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Genetic differences between geographic populations of the Crown-of-thorns starfish throughout the Pacific region

M. Nishida [1] and J. S. Lucas [2]

[1] **Department of Marine Sciences, University of the Ryukyus, Okinawa 903-01, Japan**
[2] **Department of Zoology, James Cook University, Queensland 4811, Australia**

Abstract

Ten population samples of the Crown-of-thorns starfish *Acanthaster planci* were collected between March 1982 and August 1983 from localities across the Pacific and were examined for variation at 14 enzyme loci using starch-gel electrophoresis. A sample of *A. brevispinus* was also examined for comparison. In contrast to the considerable genetic differentiation between *A. brevispinus* and *A. planci* populations ($D = 0.20 \pm 0.02$), the genetic differences between geographic populations of *A. planci* were small ($D = 0.03 \pm 0.00$; $F_{ST} = 0.07 \pm 0.02$), in spite of the great distances separating them. A positive correlation was observed between degree of genetic differentiation and geographic scale, suggesting that the genetic homogeneity among *A. planci* populations is due to gene flow by planktonic larval dispersion. In view of such macro-geographic homogeneity, it is striking that significant allele frequency differences were observed between adjacent populations separated by approximately only 10 km. The Hawaiian population was most differentiated from other populations. Treating the morphologically-distinctive, eastern Pacific *Acanthaster* as a separate species, *A. ellisii,* is not supported by these data. The lack of unique alleles in these two central and eastern Pacific populations suggests that they were derived from those in the western Pacific.

Introduction

Wide geographical distribution is characteristic of many marine animals, which typically have a sedentary adult and a planktonic larval phase in their life-cycles. The wide distribution is generally attributed to dispersal by planktonic larvae; and the planktonic phase should also promote gene exchange between widely separated populations and thus reduce genetic variation among them. On the other hand, localised selective pressures on the sedentary adult phase could facilitate genetic differentiation between nearby populations. The two phases are likely to have opposite effects on the genetic structuring of populations.

During the last decade, many attempts have been made to examine the level of genetic differentiation among widely separated populations of marine animals with sedentary or coastal adults (Johnson 1974, Campbell et al. 1975, Koehn et al. 1976, Levinton and Suchanek 1978, Buroker et al. 1979, Hedgecock 1979, Vawter et al. 1980, Winans 1980, Buroker 1983, Buroker et al. 1983, Yoshiyama and Sassaman 1983, Grant et al. 1984, Shaklee 1984, Rosenblatt and Waples 1986). However, most studies treat populations along a continental coastline or an archipelago. There have only been few studies of populations across wide ocean basins (e.g. Campbell et al. 1975, Vawter et al. 1980, Winans 1980, Buroker et al. 1983, Rosenblatt and Waples 1986). There is, thus, little information on genetic relationships and structuring among trans-oceanic populations of marine species with a planktonic larval phase and sedentary adult.

The Crown-of-thorns starfish *Acanthaster planci* (L.) (Acanthasteridae) is a widely distributed coral reef asteroid. At times, population explosions of this coral-predating starfish have been observed on coral reefs in various parts of the Pacific. It is a very fecund starfish, producing millions of eggs each breeding season. The subsequent planktonic larvae are morphologically typical of asteroid larvae and are planktonic for several weeks or more (Yamaguchi 1977, Lucas 1982). This species has a wide distribution throughout the tropical Pacific and Indian Oceans, and some macro-geographic variation in morphology has been observed (Madsen 1955). For example, the spines and arms of eastern Pacific starfish are distinctly short, leading to these starfish being treated as a different species, *A. ellisii* (Gray), by some authors (e.g. Madsen 1955, Caso 1961); although others have questioned the specific status (e.g. Glynn 1973, 1974). The other *Acanthaster* species *A. brevispinus* Fisher, the only other species

