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Authors

Ayala, FJ
Valentine, JW
Barr, LG
et al.

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Genetic Variability in a Temperate Intertidal Phoronid, *Phoronopsis viridis*

Francisco J. Ayala,¹ James W. Valentine,² Lorraine G. Barr,¹ and Gary S. Zumwalt²

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*The Phoronida are a coelomate phylum consisting of only two genera and about 12–15 described species. Phoronids probably represent the common ancestral stock of all lophophorates, and may be the most primitive living deuterostomes. Using the techniques of starch gel electrophoresis, we have studied genetic variation at 39 loci in 120 individuals of Phoronopsis viridis collected in Bodega Harbor, Bodega Bay, California. Allelic variation was found at 27 (69.2%) loci. If a locus is considered polymorphic when the frequency of the most common allele is no greater than 0.99, the proportion of polymorphic loci in the total sample is 48.7%. The average number of alleles per locus is 2.23. The expected frequency of heterozygous loci per individual on the assumption of Hardy–Weinberg equilibrium is 9.4%. There is evidence of inbreeding; the mean value of F , Wright's fixation index, is 0.21 ± 0.02 . Genetic variability in *P. viridis* is intermediate among marine invertebrates. The tropical clam, *Tridacna maxima*, has on the average 20.2% heterozygous loci per individual. At the other extreme, a brachiopod from Antarctica, *Liothyrella notorcadensis*, has an average of 3.9% heterozygous loci per individual. Among marine invertebrates, there seems to be a gradient of decreasing genetic variability from low to high latitudes, which may reflect their different adaptive strategies.*

KEY WORDS: enzyme polymorphism; biochemical evolution; adaptive strategies; Phoronida.

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¹ Department of Genetics, University of California, Davis, California.

² Department of Geology, University of California, Davis, California.

INTRODUCTION

From previous studies on the genetic variability of marine populations (Selander *et al.*, 1970; Ayala *et al.*, 1973, 1974), it has been possible to formulate models of the relations between environmental and genetic variability. In order to test these models, we are examining a variety of taxa from distinctive environmental regimes by the techniques of gel electrophoresis. Here we report on populations of intertidal phoronids from the temperate Mendocinan subprovince of the Oregonian province (Valentine, 1966) of the eastern Pacific. No intertidal temperate populations of any taxon, or any species of the phylum Phoronida, have previously been examined genetically at a large number of loci. A recent worldwide account of the taxonomy of phoronids is given by Emig (1971); northeastern Pacific species are reviewed by Marsden (1959).

MATERIALS AND METHODS

Populations Studied

A large species of *Phoronopsis* with a pale greenish lophophore inhabits bays and estuaries in California. It has sometimes been assigned to *Phoronopsis viridis* (Hilton, 1930), which is based on material from Moro Bay, but it has also been assigned to *Phoronopsis harmeri* (Pixel, 1912), described from Vancouver Island, British Columbia. If Canadian and Californian populations are specifically distinct, then the Californian form is to be called *P. viridis*, and we shall employ this name, although the relationships are not known for certain.

Although some phoronids are dioecious, others are hermaphroditic and some reproduce extensively by fission. Reproduction in *P. viridis* has been studied by Rattenbury (1953; see also Marsden [Rattenbury], 1959), who concludes that this species is probably dioecious and at any rate reproduces sexually without selfing. There is, however, a single report of fission in *P. viridis* (Hamby in Johnson, 1967).

P. viridis is reported to be exclusively intertidal in Tomales Bay (Johnson, 1967) and is known from widely scattered intertidal localities in California embayments (Marsden, 1959). The specimens that we have studied were collected from intertidal sand flats along northwestern Bodega Harbor, Bodega Bay, California (Fig. 1). Three localities were sampled on September 21, 1973, from an offshore-onshore transect approximately along the western boundary of the University of California marine reserve. Locality 1 is on the bank of the dredged boat channel which leads from Bodega Harbor to the sea, in medium-grained sand, just inshore of stands of the eel grass,

Fig. 1. (A-C) Location of sampling localities for *Phoronopsis viridis*. (D) Sampling pattern of specimens actually analyzed from each locality; six samples were taken in a 1-m grid pattern, and five or seven individuals were analyzed from each sample.

