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GENETIC DIFFERENTIATION DESPITE TELEPLANIC LARVAL DISPERSAL: ALLOZYME VARIATION IN SIPUNCULANS OF THE *APIONSOMA MISAKIANUM* SPECIES-COMPLEX

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ABSTRACT

We examined the genetic evidence for dispersal in the sipunculan *Apionsoma misakianum* (Ikeda 1904) by analysis of allozymes (using a total of nine polymorphic loci) in larval and adult samples. Genotypes of adults reared from late-stage larvae collected from the Florida Current were compared with genotypes of adults from three locations: eastern central Florida, the Florida Keys and the Bahamas. Genetic differences were found between individuals from the southern localities and the majority of specimens from the northern locality. The multilocus genotypes of the specimens reared from larvae of the Florida Current were characteristic of specimens from the Florida Keys and the Bahamas. Despite evidence for larval influx northward into the central-Florida area via the Florida Current, the southern genotypes were present in only a few adults collected (4.5%) at a northern site, with no indication of "hybrids" occurring between northern and southern genotype based on allozymes (i.e., heterozygotes). Furthermore, genetic separation of the northern and southern genotypes coincides with a faunal boundary noted for other species.

The impact of long-lived, planktotrophic larvae on dispersal and speciation of marine invertebrates (Crisp, 1978; Jablonski and Lutz, 1983; Scheltema, 1971, 1975, 1978, 1986; Shuto, 1974) has been a central question in the field of larval ecology since Thorson (1950). Many studies have inferred that the genetic homogeneity within a marine species resulted from high levels of larval transport over large geographic distances (e.g., Campton et al., 1992; Johnson and Black, 1982; 1984ab; Nash et al., 1988; Nishida and Lucas, 1988; Watts et al., 1990). A few studies of invertebrates have attributed homogeneous or heterogeneous genetic patterns over a geographic range to long- or short-dispersing larvae within each species to a greater (McMillan et al., 1992) or lesser degree (Hellberg, 1996). To date, however, few data examine the direct effect of teleplanic (far-wandering) larvae on the phylogeography within a marine species.

Sipunculan worms exhibit a great variety of developmental types that might be used to test directly the effects of differential larval dispersal. These types include: direct development without a pelagic stage, short-duration pelagic development (which in one species is parthenogenic), or long-duration pelagic development (produced by strictly outcrossing species) which may include teleplanic larvae (cf Rice, 1981). Teleplanic larvae of sipunculans are abundant across entire ocean basins (Scheltema, 1975; Scheltema and Rice, 1990). However, teleplanic larvae alone may not prevent genetic isolation and speciation from occurring in marine invertebrates. The ultimate effect of variation among these developmental types on dispersal can be addressed only through detailed genetic studies of these species. Previous population-genetic research on sipunculans has been limited to examination of variation within a single population (Balakirev and Manchenko, 1983).

One species of sipunculan, *Apionsoma misakianum* (Ikeda 1904), is reported from both the Atlantic and Pacific Oceans (Cutler, 1979; Cutler and Cutler, 1980), although the

species is not abundant at every location. In the Atlantic, this species is reported in the Bahamas (Cutler and Cutler, 1980), the Gulf of Mexico (Murina, 1967), east coast of Florida as far north as Sebastian (Rice, 1986) and from the Mediterranean (Murina 1964). A teleplanic larval stage, identified as *A. misakianum* (Rice, 1978), has been collected in all of the major currents of the Atlantic Ocean (Scheltema, 1975). The adults are particularly well-studied along the central east coast of Florida, and their larvae have been reported as common constituents of the north-flowing Florida Current, a component of the Gulf Stream (Rice, 1978, 1981, 1986).

Northward transport of large numbers of teleplanic larvae from the Florida Keys and Caribbean could provide recruitment to more northerly populations of benthic adults. Although adults collected near central Florida and adults reared from larvae collected in the Florida Current were morphologically indistinguishable, early development of laboratory spawning from the two groups was found to differ in egg length, position of the first meiotic metaphase spindle, gut pigmentation of the trochophore, and length of time from fertilization to the pelagosphera stage (Rice, 1981). The length of developmental period is not precisely known for these worms, as culture through the complete life cycle has not yet been possible in the laboratory. After 2 mo, neither pure-bred nor hybrid crosses between the two types progressed beyond early developmental phases (Rice, unpubl. data). These differences in early development led us to an investigation of the genetic variation in adults and larvae of *Apionsoma misakianum* in the Florida Current.