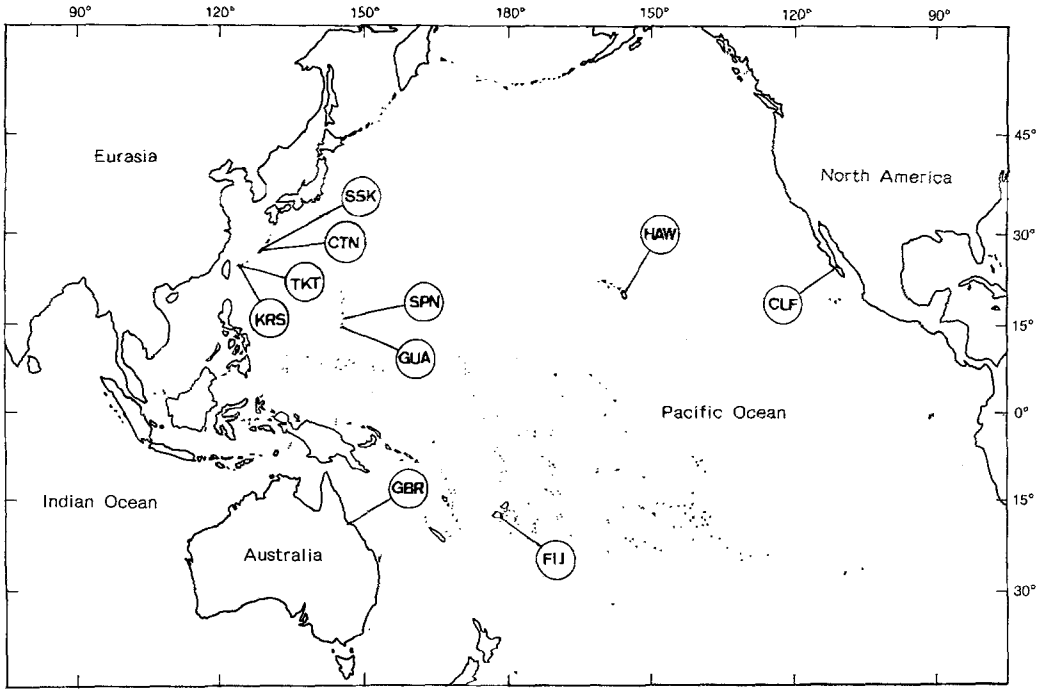

Fig. 1. Collection sites of *Acanthaster planci* populations in the Pacific. SSK: Sesoko Island, the Ryukyus; CTN: Chatan, Okinawa Island, the Ryukyus; TKT: Taketomi Island, the Ryukyus; KRS: Kuroshima Island, the Ryukyus; SPN: Saipan Island; GUA: Guam Island; GBR: Lodestone Reef, off Townsville, Great Barrier Reef; FIJ: fringing reef near Suva, Viti Levu; Haw: Kona Coast, Hawaii Island; CLF: La Paz area, Gulf of California (Sea of Cortez)

in the family, is known from Philippine and Australian waters. This species is characterized by very short and numerous spines, and is an omnivore inhabiting sandy bottoms in deep water near reefs. This is in contrast to the specialized characteristics of *A. planci* for life on coral reefs and feeding on scleractinian corals (Lucas 1986). Lucas and Jones (1976) and Lucas et al. (1985) have hypothesized that *A. planci* evolved from an *A. brevispinus*-like ancestor.

Its typical life cycle and wide distribution range, with macro-geographic morphological variation as well as sporadic population explosions, make the Crown-of-thorns starfish an interesting marine species for the study of genetic population structuring. In this study, we examined the degree of genetic variation among populations of this species from various localities in the Pacific region by using starch-gel electrophoresis. The aims were to determine what genetic differences exist between widely separated populations, and to assess population structuring by comparing the genetic differences between distantly separated populations with those between adjacent populations. Systematic and evolutionary implications of the results are also considered.

Materials and methods

Collections

Ten population samples of *Acanthaster planci* were collected between March 1982 and August 1983. Sampling sites and sample designations are shown in Fig. 1. Six of the ten populations sampled were from the northwestern Pacific region, including two pairs of samples collected from very close localities within the Ryukyu Islands, TKT/KRS and SSK/CTN, 10 and 40 km apart, respectively; and the other two (SPN, GUA) were from the Mariana Islands. Two populations were sampled from distantly separated localities (GBR, FIJ) in the southwestern Pacific; one from the Hawaiian Islands (HAW); and one from the Gulf of California (CLF) (="*A. ellisii*"). This sampling scheme permitted comparisons over geographical scales from 10 to more than 10 000 km.

