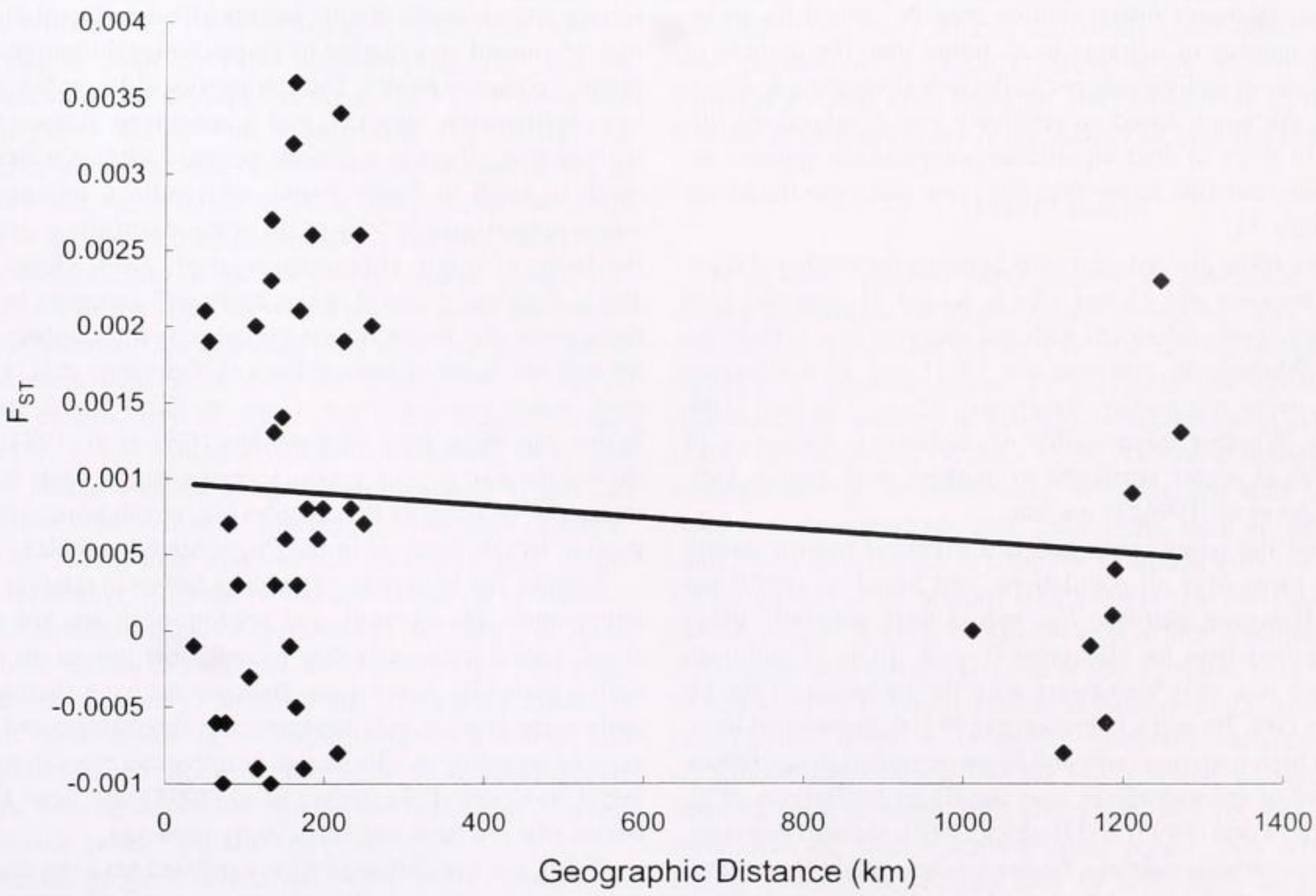

Figure 3. *Panopea abrupta* sample site pairwise genetic and geographic distances: Genetic distance, indicated as F_{ST}, over all microsatellite loci for each population pair including the SE Alaska group, as a function of watercourse distance (km) between sites. Trendline is nonsignificant.

frequency of null alleles in microsatellite loci; for all heterozygote deficient loci the expected null frequency exceeded that of the most common visible allele (Vadopalas, unpublished data). The genotypic bias recognized here is presumed to affect all populations evenly.