Materials and Methods

Collection of Adults and Culture of Larvae.—Adults of *Apionsoma misakianum* were collected from the Sebastian Pinnacles off east-central Florida; Pickles Reef, Key Largo, Florida; and Chub Cay, Bahamas (Fig. 1; Table 1). Adults of this species are small (approximately 1–2 mm in diameter and <4 mm long when contracted) and burrow in scleractinid coral rubble (e.g., *Oculina* spp., *Porites* spp.). Pieces of rubble were collected errantly by SCUBA divers from submerged reefs of the Florida Keys and Bahamas or by dredge at the Sebastian Pinnacles. Living adults were extracted individually from random coral pieces, then frozen at −80°C in 20 µL of grinding buffer (10 mM Tris, 1 mM EDTA, and 0.1% (v/v) Tween 80) for subsequent homogenization for allozyme electrophoresis.

Pelagosphera larvae, identified as the "Type C" larvae of *A. misakianum* described by Hall and Scheltema (1975), were collected on two dates within 10 d from sites in the Gulf Stream off Ft. Pierce, Florida (Fig. 1, Table 1), with a 202-mm mesh plankton net (0.75 m diam opening; 2.25 m length). Larvae were then divided into three groups (GSL1-3) as part of another study on delay of metamorphosis. Metamorphosis was induced (Rice, 1986) by transfer of larvae to containers with organic sediment (dredged from areas near the larval collections) and seawater previously exposed to adults collected from the Sebastian Pinnacles locality. In order to increase tissue for allozyme analysis, juveniles were reared on the organic sediment for approximately 16 mo at 22°C until they attained maturity. These adults, morphologically diagnosed as *A. misakianum*, were stored at −80°C as described above.

Extracts of whole worms were prepared and subsequently allozymes analyzed by starch-gel electrophoresis using standard methods (e.g., Richardson et al., 1986; Murphy et al., 1990). Enzyme systems screened during this study are listed in Table 2; names and abbreviations for enzymes are those of Murphy et al. (1990). All scorable enzymes gave optimal results on Tris-citrate buffer (pH 8.0). Multiple isozymic loci were numbered sequentially with "1" representing the fastest migrating system. Alleles were designated with letters; "a" represents the fastest anodally migrating allele within a system. There were no electromorphs that migrated cathodally in this study.

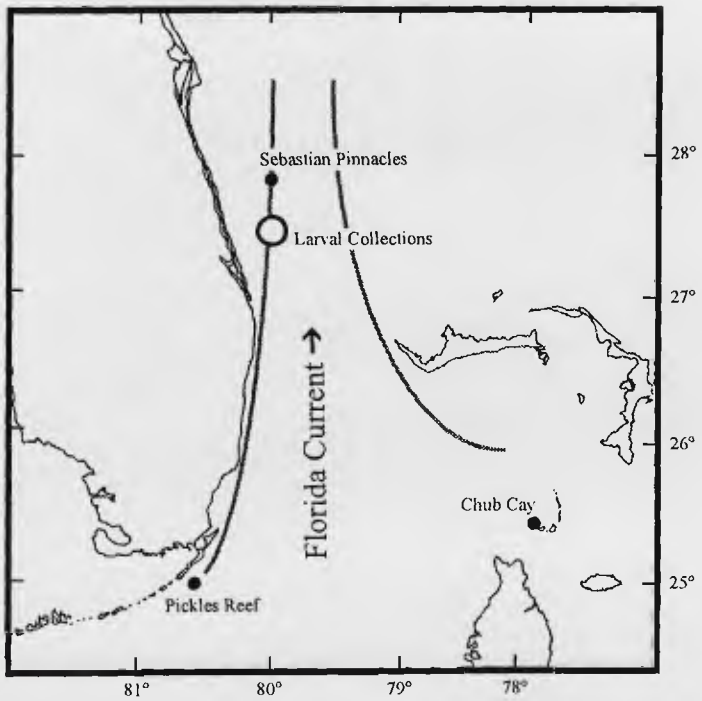

Figure 1. *Apionsoma misakianum*: Sampling locations of adults and larvae. Black dots are locations of adults sampled; open circle is location of larval collections. Dates and precise locality information for samples are listed in Table 1.

Table 1. *Apionsoma misakianum*: data for the number of individuals (n) from each locality and the date of collection.