Zostera, which line the channel. The phoronids there were densely packed and small, with medium-grained arenaceous tubes. Locality 2 is about 100 yards shoreward of the channel, in medium sand black and anoxic with hydrogen sulfide odors just below the surface. The surface was filmed with benthic diatoms and supported green algal mats. These phoronids were larger, in finer-grained tubes, and generally less dense, although local clumps were of high densities. Locality 3 is about 350 yards inshore of the channel

in clean medium sand near the high tide line and represents the most shallow-water population of phoronids observed in this region. The phoronids there were of intermediate size and low density. The channel bank sands are frequently disturbed by boat traffic, while the inshore locality is frequently exposed for long intervals. Locality 2 lies in a broad, shallow depression and appears to be less disturbed than the others. Six samples spaced at 1-m intervals in a 2×3 grid were collected at each locality, and five or seven specimens were used from each sample (see Fig. 1), for a total of 40 individuals at each locality and 120 individuals for the whole study.

Sample Preparation and Assay Techniques

The phoronids were brought directly after collection to our laboratory in Davis, where they were immediately homogenized whole in about 2 vol of tris-HCl-EDTA buffer while submerged in an ice-water bath. Centrifugation of the homogenates was done under refrigeration at 17,000 rpm for 20 min. The supernatants were stored at -70 C until used for electrophoresis. For the electrophoresis runs, a small amount of thawed supernatant was absorbed with one 4- by 10-mm piece of Whatman No. 3 filter paper. The remainder of the homogenate was stored again at -70 C for eventual retesting. Nearly all electrophoresis runs were done within a week after the time of collection.

Gels were prepared according to procedures already described (Ayala *et al.*, 1972). Four buffer systems were used. (A) Gel buffer: 76 mM tris and 5 mM citric acid, pH 8.65; electrode buffer: 300 mM boric acid and 60 mM NaOH, pH 8.1. (B) Gel and electrode buffer: 87 mM tris, 8.7 mM boric acid, 1 mM EDTA, pH 9.1. (C) Gel buffer: 9 mM tris, 3 mM citric acid, and 1.2 mM EDTA, pH 7.0; electrode buffer: 135 mM tris, 45 mM citric acid, and 1.2 mM EDTA, pH 7.0. (D) Gel buffer: 214 mM K₂HPO₄ and 27 mM citric acid, pH 7.0; electrode buffer: 7.8 mM K₂HPO₄ and 1.3 mM citric acid, pH 7.0. The buffer system and the kind of starch used for each enzyme assay are specified in Table I.

The procedures used in setting up the samples, the specifications for the electrophoretic runs, the techniques of gel fixation, and most enzyme assays are as reported in Ayala *et al.* (1972). The following additional assays were used. *Fumarase*: 25 mg β -NAD⁺, 20 mg NBT, 50 mg fumaric acid in 100 ml buffer B; incubate 1 hr at 37 C, then add 5 mg PMS. *Glucose 6-phosphate dehydrogenase*: 10 ml 0.5 tris-HCl buffer, pH 7.1, in 90 ml H₂O, 20 mg TPN⁺, 20 mg NBT, 20 mg glucose 6-phosphate, 25 mg EDTA; incubate for 1 hr at 37 C, then add 5 mg PMS. *Phosphoglucose isomerase*: 10 mg TPN⁺, 20 mg NBT, 200 mg MgCl₂, 25 mg EDTA, 20 mg fructose 6-phosphate, 80 units glucose 6-phosphate dehydrogenase, in 100 ml 0.1 M tris-HCl, pH 7.1; incubate 1 hr at 37 C, then add 5 mg PMS.

Table I. Enzymes Assayed and Procedures Used in a Study of *Phoronopsis viridis*

Enzyme	Abbreviation	Buffer system	Starch ^a	Number of loci scored
Acid phosphatase (3.1.3.2)	Acph	A	Sigma	2
Adenylate kinase (2.7.4.3)	Adk	C	Sigma	2
α -Glycerophosphate dehydrogenase (1.1.99.5)	α Gpdh	C	Sigma	1
Esterase (3.1.1.2)	Est	A	Sigma	7
Fumarase (4.2.1.2)	Fum	C	Sigma	1
Glucose 6-phosphate dehydrogenase (1.1.1.49)	G6pdh	B	E-371	1
Glyceraldehyde 3-phosphate dehydrogenase (1.1.1.8)	G3pdh	B	E-371	2
Hexokinase (2.7.1.1)	Hk	D	Sigma	2
Isocitrate dehydrogenase (1.1.1.41)	Idh	D	Sigma	1
Leucine aminopeptidase (3.4.1.1)	Lap	A	Sigma	5
Malate dehydrogenase (1.1.1.37)	Mdh	C and D	Sigma	1
Malic enzyme (1.1.1.40)	Me	C	Sigma	3
Octanol dehydrogenase (1.1.1.1)	Odh	B	E-371	2
Phosphoglucomutase (2.7.5.1)	Pgm	C	Sigma	3
Phosphoglucose isomerase (5.3.1.9)	Pgi	C	Sigma	1
Tetrazolium oxidase	To	B	E-371	2
Triose phosphate isomerase (5.3.1.1)	Tpi	D	Sigma	2
Xanthine dehydrogenase (1.2.3.2)	Xdh	B	E-371	1

^a *Sigma*: from Sigma Chemical Co., St. Louis, Mo.; E-371: lot No. 371 from Otto Hiller Electrostarck Co., Madison, Wis.