Also, for comparison, a sample of 10 *A. brevispinus* was obtained from the Great Barrier Reef region near Townsville in 1980/1981. This species is sparsely distributed and it took some time to accumulate even this small sample. Samples of pyloric caeca taken from each starfish were kept at −70 °C until use.

Electrophoresis

The pyloric caeca samples were homogenized in 0.01 M Tris-HCl buffer (pH 7.1) containing 0.001 M ethylenediaminetetraacetate (EDTA). The homogenate was absorbed onto filter paper wicks (Whatman No. 3) and subjected to horizontal starch-gel electrophoresis. Gels were made from Electrostarch Lot 307 (Otto Hiller) and Connaught starch Lot 383-1 (Connaught Lab.) in a 3:1 ratio at a starch concentration of 12%. The fourteen

Table 1. Enzyme examined, their loci and buffer systems used in electrophoresis

Enzyme (abbreviation, E.C. number)	Locus	Buffer system [a, b]
Alkaline phosphatase (ALP, 3.1.3.1)	*Alp*	CAPM (TCE)
Glucosephosphate isomerase (GPI, 5.3.1.9.)	*Gpi*	CAEA
Hexokinase (HK, 2.7.1.1)	*Hk-2*	LiOH
Isocitrate dehydrogenase (IDH, 1.1.1.42)	*Idh*	CAEA
Malate dehydrogenase (MDH, 1.1.1.37)	*Mdh-1*	CAPM
	Mdh-2	CAPM
Mannosephosphate isomerase (MPI, 5.3.1.8)	*Mpi-1*	LiOH
Leucyl-glycine peptidase (PEP (Lg), 3.4.13.–)	*Pep (Lg)*	CAPM
Leucyl-proline peptidase (PEP (Lp), 3.4.13.–)	*Pep (Lp)*	CAPM
Phosphogluconate dehydrogenase (PGDH, 1.1.1.44)	*Pgdh*	CAEA
Phosphoglucomutase (PGM, 2.7.5.1)	*Pgm*	TCE
Superoxide dismutase (SOD, 1.15.1.1)	*Sod-1*	CAEA (TCE)
	Sod-2	TCE (CAEA)
Triosephosphate isomerase (TPI, 5.3.1.1)	*Tpi*	LiOH

[a] CAPM: Citric acid – aminopropyl morpholine, pH6.0 (Clayton and Tretiak 1972); CAEA: Citric acid – aminopropyl diethanolamine, pH7.0 (Clayton and Tretiak 1972); TCE: Tris – citric acid – EDTA, pH7.0 (Ayala et al. 1973); LiOH: Tris – citric acid, pH8.5 (gel), Lithium hydroxide – boric acid, pH8.1 (electrode) (Ridgway et al. 1970)

[b] Second buffer system in parentheses

enzyme systems included in this study and the buffer systems used are shown in Table 1. These are the outcome of screening for more than 30 enzymes. The staining procedures for specific enzymes were similar to those of Shaw and Prasad (1970), Harris and Hopkinson (1976), and Redfield and Salini (1980).

Alleles were lettered alphabetically in order of decreasing anodal mobility of their protein products. Given also in Table 2 is the mobility relative to that of the most common alleles in Sesoko population in the Ryukyu Islands when using the standard buffer system. Each locus was named by an abbreviation of the enzyme name, with numerical suffix in the same order as above, when multiple loci were included.

Data analyses

Departures of genotypic frequencies from Hardy-Weinberg equilibrium were assessed by F_i (Wright 1922, Crow and Kimura 1970). The expected proportions of heterozygotes were corrected for small sample size using Levene's (1949) method. Significance of F_i was tested with χ^2 (Li and Horvitz 1953). This test was employed to avoid statistical problems arising from small expected numbers, though some information is lost.