Locality Name	n	Position		Depth	Date
Adult collections					
Sebastian Pinnacles	302	27° 49.23′N	79° 57.66′W	80 m	24 Feb. 1992
Pickles Reef	44	24° 59.20′N	80° 24.90′W	3.5 m	9 Mar. 1993
Chub Cay, Berry Is., Bahamas	36	25° 24.50′N	77° 55.00′W	4.5 m	13 May 1993
Larval collections					
GSL 1—three transects	228	27° 27.54′N	79° 57.44′W to		
		27° 27.64′N	79° 57.24′W	surface	31 Dec. 1991
		27° 27.70′N	79° 57.2′W to		
		27° 27.84′N	79° 56.97′W	surface	31 Dec. 1991
		27° 28.18′N	79° 57.36′W to		
		27° 28.36′N	79° 57.04′W	0–70 m	31 Dec. 1991
GSL 2—two transects	131	27° 28.44′N	79° 54.05′W to		
		27° 29.60′N	79° 53.60′W	0–70 m	9 Jan. 1992
		27° 30.02′N	79° 53.51′W to		
		27° 30.93′N	79° 53.71′W	0–70 m	9 Jan. 1992
GSL 3	92	collection data same as for GSL 1			

Table 2. *Apionsoma misakianum*: Enzymes examined in this study.

	E. C. no.	Symbol[a]	No. of loci[b]
Acid phosphatase	3.1.3.2	*Acp*	1
Alanopine dehydrogenase	1.5.1.17	*Alpdh*	1
Alkaline phosphatase	3.1.3.1	*Alp*	3 [c]
Arginine kinase	2.7.3.3	*Ark*	1
Aspartate aminotransferase	2.6.1.1	*Aat*	1 [c]
Esterase (α-naphthyl acetate substrate)	3.1.1. -	*Est*	2 [c]
Fructose bisphosphate aldolase	4.1.2.13	*Fba*	1
α-galactosidase	3.2.1.22	*α-Gal*	1
β-galactosidase	3.2.1.23	*β-Gal*	1
Glucose-6-phosphate isomerase	5.3.1.9	*Gpi*	1 [c]
l-Iditol dehydrogenase	1.1.1.14	*Iddh*	1
Isocitrate dehydrogenase	1.1.1.42	*Idh*	2
l-Lactate dehydrogenase	1.1.1.27	*Ldh*	1
Malate dehydrogenase	1.1.1.37	*Mdh*	2
α-mannosidase	3.2.1.24	*α-Man*	1
Peptidase (specific to Leu-Ala)	3.4.13.11	*Pep-LA*	3 [c]
Peptidase (specific to Leu-Gly-Gly)	3.4.11.4	*Pep-LGG*	2 [c]
Phosphoglucomutase	5.4.2.2	*Pgm*	1 [c]
Superoxide dismutase	1.15.1.1	*Sod*	1

[a]Genetic abbreviation used in Table 2.
[b]Number of total isozymic loci resolved.
[c]Systems with polymorphic loci

Enzyme systems were screened using adults collected at Sebastian Pinnacles to identify polymorphic loci. Given their small adult size, only one slab gel (1.3-cm thick) could be run for each individual. Seven replicates could be sliced from each run to analyze a total of seven enzyme systems for each individual. Given this constraint, seventeen loci that were monomorphic for adults from the Sebastian Pinnacles were not studied further. Ten polymorphic loci (in seven enzyme systems) were resolvable for adult specimens from Sebastian Pinnacles; one (*Alp*) was inconsistently scorable and was omitted from the final data analysis. The remaining nine polymorphic loci were analyzed for 117 field-collected adults (36 from Chub Cay, 44 from Pickles Reef and 37 from Sebastian Pinnacles) and 76 adults reared from larvae. Statistics (allele frequencies and observed and expected heterozygosities) were calculated using standard formulae (Murphy et al., 1990). Genetic distances were calculated by the unbiased method of Nei (1978), and variances were calculated by the approximate formulae of Nei (1987).

A principal components analysis (PCA) was performed on alleles from polymorphic loci as described by Liu et al. (1991). PCA is a multivariate data reduction tool that allows genetic patterns to be examined from the perspective of the individual. While measures of population subdivision are traditionally calculated using Wright's F-statistics or Nei's G-statistics, these methodologies require assumptions (random breeding individuals within subpopulations, no selection or genetic drift occurring, etc.) that we could not test for our system. It would be artificial to treat the larval pool as a population for genetic comparison to the adult populations in any of these methods as there is no a priori reason to assume that they are propagules from a single, randomly interbreeding population (two important assumptions required to be met); therefore, we limited our treatment to PCA of the multi-allelic data for individuals.