Samples of 20–22 phoronids were run in each gel together with two control samples. As controls, we used our standard stocks of *Drosophila willistoni* flies. Samples of these stocks, and the mobility of the *Phoronopsis viridis* enzymes relative to the controls, are available on request from F. J. Ayala.

RESULTS

The 18 enzymes assayed in our study and the abbreviations used to designate the enzymes are given in Table I. A total of 39 zones of activity were scored. Variation was observed in 27 (69.2%) of the 39 zones. The patterns observed in each of these variable zones conform to those expected for diploid organisms according to Mendelian heredity. The zymogram patterns of all 120 individuals were not identical in any two of the 27 variable zones. That is, we did not find any pair of zones of activity such that every individual heterozygous in one zone would also be heterozygous in the other zone. Therefore, we assume that the 27 variable zones of activity are controlled by 27 different gene loci. We have also assumed that the 12 invariant zones are determined by 12 different gene loci, even though we cannot be completely certain that

we are not scoring products of the same locus more than once. This possibility, however, is not likely to affect our results to a substantial degree.

The abbreviations for the enzymes as given in Table I, italicized, are used to designate the corresponding gene loci. When several zones of activity exist for a given enzyme, a hyphenated numeral is added to the symbol of the enzyme to designate each zone and the gene coding for it. The zone of activity with the least anodal migration is designated 1, the next is designated 2, and so on. At each locus one allele, usually the most common, has been arbitrarily named 100. All other alleles are designated by reference to that standard, adding to, or subtracting from, 100 the number of millimeters by which the migration of the enzyme coded by each allele differs from the standard.

The main results of our study of *Phoronopsis viridis* are given in Table II. For each locality, and for the total sample, the table gives the sample sizes, the allelic frequencies, and the heterozygosities. Sample size is the number of genes sampled, i.e., twice the number of individuals. The observed frequencies of heterozygous individuals at each locus are followed, in parentheses, by their expected frequencies according to the Hardy-Weinberg formula. The "total" allelic frequencies and heterozygosities have been calculated by pooling the data, that is, as if all samples came from the same Mendelian population, or from different Mendelian populations with identical gene frequencies. The allelic frequencies are quite similar in the three populations, although some very slight differentiation between them may exist (see below).

A summary of genetic variation in *P. viridis* is given in Table III. Averages for all three localities as well as totals for the pooled data have been included in the summary. On the average, 1.79 ± 0.04 alleles per locus per population have been found, and 2.23 ± 0.21 alleles per locus in the total sample. Two criteria of polymorphism are used (Ayala *et al.*, 1972). The first, more restrictive criterion considers a population polymorphic at a given locus when the frequency of the most common allele is no greater than 0.95; by the second, more relaxed criterion a population is considered polymorphic at a given locus when the frequency of the most common allele is no greater than 0.99. The proportion of polymorphic loci per population is $25.9 \pm 2.7\%$ by the first criterion and $48.2 \pm 4.3\%$ by the second criterion. These estimates are only slightly increased when the data for all three populations are pooled, reflecting the fact that, in general, any locus polymorphic in one population is also polymorphic in the other two.

The expected frequency of heterozygous loci per individual (obtained by averaging over all loci the expected frequency of heterozygous individuals at each locus) is $8.8 \pm 0.5\%$ on the average. There is some indication that population 1 has less genetic variation than population 2, while population 3 is intermediate, although the differences between the populations are not statistically significant. Estimates of heterozygosity in the individual popula-

Table II. Allelic Frequencies (in *italics*) at 27 Variable Loci in Three Natural Populations of *Phoronopsis viridis* from Bodega Bay^a