The degree of genetic differentiation among samples of *Acanthaster planci* was quantified by standardized genetic variance, F_{ST} (Wright 1965), as modified by Nei (1977) for more than two alleles. The F_{ST} value was corrected for the binomial sampling variance by the method of Workman and Niswander (1970). Significance of F_{ST} was tested with χ^2 analyses (Workman and Niswander 1970).

Unbiased genetic identity (I) and distance (D) were calculated for pairwise combinations of all samples according to Nei (1978). A dendrogram based on genetic distance was constructed using the unweighted pair-group method with arithmetic means (UPGMA, Sneath and Sokal 1973).

Results

Allele frequencies

The allele frequency data for all *Acanthaster* samples are given in Table 2. In *A. planci*, three loci (*Hk-2, Mpi,* and *Sod-2*) out of 14 loci examined were monomorphic or nearly monomorphic for the same allele in all samples. The remaining 11 loci were polymorphic (95% level) in at least one sample of *A. planci.* The most frequent alleles were the same at all 11 polymorphic loci of the western Pacific populations. The HAW population had the same alleles as the most frequent ones except for the *Pgdh* locus, and this population was monomorphic or nearly monomorphic at 9 loci of the 11 typically polymorphic ones. The most common alleles in the CLF population were also the same as those in the western Pacific populations except for the *Pep(Lg)* and *Pgm* loci.

Two other regional features were notable in the allele frequency patterns. Firstly, the *Mdh-2*^{b} allele, fairly frequent in all populations in the northwestern Pacific, was not found in those of the southwestern Pacific. Secondly, all the alleles in populations of the central and eastern Pacific (HAW and CLF) were found in those of the western Pacific, whereas the converse was not the case.

Genetic variation within populations

Of the 98 χ^2 tests for F_i in polymorphic loci in *Acanthaster planci* populations, there were eight instances where F_i significantly ($P < 0.05$) deviated from zero. In each instance the F_i value was positive, indicating that departure from Hardy-Weinberg expectation was due to a deficiency of heterozygotes. These were *Alp* in SSK, *Gpi* in GBR, *Mdh-1* in SSK, *Pep(Lg)* in HAW, *Pgdh* in GUA and CLF, and *Sod-1* in SSK and TKT. Given 98 tests and a $P = 0.05$, one would expect 4.9 significant outcomes due to chance alone. The eight cases were more frequent than expected by chance, but not significant (one-tailed χ^2-test, $P > 0.05$). Fifty-five of the 98 F_i estimates were positive and 43 negative, and this was not significantly different from 1:1 (two-tailed χ^2-test, $P > 0.1$).

Table 2. *Acanthaster planci* and *A. brevispinus*. Allele frequencies at 14 loci in populations. BR: *A. brevispinus*. Sample abbreviations for *A. planci* as in Fig. 1. Number of individuals examined in parentheses. nd: not scored