The mean expected heterozygosities for microsatellites (0.94) and allozymes (0.390) (Tables 2 and 3) were similar to those found in other marine bivalves. For example, the overall mean expected heterozygosity was 0.261 for 24 allozyme loci in the razor clam *Siliqua patula* (LeClair & Phelps 1994), 0.158 to 0.261 (mean 0.214) for seven allozyme loci in the greenshell mussel *Perna canaliculus* (Apte & Gardner 2001), and 0.114 to 0.696 (mean 0.413) for nine allozyme loci in the bivalve *Spisula ovalis* (David et al. 1997). For microsatellites, Huvet et al. (2000) reported a range of 0.83 to 0.96 (mean 0.88) at four loci in the Pacific oyster *Crassostrea gigas*.

Among-population Genetic Diversity

We detected similar patterns of genetic differentiation with microsatellites and allozymes. Significant differences between site 15 and all other common collections except for sites 1 and 13 were revealed by multilocus genotypic and allelic analyses using the two marker classes separately and combined (Table 1). Genetic differences between site 15 and collections within the Southern Basin were similar: no differentiation was detected between site 15 and site 1 with either marker class, whereas differences were detected between site 15 and the proximate collections from sites 3/4 and 2 with allozymes and microsatellites, respectively. The general concordance between the two marker classes in this study corroborates the findings of others (Scribner et al. 1998, Ross et al. 1999, Allendorf & Seeb 2000, De Woody & Avise 2000) and supports the notion of selective neutrality of allozymes.

Four significant pairwise exact test comparisons with all markers combined were not significant for either marker class alone. Greater power to detect differentiation may be gained by an increase in the number of markers used, rather than the number of alleles per locus or individuals per collection (Ferguson & Danzmann 1998). Although based on relatively low P values, the differentiation in three of four significant comparisons appears derived from microsatellite rather than allozyme genotype frequency variation (Table 1).

There were some discordant results between the marker classes. Differences between site 15 and sites 6, 8, and 11, detected with microsatellites, were undetected with the allozyme loci in pairwise exact tests, although the pairwise site 15-11 and 15-8 allozyme comparisons were marginally significant ($P = 0.06$ and 0.04, respectively). Whether this possible discordance is indicative of greater statistical power attributed to markers with greater variability (Goudet et al. 1996) is unclear.

We plotted the relationship between expected heterozygosity and F_{ST} per locus over all populations, and found no significant correlation. However, pairwise F_{ST} values were generally lower for microsatellites than for allozymes (Fig. 2, Table 1), although this difference was only significant with the inclusion of the allozyme locus GPI. Jin and Chakraborty (1995) demonstrated theoretically that high mutation rates and stepwise mutation can reduce F_{ST}, a feature of microsatellites also simulated by Balloux et al. (2000). Charlesworth (1998) and Hedrick (1999) showed that compared with less variable markers, higher marker variability reduced F_{ST} values. Nevertheless, highly variable markers are powerful in exact tests of differentiation not just by virtue of their sensitivity to gene flow (Ross et al. 1999) but also because their high variability imparts greater statistical power (Estoup et al. 1998). The paradox of lower F_{ST} parameter estimates but greater power to detect differentiation in exact tests among highly variable loci was discussed by Hauser and Ward (1998), who concluded that F_{ST} values from microsatellites can be poor estimators of genetic differentiation, and call for the use of multiple marker classes in studies of genetic structure such as used in the present study.

Biological Significance

Whether the statistically significant genetic differences among populations detected with both sets of markers together and independently are biologically significant is difficult to assess, because the pattern is inconsistent. With both allozymes and microsatellites, the differentiated pairs include site 15 and 5 other sites: 4 within Puget Sound, and the single Georgia Strait sample (16). The significant genetic differences between sites 15 and 12 and Southern Basin collections correlate with watercourse distance, whereas the differences between the outer Straits collections do not. In addition, the differences detected with microsatellites between the site 2 collection in the Southern Basin and collections in nonadjacent basins/inlets, including S.E. Alaska seem to correspond to IBD, yet with these same markers we were unable to detect differentiation between the S.E. Alaska collection and any other population surveyed. Greater differences were detected on a scale of 100 km than 1000 km; the lack of correlations between F_{ST} and distance for either class of marker (Figs. 2, 3) and the lack of differentiation between site 17 (S.E. Alaska) and all other collections in the complex except site 2 reinforce our conclusion that a simple IBD model does not apply to the current dataset.