Locus	Alleles	Locality			Total ^b
		1	2	3	
<i>Est-2</i>	Sample size	78	80	80	238
	99	<i>0.000</i>	<i>0.000</i>	<i>0.025</i>	<i>0.008</i>
	100	<i>1.000</i>	<i>1.000</i>	<i>0.975</i>	<i>0.992</i>
	Heterozygosity	0.000 (0.000)	0.000 (0.000)	0.050 (0.049)	0.017 (0.017)
<i>Est-3</i>	Sample size	78	80	80	238
	99	<i>0.064</i>	<i>0.125</i>	<i>0.038</i>	<i>0.076</i>
	100	<i>0.936</i>	<i>0.875</i>	<i>0.962</i>	<i>0.924</i>
	Heterozygosity	0.128 (0.120)	0.250 (0.219)	0.075 (0.072)	0.151 (0.140)
<i>Est-5</i>	Sample size	78	74	78	230
	98	<i>0.372</i>	<i>0.581</i>	<i>0.500</i>	<i>0.483</i>
	100	<i>0.423</i>	<i>0.284</i>	<i>0.474</i>	<i>0.396</i>
	102	<i>0.205</i>	<i>0.135</i>	<i>0.026</i>	<i>0.122</i>
	Heterozygosity	0.385 (0.641)	0.459 (0.564)	0.487 (0.524)	0.443 (0.596)
<i>Est-6</i>	Sample size	80	80	80	240
	97	<i>0.013</i>	<i>0.000</i>	<i>0.013</i>	<i>0.010</i>
	100	<i>0.987</i>	<i>0.975</i>	<i>0.975</i>	<i>0.979</i>
	103	<i>0.000</i>	<i>0.025</i>	<i>0.013</i>	<i>0.012</i>
	Heterozygosity	0.025 (0.025)	0.000 (0.049)	0.050 (0.049)	0.025 (0.041)
<i>Est-7</i>	Sample size	50	20	26	96
	98	<i>0.020</i>	<i>0.000</i>	<i>0.000</i>	<i>0.010</i>
	100	<i>0.980</i>	<i>1.000</i>	<i>1.000</i>	<i>0.990</i>
	Heterozygosity	0.040 (0.039)	0.000 (0.000)	0.000 (0.000)	0.021 (0.021)
<i>Lap-3</i>	Sample size	6	Not assayed	20	26
	98	<i>0.000</i>		<i>0.050</i>	<i>0.038</i>
	100	<i>1.000</i>		<i>0.950</i>	<i>0.962</i>
	Heterozygosity	0.000 (0.000)		0.100 (0.095)	0.077 (0.074)
<i>Lap-4</i>	Sample size	74	66	76	216
	97	<i>0.014</i>	<i>0.015</i>	<i>0.013</i>	<i>0.014</i>
	100	<i>0.986</i>	<i>0.985</i>	<i>0.987</i>	<i>0.986</i>
	Heterozygosity	0.027 (0.027)	0.030 (0.030)	0.026 (0.026)	0.028 (0.027)
<i>Lap-5</i>	Sample size	78	78	80	236
	98	<i>0.000</i>	<i>0.013</i>	<i>0.000</i>	<i>0.004</i>
	100	<i>0.577</i>	<i>0.474</i>	<i>0.600</i>	<i>0.551</i>
	102	<i>0.269</i>	<i>0.359</i>	<i>0.350</i>	<i>0.326</i>
	103	<i>0.154</i>	<i>0.154</i>	<i>0.050</i>	<i>0.119</i>
	Heterozygosity	0.359 (0.571)	0.744 (0.622)	0.525 (0.515)	0.542 (0.576)
<i>Acph-1</i>	Sample size	78	38	80	196
	100	<i>1.000</i>	<i>1.000</i>	<i>0.987</i>	<i>0.995</i>
	104	<i>0.000</i>	<i>0.000</i>	<i>0.013</i>	<i>0.005</i>
	Heterozygosity	0.000 (0.000)	0.000 (0.000)	0.025 (0.025)	0.010 (0.010)
<i>Acph-2</i>	Sample size	76	58	78	212
	96	<i>0.000</i>	<i>0.034</i>	<i>0.000</i>	<i>0.009</i>
	98	<i>0.026</i>	<i>0.000</i>	<i>0.154</i>	<i>0.066</i>
	100	<i>0.921</i>	<i>0.914</i>	<i>0.821</i>	<i>0.882</i>
	102	<i>0.013</i>	<i>0.000</i>	<i>0.026</i>	<i>0.014</i>
	104	<i>0.000</i>	<i>0.017</i>	<i>0.000</i>	<i>0.005</i>
	106	<i>0.039</i>	<i>0.034</i>	<i>0.000</i>	<i>0.024</i>
	Heterozygosity	0.105 (0.149)	0.172 (0.162)	0.205 (0.302)	0.160 (0.217)