An UPGMA dendrogram was constructed from genetic distances D (Nei, 1978) according to procedures outlined by Sneath and Sokal (1973). All statistics were calculated using either SAS programming (SAS Institute Inc., 1989, 1992) or the GeneStrut genetics analysis package (Constantine et al., 1994).

Table 3. *Apionsoma misakianum*: Survivorship data from larval settlement groups.

Culture ID	No. of Larvae Set (Date)	No. of metamorphs (Date)	No. of Adults (Date)
GSL1	228 (23 Jan 1992)	120 (11 Feb 1992)	53 (7 May 1993)
GSL2	131 (23 Jan 1992)	106 (11 Feb 1992)	47 (11 Feb 1993)
GSL3	92 (12 Feb 1992)	27 (20 Feb 1992)	7 (20 Feb 1993)

Results

Initial metamorphic success of larvae in culture ranged from 80.9 to 29.3%, but survivorship to adult size was 35.9 to 7.6% (Table 3). Heaviest mortality occurred in GSL 3, which was a subset of the same larval collection as GSL 1 but differed only in onset of metamorphosis by 1 mo.

Allele frequencies for the nine polymorphic loci which were used in the principal components analysis (PCA) and distance calculations are reported in Table 4. The disparity in overall observed and theoretical expected heterozygosities (H_{OBS} and H_{EXP}) arises from rare alleles within populations. In the case of the Sebastian Pinnacles population, all rare alleles for *Pep-LGG-2* and *Pep-LA-2* occurred in two individuals that appear reproductively isolated from the remainder of the population. A few rare alleles also occurred in the reared larvae that were not seen in the adult populations. After pooling rare alleles within each sample into a single category within each locus, no loci were significantly different from Hardy-Weinberg expected frequencies (χ^2 test, $\alpha = 0.05$), although smaller sample sizes limit potential for deviation from Hardy-Weinberg and discovery of rare alleles. The variation in number of individuals for each locus (n) arises from data omitted for individuals possessing ambiguous or uninterpretable allozyme profiles for each locus.

PCA demonstrates that there are two distinct genetic entities based on the 44 combined alleles of the polymorphic loci (Fig. 2). The first and second PCs explain 12.1% and 5.7% of the variation in the data set, respectively, with each subsequent PC explaining ≤5.5% of the remaining variation. The left cluster represents the "tropical" genotype which was found in all adults from Chub Cay, Bahamas, and Pickles Reef, Florida Keys, and all adults reared from larvae collected in the Florida current just south of Sebastian Pinnacles, the temperate site (Fig. 1). Adults collected from the Sebastian Pinnacles displayed genotypes of the "temperate" type, except two that displayed genotypes in the "tropical" cluster (these two individuals overlap completely as a single point in Fig. 2). Any "hybrids" (i.e., heterozygotes) between the two types would appear as points scattered between the two clusters. The primary loci contributing to the first principal component were (in order of descending weight): *Pep-LGG-2*, *Pep-LA-2*, *Est-2*, *Pgm*, and *Gpi*. Additional individuals which were run in the preliminary screening process did demonstrate tropical allele types, but were not scored for all alleles used in the overall PCA and, and therefore could not be included. A total of five out of 110 adults (4.5%) from Sebastian Pinnacles screened for relevant loci (inclusive of those in the PCA) possessed "tropical-" genotype alleles. No "tropical" types present in the "temperate" locality showed any signs of intermediate heterozygotes between "tropical" and "temperate" genotypes.

Table 4. *Apionsoma misakianum*: Allele frequencies, expected (H_{EXP}) and observed (H_{OBS}) heterozygosities, and numbers of individuals (n) sampled for the six groups (see Table 1).