Aside from general panmixia, there are a number of alternate explanations for our failure to find IBD. First, it may be that neither the weighted nor the unweighted distance matrix sufficiently approximates abiotic factors affecting larval dispersal, and that we missed an isolation by distance signal through an inappropriate circulation model. The complexity of Puget Sound's hydrology, bathymetry, currents, and wind-driven surface waters notwithstanding, there is a general seaward surface water flow from south to north in Puget Sound, with vertical mixing at the sills where approximately 50% of the surface outflow is cycled back to the basin of origin (Ebbesmeyer et al. 1984, Geyer & Cannon 1982). Particles released in one basin will generally be exchanged throughout the Puget Sound complex before exiting the system through the Strait of Juan de Fuca (Ebbesmeyer et al. 1988). However, water mass retention times in Hood Canal and Whidbey basins can be as high as 9 months (Cox et al. 1984) and easily exceed the 4 to 6 week geoduck pelagic stage. Thus both distance matrices oversimplify the complexities of estuarine circulation and passive larval dispersal in the Puget Sound complex.

Second, the behaviors of geoduck larvae in relation to currents, temperature, photoperiod, and phototropism are not well understood. Larval phototaxis may have a large impact on associations with a particular water mass. Because we have attempted to integrate some gross circulation rates, larval behaviors and differential rates of recycling at sills may play important roles in the degree of larval transport (Ebbesmeyer et al. 1998) and gene flow among basins that we have not sufficiently modeled.

Third, the low differentiation exhibited between the SE Alaska collection and those in Washington may be due not to high gene

flow, but instead to the tendency of highly variable microsatellites to underestimate population structure in cases of low gene flow, as demonstrated both empirically and via simulations by (Balloux et al. 2000). A combination of array size constraints (Nauta & Weissing 1996, Calabrese et al. 2001), size homoplasy/binning (Viard et al. 1998, Angers et al. 2000), and statistical noise associated with the allele:specimen ratio, perhaps strongly associated with our highly variable microsatellite loci, may confirm observations that microsatellites can underestimate genetic differentiation (Hauser & Ward 1998), especially in cases where the separation is recent.

The genetic differences found may be due to some hydrographic factors affecting geoduck genetic population structure. Differences in geoduck settlement have been hypothesized to be due to localized hydrodynamics (Zhang & Campbell 2004). The differentiation of the site 15 collection may be due to difficult emigration caused by strong oceanographic conditions that occur in the Strait of Juan de Fuca. A sill, roughly between Victoria, BC and Dungeness Spit, Washington, separates the inner and outer Strait (Fig. 1). Intense homogenization of surface and deep waters occurs at the sill, where summer water temperatures are colder than they are elsewhere in the region, perhaps serving as a barrier to successful larval immigration. A possible isolating mechanism in this general locality is suggested by the allele frequency divergence in Butter clams (*Saxidomus giganteus*) found by Johnson and Utter (1973).

The relatively few differentiated collections may reflect selection, because the differences may be primarily driven by a minority of loci linked to genes under selection. Among the allozyme loci, the significant differentiation of site 15 from other collections was driven primarily by the allozyme locus *GPI**. GPI seems to be under temperature selection in *Mytilus edulis* (Hall 1985) with a latitudinal gradient among alleles (Koehn et al. 1976, Koehn et al. 1984). Both temperature and salinity vary less at site 15 compared with other more estuarine localities; a similar mild selective effect might explain the observed differences in *GPI** allele frequencies in geoducks. Without *GPI**, jackknifing over collections resulted in a global F_{ST} of 0.00175 (Table 4), indicating *GPI** has the strongest effect among allozymes loci on global F_{ST}. Without site 15, jackknifing over loci resulted in an overall F_{ST} for *GPI** of 0.00088, further indication that the differentiation may be due to this locus. However, no evidence that this locus is under selection was detected using the method of Beaumont and Nichols (1996).