Table II. Continued

Locus	Alleles	Locality			Total ^b
		1	2	3	
<i>Mdh</i>	Sample size	80	78	80	238
	94	0.000	0.013	0.013	0.008
	100	1.000	0.974	0.987	0.987
	106	0.000	0.013	0.000	0.004
	Heterozygosity	0.000 (0.000)	0.051 (0.050)	0.025 (0.025)	0.025 (0.025)
<i>αGpdh</i>	Sample size	72	70	80	220
	96	0.014	0.000	0.000	0.005
	100	0.986	1.000	1.000	0.995
	Heterozygosity	0.028 (0.027)	0.000 (0.000)	0.000 (0.000)	0.009 (0.009)
<i>G3pdh-1</i>	Sample size	46	50	80	176
	98	0.022	0.000	0.075	0.040
	100	0.957	0.940	0.875	0.915
	102	0.022	0.000	0.025	0.017
	104	0.000	0.000	0.025	0.011
	106	0.000	0.040	0.000	0.011
	110	0.000	0.020	0.000	0.006
	Heterozygosity	0.087 (0.084)	0.100 (0.114)	0.250 (0.228)	0.159 (0.161)
<i>G6pdh</i>	Sample size	80	80	70	230
	97	0.038	0.038	0.057	0.043
	100	0.962	0.825	0.914	0.900
	103	0.000	0.137	0.029	0.057
	Heterozygosity	0.075 (0.072)	0.200 (0.299)	0.114 (0.160)	0.130 (0.185)
<i>Fum</i>	Sample size	80	80	52	212
	100	0.975	1.000	0.981	0.986
	108	0.025	0.000	0.019	0.014
	Heterozygosity	0.050 (0.049)	0.000 (0.000)	0.038 (0.038)	0.028 (0.028)
<i>Idh</i>	Sample size	80	80	80	240
	100	0.987	0.987	1.000	0.992
	104	0.013	0.013	0.000	0.008
	Heterozygosity	0.025 (0.025)	0.025 (0.025)	0.000 (0.000)	0.017 (0.017)
<i>Odh-1</i>	Sample size	80	80	80	240
	100	1.000	1.000	0.975	0.992
	106	0.000	0.000	0.025	0.008
	Heterozygosity	0.000 (0.000)	0.000 (0.000)	0.050 (0.049)	0.017 (0.017)
<i>Me-2</i>	Sample size	80	80	80	240
	100	0.975	0.987	0.975	0.979
	104	0.025	0.013	0.025	0.021
	Heterozygosity	0.050 (0.049)	0.025 (0.025)	0.050 (0.049)	0.042 (0.041)
<i>Me-3</i>	Sample size	58	52	66	176
	98	0.052	0.000	0.000	0.017
	100	0.707	0.808	0.939	0.824
	105	0.241	0.192	0.061	0.159
	Heterozygosity	0.172 (0.439)	0.077 (0.311)	0.121 (0.114)	0.125 (0.296)

Table II. Continued

Locus	Alleles	Locality			Total ^b
		1	2	3	
<i>Tpi-1</i>	Sample size	12	6	10	28
	100	1.000	1.000	0.800	0.929
	110	0.000	0.000	0.200	0.071
	Heterozygosity	0.000 (0.000)	0.000 (0.000)	0.000 (0.320)	0.000 (0.133)
<i>Tpi-2</i>	Sample size	80	78	80	238
	92	0.000	0.026	0.000	0.008
	95	0.000	0.013	0.000	0.004
	100	1.000	0.923	0.963	0.962
	106	0.000	0.026	0.012	0.013
	110	0.000	0.013	0.025	0.013
	Heterozygosity	0.000 (0.000)	0.154 (0.146)	0.075 (0.073)	0.076 (0.074)
<i>Pgm-1</i>	Sample size	80	66	80	226
	95	0.112	0.242	0.137	0.159
	100	0.875	0.727	0.863	0.827
	105	0.013	0.030	0.000	0.013
	Heterozygosity	0.200 (0.222)	0.303 (0.411)	0.175 (0.237)	0.221 (0.290)
<i>Pgm-3</i>	Sample size	80	78	80	238
	96	0.025	0.038	0.050	0.038
	100	0.937	0.833	0.850	0.874
	103	0.013	0.115	0.087	0.071
	105	0.025	0.013	0.013	0.017
	Heterozygosity	0.125 (0.112)	0.231 (0.291)	0.200 (0.267)	0.185 (0.229)
<i>Pgi</i>	Sample size	42	80	80	202
	100	1.000	1.000	0.988	0.995
	108	0.000	0.000	0.013	0.005
	Heterozygosity	0.000 (0.000)	0.000 (0.000)	0.025 (0.025)	0.010 (0.010)
<i>Adk-1</i>	Sample size	14	36	56	106
	96	0.357	0.528	0.464	0.472
	100	0.643	0.472	0.536	0.528
	Heterozygosity	0.429 (0.459)	0.139 (0.498)	0.286 (0.497)	0.224 (0.496)
<i>Hk-1</i>	Sample size	80	78	80	238
	100	0.987	1.000	1.000	0.996
	110	0.013	0.000	0.000	0.004
	Heterozygosity	0.025 (0.025)	0.000 (0.000)	0.000 (0.000)	0.008 (0.008)
<i>Hk-2</i>	Sample size	80	28	76	184
	98	0.000	0.000	0.013	0.005
	100	0.975	1.000	0.974	0.978
	102	0.025	0.000	0.013	0.016
	Heterozygosity	0.050 (0.049)	0.000 (0.000)	0.051 (0.052)	0.043 (0.043)