Locus		Adults			Larvae		
		Chub Cay	Pickles Reef	Sebastian Pinnacles	GSL 1	GSL 2	GSL 3
Aat	a	0.00	0.00	0.00	0.02	0.00	0.00
	b	0.97	0.99	1.00	0.91	1.00	1.00
	c	0.03	0.01	0.00	0.07	0.00	0.00
	H_{EXP}	0.05	0.02	0.00	0.17	0.00	0.00
	H_{OBS}	0.06	0.02	0.00	0.18	0.00	0.00
	n	36	44	37	33	32	7
Est-1	a	0.11	0.10	0.08	0.03	0.17	0.00
	b	0.25	0.23	0.40	0.27	0.22	0.21
	c	0.36	0.23	0.32	0.30	0.30	0.14
	d	0.26	0.43	0.19	0.32	0.31	0.64
	e	0.02	0.01	0.01	0.08	0.00	0.00
	H_{EXP}	0.74	0.71	0.71	0.74	0.75	0.56
	H_{OBS}	0.63	0.43	0.38	0.70	0.56	0.71
	n	32	44	34	30	32	7
Est-2	a	0.03	0.10	0.11	0.06	0.16	0.14
	b	0.00	0.00	0.00	0.00	0.00	0.07
	c	0.68	0.43	0.15	0.44	0.56	0.57
	d	0.18	0.42	0.32	0.38	0.26	0.22
	e	0.11	0.05	0.42	0.12	0.02	0.00
	H_{EXP}	0.50	0.63	0.70	0.66	0.60	0.65
	H_{OBS}	0.45	0.53	0.39	0.55	0.58	0.57
	n	31	43	31	33	31	7
Gpi	a	0.00	0.00	0.04	0.00	0.02	0.00
	b	0.01	0.05	0.19	0.01	0.08	0.00
	c	0.64	0.72	0.42	0.82	0.65	0.64
	d	0.34	0.18	0.15	0.16	0.14	0.00
	e	0.01	0.04	0.12	0.01	0.09	0.36
	f	0.00	0.01	0.08	0.00	0.02	0.00
	H_{EXP}	0.49	0.46	0.75	0.31	0.54	0.49
	H_{OBS}	0.50	0.43	0.59	0.25	0.50	0.14
	n	36	44	37	36	32	7
Pep-LA-2	a	0.01	0.00	0.92	0.11	0.03	0.00
	b	0.00	0.00	0.00	0.00	0.02	0.14
	c	0.92	0.98	0.08	0.89	0.93	0.57
	d	0.07	0.02	0.00	0.00	0.02	0.29
	H_{EXP}	0.16	0.05	0.15	0.20	0.12	0.61
	H_{OBS}	0.11	0.00	0.05	0.17	0.06	0.00
	n	36	42	37	36	32	7

Genetic distance (D) was calculated and populations clustered by UPGMA (Sneath and Sokal, 1973) for comparison with the PCA and other genetic studies. The UPGMA dendrogram again reveals two distinct genetic clusters: the adults at Sebastian Pinnacles and the adults and larvae with "tropical" genotypes (Fig. 3). An apparent divergence of

Table 4. Continued.

Locus		Adults			Larvae		
		Chub Cay	Pickles Reef	Sebastian Pinnacles	GSL 1	GSL 2	GSL 3
Pep-LA-3	a	0.00	0.02	0.00	0.00	0.00	0.00
	b	0.00	0.00	0.03	0.00	0.00	0.00
	c	0.39	0.16	0.35	0.15	0.12	0.00
	d	0.61	0.73	0.58	0.69	0.72	0.86
	e	0.00	0.09	0.04	0.16	0.16	0.14
	H_{EXP}	0.48	0.44	0.54	0.49	0.45	0.26
	H_{OBS}	0.28	0.16	0.19	0.26	0.13	0.00
	n	32	44	37	27	32	7
Pep-LGG-1	a	0.00	0.00	0.00	0.00	0.06	0.00
	b	0.04	0.18	0.05	0.06	0.16	0.00
	c	0.33	0.63	0.14	0.45	0.47	0.85
	d	0.60	0.15	0.35	0.31	0.28	0.14
	e	0.03	0.00	0.46	0.02	0.03	0.00
	f	0.00	0.04	0.00	0.16	0.00	0.00
	H_{EXP}	0.54	0.56	0.65	0.68	0.68	0.26
	H_{OBS}	0.20	0.15	0.16	0.32	0.06	0.00
	n	35	39	37	31	32	7
Pep-LGG-2	a	0.00	0.00	0.95	0.00	0.00	0.00
	b	0.87	0.90	0.05	0.83	0.84	0.64
	c	0.13	0.10	0.00	0.17	0.14	0.36
	d	0.00	0.00	0.00	0.00	0.02	0.00
	H_{EXP}	0.23	0.19	0.10	0.29	0.27	0.49
	H_{OBS}	0.09	0.07	0.00	0.11	0.19	0.42
	n	35	43	37	35	32	7
Pgm	a	0.00	0.00	0.02	0.00	0.00	0.00
	b	0.00	0.09	0.24	0.02	0.00	0.00
	c	0.40	0.38	0.44	0.56	0.33	0.28
	d	0.50	0.36	0.27	0.30	0.45	0.28
	e	0.07	0.11	0.03	0.06	0.14	0.37
	f	0.03	0.06	0.00	0.06	0.08	0.07
	H_{EXP}	0.59	0.71	0.68	0.60	0.67	0.76
	H_{OBS}	0.66	0.70	0.73	0.42	0.47	0.43
	n	35	42	33	33	32	7