Locus	Allele	Relative mobility	SSK	CTN	TKT	KRS	SPN	GUA	GBR	FIJ	HAW	CLF	BR
Alp	*a*	110	0.009	0.024	0.012	0.012	–	–	0.048	0.146	–	0.010	–
	b	100	0.943	0.915	0.890	0.869	0.925	0.974	0.881	0.780	1.000	0.870	0.650
	c	93	–	0.037	0.085	0.095	0.063	0.013	0.036	0.061	–	–	–
	d	85	0.047	0.024	0.012	0.024	0.013	0.013	0.036	0.012	–	0.120	0.350
			(53)	(41)	(41)	(42)	(40)	(38)	(42)	(41)	(32)	(50)	(10)
Gpi	*a*	113	0.083	0.073	–	–	0.025	–	0.024	0.012	0.016	0.183	–
	b	100	0.881	0.841	0.951	0.958	0.938	0.988	0.940	0.610	0.969	0.817	1.000
	c	93	–	0.024	0.012	–	–	0.013	0.036	0.341	–	–	–
	d[a]	87	0.012	0.061	0.037	0.042	0.038	–	–	0.037	0.016	–	–
	e	74	0.024	–	–	–	–	–	–	–	–	–	–
			(42)	(41)	(41)	(36)	(40)	(40)	(42)	(41)	(32)	(52)	(10)
Hk-2	*a*	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
			(19)	(11)	(18)	(13)	(22)	(23)	(42)	(41)	(32)	(53)	(10)
Idh	*a*[a]	120	0.130	0.110	0.061	0.038	0.051	0.063	0.131	0.063	–	0.296	–
	b	100	0.848	0.841	0.915	0.949	0.949	0.938	0.833	0.925	1.000	0.704	1.000
	c	85	–	0.012	–	–	–	–	0.012	–	–	–	–
	d	70	0.022	0.037	0.024	0.013	–	–	0.024	0.013	–	–	–
			(46)	(41)	(41)	(39)	(39)	(40)	(42)	(40)	(32)	(49)	(5)
Mdh-1	*a*	115	0.057	0.012	–	0.012	–	0.014	0.071	0.085	–	–	–
	b	100	0.943	0.988	1.000	0.988	1.000	0.986	0.929	0.915	1.000	1.000	1.000
			(53)	(41)	(39)	(42)	(40)	(37)	(42)	(41)	(32)	(53)	(10)
Mdh-2	*a*	124	–	–	–	0.012	–	–	–	–	–	–	–
	b	120	0.130	0.159	0.268	0.095	0.025	0.014	–	–	0.032	–	–
	c	100	0.770	0.756	0.683	0.821	0.863	0.824	0.929	0.854	0.968	0.774	–
	d	80	–	–	–	–	–	–	–	0.085	–	–	–
	e	70	0.100	0.085	0.049	0.071	0.113	0.162	0.071	0.061	–	0.226	1.000
			(50)	(41)	(41)	(42)	(40)	(37)	(42)	(41)	(31)	(53)	(10)
Mpi-1	*a*	100	1.000	1.000	1.000	1.000	1.000	0.972	0.986	0.974	nd	1.000	1.000
	b	97	–	–	–	–	–	0.028	0.014	0.026	nd	–	–
			(43)	(40)	(41)	(41)	(33)	(18)	(37)	(39)	nd	(52)	(10)
Pep(Lg)	*a*	107	–	–	–	0.048	–	–	–	–	–	0.516	–
	b	100	0.700	0.667	0.568	0.619	0.525	0.614	0.500	0.585	0.813	0.484	0.100
	c[a]	94	0.200	0.333	0.386	0.286	0.463	0.273	0.274	0.415	0.187	–	0.250
	d	89	0.100	–	–	–	–	0.114	0.202	–	–	–	0.050
	e	84	–	–	0.045	0.048	–	–	0.024	–	–	–	0.450
	f	75	–	–	–	–	0.013	–	–	–	–	–	0.150
			(30)	(6)	(22)	(21)	(40)	(22)	(42)	(41)	(32)	(31)	(10)
Pep(Lp)	*a*	130	–	0.071	–	–	–	–	0.014	–	–	0.020	–
	b[a]	115	0.071	–	0.071	0.050	–	–	0.167	0.149	–	0.147	–
	c	100	0.738	0.714	0.786	0.850	1.000	0.875	0.778	0.838	1.000	0.706	1.000
	d[a]	85	0.190	0.214	0.143	0.100	–	0.125	0.042	0.014	–	0.127	–
			(21)	(7)	(21)	(10)	(6)	(4)	(36)	(37)	(31)	(51)	(10)
Pgdh	*a*[a]	100	0.638	0.538	0.622	0.581	0.784	0.575	0.512	0.683	–	0.600	1.000
	b	88	0.103	0.192	0.171	0.242	0.068	0.238	0.238	0.232	1.000	0.400	–
	c	78	0.103	0.090	0.049	0.032	0.054	0.113	0.071	0.024	–	–	–
	d	72	0.121	0.115	0.061	0.081	0.054	0.050	0.143	0.037	–	–	–
	e	64	0.017	0.064	0.098	0.065	0.041	0.025	0.036	0.024	–	–	–
	f	58	0.017	–	–	–	–	–	–	–	–	–	–
			(29)	(39)	(41)	(31)	(37)	(40)	(42)	(41)	(32)	(35)	(7)
Pgm	*a*	120	0.071	–	0.024	–	–	–	–	–	–	0.010	–
	b	100	0.898	0.646	0.598	0.845	0.763	0.913	0.881	0.750	1.000	0.442	0.250
	c	80	0.031	0.354	0.378	0.155	0.238	0.088	0.107	0.250	–	0.548	0.750
	d	60	–	–	–	–	–	–	0.012	–	–	–	–
			(49)	(41)	(41)	(42)	(40)	(40)	(42)	(38)	(20)	(52)	(6)
Sod-1	*a*	100	0.837	0.866	0.854	0.861	0.500	0.667	0.845	0.988	0.979	0.875	1.000
	b	80	0.163	0.134	0.146	0.139	0.500	0.333	0.155	0.012	0.021	0.125	–
			(52)	(41)	(41)	(36)	(40)	(39)	(42)	(41)	(24)	(52)	(10)