^a The observed (and expected, in parentheses) frequencies of heterozygous individuals are given for each locus. The sample size is twice the number of individuals sampled.

^b The "total" allelic frequencies are those observed by pooling the data for the three localities. The expected frequencies of heterozygotes for the "total" sample are calculated according to the Hardy-Weinberg expectations from the "total" allelic frequencies.

Table III. Summary of Genetic Variation in Three Natural Populations of *Phoronopsis viridis*^a

Locality	Number of loci studied	Number of genes sampled per locus	Number of alleles per locus	Percent polymorphic loci ^b		Percent heterozygous loci per individual ^c	$\bar{F}^d$
				(1)	(2)		
1	39	66.7 ± 3.6	1.72 ± 0.14	20.5	46.2	8.0 ± 2.7	0.26 ± 0.09
2	38	63.6 ± 3.5	1.79 ± 0.18	28.9	42.1	9.8 ± 2.9	0.19 ± 0.12
3	39	70.6 ± 2.8	1.87 ± 0.14	28.2	56.4	8.5 ± 2.4	0.18 ± 0.08
Average	38.7	67.0 ± 2.0	1.79 ± 0.04	25.9 ± 2.7	48.2 ± 4.3	8.8 ± 0.5	0.21 ± 0.02
Total ^e	39	197.2 ± 9.0	2.23 ± 0.21	28.2	48.7	9.4 ± 2.5	0.26 ± 0.06

^a The standard errors of each estimate are given where appropriate.

^b A locus is considered polymorphic when the frequency of the most common allele is (1) ≤ 0.95 ; (2) ≤ 0.99 .

^c Expected frequencies calculated on the assumption of Hardy-Weinberg equilibrium. Only 34 loci with sample size greater than 20 in all three localities have been used to calculate the expected frequencies of heterozygotes in each indicated population, or their average. All 39 loci are included in the "Total."

^d Calculated as the average value for seven loci (*Est-5*, *Lap-5*, *Acph-2*, *Me-3*, *Pgm-1*, *Pgm-3*, *Adk-1*) with an expected frequency of heterozygotes of at least 10% in all three localities.

^e Includes the three samples and treats them as if all three came from one single population.

tions are calculated using only the 34 loci for which the sample size is 20 or greater in each population. The use of very small samples to estimate the heterozygosities may introduce a bias. However, if all 39 loci are used, the expected average frequency of heterozygous loci per individual is $9.3 \pm 1.0\%$. This value is very similar to the expected heterozygosity calculated for the pooled data (9.4%), which also includes all 39 loci.

It can be seen in Table II that at several loci, particularly the most polymorphic ones, the observed frequency of heterozygous individuals is less than the frequency expected on the assumption of Hardy-Weinberg equilibrium. For the pooled data, the observed frequency of heterozygous loci per individual is $7.1 \pm 1.9\%$, while the expected frequency is $9.4 \pm 2.5\%$. Such a deficiency of heterozygotes could conceivably be due to the Wahlund effect, i.e., to the pooling of data from several Mendelian populations with different allelic frequencies. However, this is not so for our data, at least for the most part. The observed average frequencies of heterozygous loci per individual (using only the 34 loci with sample size ≥ 20 in each population) for populations 1, 2, and 3 are, respectively, $5.8 \pm 1.6\%$, $8.3 \pm 2.8\%$, and $7.7 \pm 2.2\%$. In every case, these values are lower than the expected heterozygosities as given in Table III. The average observed heterozygosity is $7.3 \pm 0.7\%$ vs. the $8.8 \pm 0.5\%$ expected heterozygosity (see Table III).

At least five conceivable hypotheses may account for the observed defi-

ency of heterozygotes. First, it might be that within each individual sample we have collected individuals from two or more Mendelian populations with different allelic frequencies. This seems unlikely, because all six subsamples were collected within a small area with high density of phoronids. In each locality the two farthest subsamples were separated from each other by only 2.24 m, and most pairs of subsamples were separated by only 1 m.