GSL 3 from other larval samples is due in large part to the presence of a single esterase allele (*Est-2b*) in one GSL-3 individual. Combined with a small sample size for this group (n = 7), this produces larger distances from the other larval samples for GSL 3. The omission of "tropical" forms present at the Sebastian Pinnacles did not change the branching pattern of the UPGMA dendrogram, but it increased the distance between the Sebastian and remaining groups by an average of 0.06. The average genetic distance (*D*) for all populations compared with Sebastian Pinnacles was 0.59.

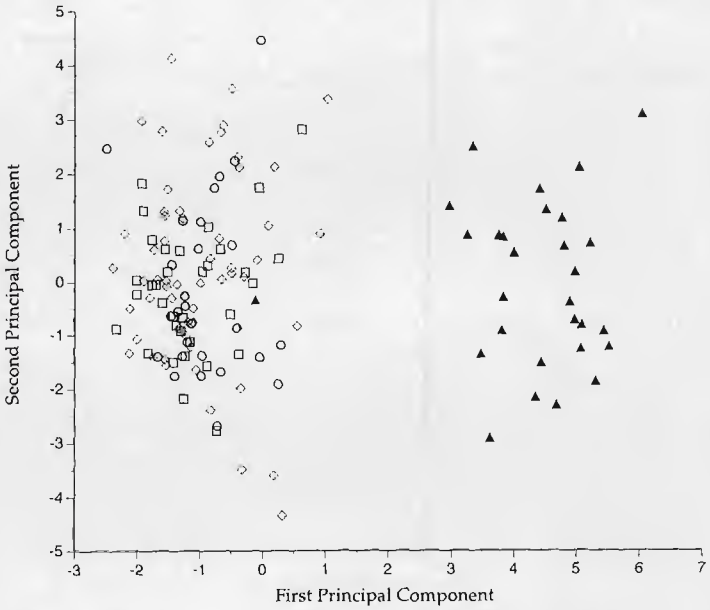

Figure 2. *Apionsoma misakianum*: a plot of the first v. second principal components based on polymorphic loci for adult populations and larval collections. Individuals from each collection are identified by the following symbols: (○) adults from Chub Cay, Bahamas, (□) adults from Pickles Reef, Florida, (▲) adults from Sebastian Pinnacles, Florida, and (◊) adults raised from pelagosphera from the Gulf Stream southwest of Sebastian Pinnacles.

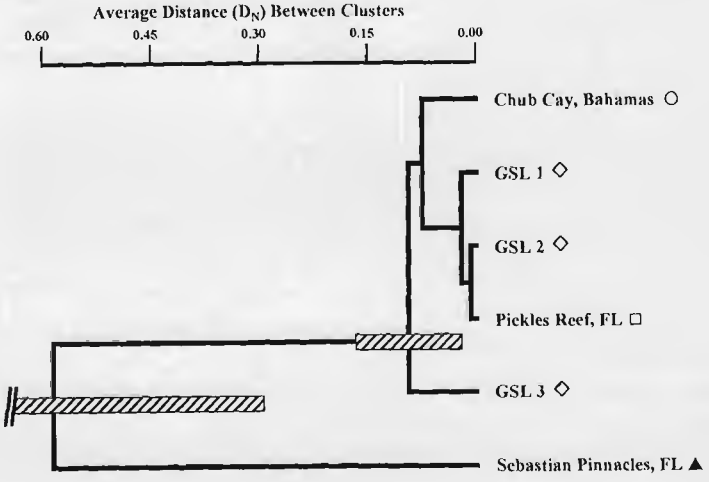

Figure 3. *Apionsoma misakianum*: a UPGMA dendrogram based on Nei's (1978) distances (*D*) between groups sampled. Benthic adult populations are denoted by locality name, whereas pools of reared larvae are identified by GSL lot number (see Table 1). Note that individuals with the "tropical" allele type are not included in the Sebastian Pinnacles group. Error bars (in diagonal hatch) are calculated as described for allozyme data by Nei et al. (1985).