Another possible cause of the seemingly random genetic differentiation could be the scale on which we are sampling. Seemingly random differentiation can exist on a very fine scale due to variable settlement patterns (Larson & Julian 1999). *Panopea abrupta* larval cohorts may aggregate in clusters to increase dispersal. If larvae in the natural environment increase their drag coefficient by rafting together using a combination of byssus and mucus as we have observed in the hatchery environment (Vadopalas, unpublished data), they may remain associated until settlement. Some separation of larvae would still occur via turbulence, postsettlement byssus drifting (Sigurdsson et al. 1976), or pedal locomotion over the substrate (Cole & Beattie 1991). Nevertheless, some degree of clustered dispersal may explain our results. If it does occur, some degree of increased relatedness within, and increased differentiation among, collections on a subkilometer sampling scale may be detectable. Studies are underway to investigate microspatial genetic variation in *P. abrupta*.

We assume the geoduck collections in this study consisted of overlapping generations, because ages range from 12 to 131 for a random sample of *P. abrupta* from Puget Sound (Goodwin & Shaul 1984). With the high longevity, early reproduction, and no apparent reproductive senility (Sloan & Robinson 1984), the mean generation time for geoduck clams is approximately 30 years; a random sample of 100 geoducks is likely to include more than 50 year classes and many overlapping generations. Because overlapping generations violate assumptions of many population genetic models (Hartl & Clark 1997), the overall effect of our random sampling of cohorts within each collection should be to minimize the effect of temporal genetic drift (Jorde & Ryman 1995). However, *P. abrupta* may use the strategy modeled by Ripley (1998) in which bivalves with high longevity and fecundity can wait many years until the relatively rare occurrence when conditions are conducive to mass reproductive success. This strategy would maintain genetic variability over long temporal scales, but may create stochastic differences on shorter temporal scales. Indeed, in another large collection for which we have age data (genotype and age data to be reported in a forthcoming study), 27% of the specimens are of a single year class. If separate year classes are genetically differentiated via sweepstakes recruitment (Hedgecock 1994) in years, intervening between episodes of strong recruitment genetic variability would be reduced within cohorts. Supporting the sweepstakes hypothesis, David et al. (1997) demonstrated significant differences between cohorts in the clam *Spisula ovalis*, Li and Hedgecock (1998) demonstrated significant genetic heterogeneity among larval *Crassostrea gigas* cohorts, and Moberg and Burton (2000) detected greater heterogeneity among recruits than among adults over similar spatial scales.

Our results are similar to those of Johnson and Black (1984) and Edmands et al. (1996) who found what appeared to be stochastic genetic differentiation in marine invertebrates (*Siphonaria jeanae* and *Strongylocentrotus purpuratus*, respectively), with genetic homogeneity on a broad spatial scale and heterogeneity on a fine scale. Similar observations for other marine taxa exist (see Shaklee & Bentzen 1998 for a review). Whether the heterogeneity detected in this study reflects temporally stable differences among local populations of geoduck clams, or are instead due to large variation in year class strength and strong bias in reproductive success among spawners is the subject of ongoing investigations.

CONCLUSION

In general, genetic homogeneity, or panmixia, was found among collections of Puget Sound geoducks, with only a few statistically significant differences that are inconsistent with an isolation by distance model. To maximize the signal of genetic differentiation relative to random noise, temporal replication is highly recommended (Waples 1998). Temporal homogeneity was assumed among our collections, but given age data currently on hand for other geoduck clam collections, such an assumption is unlikely to be accurate. A temporal Wahlund effect is possible if, for example, a large proportion of a single year class comprises the site 15 sample. Thus, it is difficult to conclude whether the genetic differences between this site and others represent long-term effects of reproductive isolation leading to separate evolutionary trajectories, thermal selection, or simply stochastic variation. Including only those collections within the Puget Sound, no significant differences were detected with allozymes, and yet four differences were detected with microsatellites. Again, this differentiation among geoduck collections may be temporally unstable over a scale much longer than a few generations. Whether such patterns

of "chaotic genetic patchiness" apply to other marine taxa in the Puget Sound region remains to be investigated. We emphasize the need to study both the spatial and temporal scales of sweepstakes recruitment, so fishery managers can incorporate genetic population structure into harvest and culture management models.

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