The second possibility is that we might have sampled more than one species of phoronids. This hypothesis is unlikely. First we note that the three local populations have similar allelic frequencies at every locus, and also that the excess of homozygotes persists after treating each locality separately. Secondly, if we had sampled two (or more) species with different genetic constitutions, it should be possible to separate all individuals within each sample into two (or more) different classes according to their genotypes. Individuals homozygous for less frequent alleles at a given locus should have less common genotypes also at other loci at which an excess of homozygotes exists. Examination of the genotype of every individual over all polymorphic loci does not give any evidence of the existence of two species with different genetic frequencies.

The third hypothesis is that, at some loci at least, homozygotes have higher fitness than heterozygotes. This hypothesis fails to account for the substantial amount of genetic variability found in *P. viridis*. The excess of observed over expected homozygotes occurs precisely at the most polymorphic loci. If natural selection favored homozygotes over heterozygotes and fitnesses remained constant, these loci would become fixed for one or another allele.

The fourth hypothesis assumes that "null" alleles exist and that some genotypes scored as homozygotes are instead heterozygous for the null and some other allele. While we cannot completely exclude this possibility, it seems unlikely that it would account for the large deficiency of heterozygotes. For that, "null" alleles would need to occur in high frequencies at most or all polymorphic loci in *P. viridis*. Moreover, no homozygotes for "null" alleles have been detected—that is, individuals with no activity at all—in any zymogram.

The fifth, and most likely, hypothesis is that the populations of *P. viridis* are somewhat inbred. As a measure of inbreeding, we have used Wright's *fixation index*, F (Wright, 1951; Li, 1955), estimated as follows:

$$F = \frac{4PQ - H^2}{(2P + H)(2Q + H)} \quad (1)$$

where P and Q are the observed frequencies of the two homozygous types and H is the frequency of heterozygotes. To calculate F for a given locus, we

have designated the frequency of homozygotes for the most common allele as P , the frequency of heterozygotes for that allele as H , and the frequency of all other genotypes as Q . We have calculated F for each of the following seven loci: *Est-5*, *Lap-5*, *Acp-2*, *Me-3*, *Pgm-1*, *Pgm-3*, and *Adk-1*. These are the only loci for each of which the expected frequency of heterozygotes is no smaller than 0.10 in each locality. The values of F calculated as averages over the seven loci are given in Table III for each locality. The average F over the three localities is 0.21 ± 0.02 . There is statistically significant evidence of inbreeding in these populations of *P. viridis*. The average fixation index, F , calculated for the pooled data is 0.26 ± 0.06 . The fact that this value is greater (although not significantly greater) than the average for the three populations, suggests that the three populations have somewhat different gene pools.

To measure the degree of genetic similarity between the populations, we have used the statistic I , defined as follows (Nei, 1972; Ayala, 1974):

$$I = I_{xy}/(I_x \cdot I_y)^{1/2} \quad (2)$$

where I_{xy} , I_x , and I_y are the arithmetic means, over all loci, of $\Sigma x_i y_i$, Σx_i^2 , and Σy_i^2 , respectively, with x_i and y_i being the frequencies of the i th allele in two given populations, X and Y, respectively.

The average genetic distance between two populations is calculated as

$$D = -\log_e I \quad (3)$$

The average genetic similarities (and genetic distances, in parentheses) between the populations are 0.9964 (0.0036), 0.9954 (0.0046), and 0.9952 (0.0048) between populations 1 and 2, 1 and 3, and 2 and 3, respectively. The average genetic similarity for all three pairwise comparisons is $99.57 \pm 0.04\%$; the average genetic distance is $0.43 \pm 0.04\%$. Little genetic differentiation exists between the populations. This is hardly surprising considering their geographic proximity. The average genetic distance between some 40 populations of *Drosophila willistoni* sampled through Central America, the Caribbean islands, and the northern half of South America is $1.5 \pm 0.6\%$ (Ayala, 1974). Similar amounts of genetic differentiation are found in other *Drosophila* species. The average genetic differentiation between Californian populations of the salamander *Taricha* is $2.9 \pm 0.9\%$, and between human populations is $1.3 \pm 0.3\%$ (Ayala, 1974).