Discussion

Past research on the development of *A. misakianum* demonstrated distinct differences in patterns of early development between offspring of adults reared from larvae collected in the Florida Current and offspring of the benthic adults from central Florida (Rice, 1981). The present study documented genetic differences between the adults reared from larvae in the Florida Current and the benthic adults in the area off central Florida. Although mortality was high for larvae reared to adulthood, the PCA showed that larvae, which survived rearing, were more similar genetically to adults collected 150 km south of the larval site than they were to adults collected near the larval sampling locality.

Results of the PCA identify that adults of the "tropical" genotype do occur, naturally, in the northern group. Despite the similarity of adult microhabitat (i.e., the coral fragments), the differences in depth of the adult populations could be a factor in the limited recruitment, as many species are found at limited depth ranges. But this depth difference does not exclude recruitment of individuals with the "tropical" genotype to the Sebastian Pinnacles, as evidenced by our study.

Several hypotheses may account for the absence of "hybrid" adults in our northern population sample: (1) competition for coral fragments may relegate migrant individuals to previously uncolonized fragments and reduce the probability of interbreeding, (2) differential response to environmental stimuli may produce asynchrony in spawning times between migrants and the remaining population (although note that both groups spawn at the same time under laboratory conditions, Rice, unpubl. data), (3) post-fertilization barriers to cross-breeding may occur between cryptic species, (4) "hybrid" larvae might occur but completely disperse from the area, (5) the temperate habitat (e.g., annual variation in temperature, change in food) on the "tropical" type may result in the formation of a "tropical" sterile isolate in the north (as suggested by Bhaud, 1989), or (6) "hybrid" larvae may have poor survivorship as compared to the "temperate" or "tropical" types. Any of these factors alone, or in combination, would produce limited numbers of hybrids in the northern locality and thereby limit our ability to observe them. Recruitment in other species of sipunculans has been demonstrated to vary under field conditions, even in the absence of any competitors (Hutchings et al., 1992; Rice, unpubl. data), so it is possible that one or more of these hypotheses has an effect on the introgression of the "tropical" genotype.

Several factors cannot be assessed by our study. Larvae of the "temperate" or "hybrid" types in our samples could have been missed. Their absence is attributable to either transport away by the predominant south–north current flow or death during the high mortality from rearing to adult size. We know only that the surviving adults reared from larvae were of the "tropical" genotype (despite the fact that they were reared on mud from the northern population). Also, larvae were collected only twice within 10 d for this study. We cannot determine based on this limited sample whether they represent a single, mass spawning of a more variable population or two (or more) cohorts of only slightly different individuals. However, the presence of rare alleles in the larval pool that are not present in the adult populations is suggestive that there are larvae from other populations present in our sample. Clearly, more data is needed on larval and adult populations to fully understand genetics of these populations.

However, findings from the metamorphosis and larval-rearing are relevant to the interpretation of our data on larval genetics. Gulf Stream larvae were competent to meta-

morphose (up to 80.9%); they metamorphosed when treated with water conditioned by adults from the northern locale (as described in Rice, 1981, 1986), and the surviving "tropical-" genotype larvae were successfully reared on sediment from the temperate area. Also, a water-soluble factor (a low molecular-weight compound of ≤500) exuded by adults has been suggested to be a species-specific cue for metamorphosis (Rice, 1981). These factors in combination suggest that selection of larvae by conditions in the northern locality is not biased against the "tropical" types, as one might expect given their low frequency in the northern population.

Differences between tropical and temperate adults might be attributable to local adaptation to regional climate and habitat. Climatic conditions of the continental shelf are influenced by the proximity of the Gulf Stream (especially minimum ocean temperatures which affect species' distributions, Bhaud, 1993), and it is within the area of 27°N that the Gulf Stream begins to diverge from the coastline of Florida. To the south, reef systems and coralline beach sediments predominate, whereas to the north the dominant substrate type is quartz sand or mud of salt-marsh estuaries.

The region along the central coast of Florida has long been recognized as a zone of faunal transition (Hayden and Dolan, 1976; Hedgpeth, 1953). Most intraspecific studies on genetic markers have focused on peninsular Florida and its role as a barrier to dispersal for marine invertebrate species. Genetic transition for these species has been analyzed using nuclear and cytoplasmic genetic markers: allozymes for horseshoe crabs (Selander et al., 1970), anemones (McCommas, 1982), oysters (Buroker, 1983), stone crabs (Bert, 1986; Bert and Harrison, 1988), oyster drills (Liu et al., 1991), ribbed mussels (Sarver et al., 1992), marsh and fiddler crabs (Felder and Staton, 1994) and ghost shrimps (Staton and Felder, 1996); and mitochondrial DNA (mtDNA) for horseshoe crabs (Saunders et al., 1986), oysters (Reeb and Avise, 1990), hermit crabs (Cunningham et al., 1992), and hydroids (Cunningham and Buss, 1993). The generalized conclusions of these studies is that Gulf and Atlantic populations are genetically distinguishable, if not by allozymes, then definitely by more sensitive mtDNA analysis (see reviews by Avise, 1992, 1994). This type of concordance across species has been argued by Avise (1998) to suggest shared historical forces shaping the genetic isolation across a region; here the disjuncture occurs between the Atlantic the Gulf coasts of Florida. However the history shaping this disjuncture may be far from a single, simple history.

Several factors suggest that, even though contingency and common history play a role in evolutionary processes in coastal Florida, history does not necessarily produce simple patterns, as causative impacts on this history may vary in time and space. The American oyster is arguably the best-examined case of genetic disjuncture for peninsular Florida: its populations have been examined for allozymes (Buroker, 1983), mtDNA (Reeb and Avise, 1990), and single-copy nuclear DNA (Karl and Avise, 1992). Even though mtDNA haplotypes differ across the same geographic region of eastern Florida (Reeb and Avise, 1990) as do the allozymes for *A. misakianum* in our study, allozymes (see reanalysis of data from Buroker [1983] by Cunningham and Collins [1994]) and nuclear markers (Karl and Avise, 1992) are segregated between populations on the western side of the Florida peninsula. Concordance across genetic markers over geographic boundaries (Avise, 1998) is not satisfied, demonstrating that there is no simple answer to the phylogeographic patterns seen here. In short, we cannot be sure if mtDNA haplotypes are "leaking" into Atlantic populations (as suggested by Reeb and Avise, 1990) or if nuclear alleles have introgressed into the Gulf population. Indeed, both may have happened at different times

or other more recent abiotic factors of climate change might contribute to the complexity of trans-Floridian species (Felder and Staton, 1994).

Teleplanic larvae are a part of the complex history connecting even distant populations of *A. misakianum*. The formation of sibling species requires a panmictic population that is subsequently subdivided either by geographic boundaries or within a region by reproductive isolating mechanisms. Even if ancestral populations were distant geographically, teleplanic larvae are still a considerable genetic link between populations to be overcome, given that a single migrant per generation will prevent genetic differentiation between populations, theoretically. Although mtDNA haplotypes can diverge even within species (given the clonal nature of their transmission), divergence in nuclear genes is direct evidence of reproductive isolation. Genetic and developmental differences between northern and southern individuals across this zone provide a concordance of data that suggest cryptic speciation has occurred in *A. misakianum* (see Knowlton, 1993).

Teleplanic larvae have been hypothesized to affect biogeographic distributions of marine invertebrates that possess these larvae. In gastropods, it has been suggested that, on average, species with planktotrophic development have larger biogeographic ranges than those with direct development (see Scheltema, 1989, for review); however, re-examination of these data has shown that a species' range may be dependent upon factors affecting adult survival (e.g., minimum sea-surface temperature) regardless of developmental mode (Bhaud, 1993). There are other reports of molluscan species that have distributions that are not correlated to length of larval development (Johannesson, 1988, Ó Foighil, 1989). Our data are consistent with the argument that an assessment of a species' biogeographic range by larval ecology and morphology alone may lead to erroneous conclusions.

In conclusion, we can state that there is strong reproductive isolation between northern and southern populations, and this occurs despite the influx into the northern area of larvae that are genetically similar to the southern type. Also, there appears to be reproductive isolation between the "temperate" and "tropical" types in the northern population that suggests cryptic speciation between the two types. Given these results, we emphasize that potential larval dispersal may be quite different from realized larval dispersal. Many marine species with teleplanic larvae may have more limited ranges than hypothesized given their potential for larval dispersal. Furthermore, potential or realized larval dispersal does not necessarily confer genetic homogeneity across a species with a large range.

In the future, we will investigate the use of other molecular markers to assess the temporal and spatial variation in larval genotypes within the lower Gulf Stream for members of the *A. misakianum* species-complex, as well as sampling other adult populations. It is hoped that by expanding this research to larger geographic scales, we can gain a fuller understanding of the interaction of genetic and geographic variation and the role of teleplanic larvae in biogeography and speciation for a marine benthic invertebrate.

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