DISCUSSION

The Phoronida are a coelomate phylum of low diversity at present, with two genera and about 12–15 described species living in shallow marine waters. As phoronids are unskeletonized, their fossil record is poor and their

time of origin obscure. It seems possible that they antedate the Cambrian, and they may well be represented by fossil burrows (such as the trace fossil *Skolithus*) in late Precambrian rocks, although we cannot be certain of this. Phoronids are believed to be closely allied to the other extant lophophorate coelomate phyla, the Brachiopoda and Ectoprocta (see, for example, Hyman, 1959; Zimmer, 1967). In fact, phoronids may well represent the common ancestral stock of the living lophophorates (Valentine, 1973; Farmer *et al.*, 1973), and it has been suggested that they are the most primitive living deuterostomes (Zimmer, 1964). It is partly because of the uniqueness of this small phylum and its possible great phylogenetic importance that we have chosen it for study. There are as yet too few data to assess the possibilities of a phylogenetic effect on genetic variation, however.

The inbreeding in *P. viridis* is an unexpected finding. Phoronids have a very characteristic and famous pelagic larval stage, the actinotroch larva, which often lasts for weeks. Indeed, the pelagic larval stages have in general been better known than the benthic adults until recently, and larval types are still known for which benthic adults have not as yet been discovered. Silén (1954) classed living phoronids into three types, which have been expanded to four by Marsden (1959), and these types appear to differ by characteristic larval durations, among other features. The northwest American species of *Phoronopsis* belong to a type that seems to have a relatively long larval stage, lasting perhaps 2 or more weeks, although precise data on the length of larval life in *P. viridis* are not available. Now the waters of Bodega Harbor are nearly all flushed to sea on low tides. Therefore, it is necessary to account for inbreeding within a life cycle that must include the removal of young from Bodega Harbor into the open ocean and the reintroduction of larvae later. The introduced larvae must presumably include many siblings, though they need not be descended from Bodega Harbor ancestors. Perhaps swarms of sibling larvae tend to remain together and to have similar settling behaviors, leading them to congregate for metamorphosis. Perhaps, also, asexual reproduction occurs occasionally among *P. viridis* populations (see Johnson, 1967), creating numbers of genetically identical individuals that on spawning produce inbred offspring. We did not, however, find any two or more individuals that were the products of asexual reproduction since no two individuals had identical genotypes at every locus studied. At any rate, our findings suggest that the reproductive and larval behaviour of *P. viridis* could be studied further with profit.

As for the adaptive significance of the levels of genetic variation that we have found, it may relate to the strategy of adaptation in an environment of intermediate stability. *Phoronopsis viridis* lies within the middle range of genetic variability as it is now known for marine invertebrates. The highest variability yet found occurs in a tropical reef population from Eniwetok

(*Tridacna maxima*), which has an average heterozygosity of about 20.2% (Ayala *et al.*, 1973). The lowest variability recorded is in a shallow subtidal brachiopod population from Antarctica (*Liothyrella notorcadensis*), which has an average heterozygosity of about 3.9% (Ayala *et al.*, 1974). The average heterozygosity of *P. viridis* is about 9%. Thus *P. viridis* is intermediate climatically and in genetic variability. Another temperate invertebrate in which genetic variability has been studied adequately is the xyphosuran *Limulus polyphemus* from the northwestern Atlantic (Selander *et al.*, 1970). *L. polyphemus* has an average heterozygosity of about 6.7%, perhaps on the low side of the intermediate range.

Data on genetic variability in all animals have been reviewed by Selander and Kaufman (1973). They indicate that vertebrates tend to be less variable than invertebrates, perhaps by half. Selander and Kaufman suggest that the larger, more mobile vertebrates tend to perceive their environments as relatively "fine-grained" (see Levins, 1968) and therefore require relatively little genetic variability to supplement their broad homeostasis. To smaller, less motile invertebrates, however, the environment appears to be more "coarse-grained," and they require larger genetic variability to equip them for the variety of environmental states that they perceive. Ayala *et al.* (1974) have employed this sort of argument to explain a possible latitudinal trend in genetic variability. Populations in the tropics, it is suggested, are able to perceive their environments as relatively coarse-grained because the stability of trophic resources permits specialization as to microhabitats and to restricted food sources. To highly specialized populations, the environment appears patchy. In high latitudes, by contrast, resource fluctuations are great and the populations are forced to be flexibly adapted and to perceive the environment as fine-grained (Levins, 1968; Valentine, 1971). Thus tropical populations might have large genetic variability, so that individuals can be specialists, while in high latitudes the entire population might be flexible and have low genetic variability.

The finding of intermediate variability in *P. viridis* from temperate shallow water fits this model very well. *Limulus polyphemus*, the other temperate marine population for which genetic variability has been estimated is somewhat less variable than *P. viridis*. This is to be expected from the reasoning of Selander and Kaufman (1973). That is, *Limulus* is larger and more motile than *Phoronopsis* and should perceive environments as finer-grained and be less genetically variable, other things being equal.

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