Table 2 (continued)

Locus	Allele	Relative mobility	SSK	CTN	TKT	KRS	SPN	GUA	GBR	FIJ	HAW	CLF	BR
Sod-2	*a*	60	–	–	–	–	–	–	–	–	–	–	0.050
	b[a]	100	1.000	1.000	1.000	1.000	1.000	1.000	0.988	1.000	1.000	1.000	0.350
	c	60	–	–	–	–	–	–	0.012	–	–	–	0.600
			(53)	(41)	(41)	(42)	(40)	(40)	(42)	(41)	(32)	(53)	(10)
Tpi	*a*	104	0.010	0.024	0.061	0.037	0.025	0.025	0.036	–	–	–	–
	b	100	0.990	0.976	0.939	0.963	0.975	0.975	0.964	1.000	1.000	1.000	1.000
			(52)	(41)	(41)	(41)	(40)	(40)	(42)	(41)	(32)	(53)	(10)

[a] Including one or two alleles with close mobility

Table 3. *Acanthaster planci* and *A. brevispinus*. Estimates of genetic variability in populations based on 14 loci. Sample abbreviations as in Fig. 1

Population	Average number of individuals	Average number of alleles per locus (A)	Proportion of polymorphic loci ($P_{0.99}$)[a]	Average heterozygosity (H)[b]
SSK	42	2.64	0.714	0.216
CTN	34	2.57	0.786	0.251
TKT	36	2.50	0.714	0.234
KRS	34	2.57	0.786	0.197
SPN	36	2.14	0.643	0.173
GUA	33	2.29	0.857	0.188
GBR	41	2.86	0.929	0.227
FIJ	40	2.50	0.786	0.226
HAW[c]	30	1.38	0.308	0.037
CLF	49	1.93	0.643	0.250
BR	9	1.57	0.714	0.155
Mean[d] ±SE		2.34 ±0.14	0.717 ±0.053	0.200 ±0.020

[a] The frequency of the most common allele ≦0.99
[b] Unbiased estimate by Nei's (1978) method
[c] Based on 13 loci
[d] For 10 *A. planci* populations

Table 4. *Acanthaster planci*. Standardized genetic variance (F_{ST}) for each polymorphic locus. Analysis was made for four geographically different scales (overall Pacific, western Pacific, northwestern Pacific, and the Ryukyu Islands). Significance of F_{ST} was assessed by Chi-square test. Maximum geographic distance in parentheses

Locus	Overall Pacific (12 500 km)	Within western (7 500 km)	Within northwestern (2 500 km)	Within the Ryukyus (400 km)
Alp	0.029***	0.021**	0.006	0.003
Gpi	0.110***	0.117***	0.018**	0.013
Idh	0.054***	0.007	0.008	0.005
Mdh-1	0.020*	0.012*	0.008	0.013
Mdh-2	0.045***	0.034***	0.020**	0.006
Pep(Lg)	0.092***	0.018	0.012	0.000
Pep(Lp)	0.037**	0.011	0.000	0.000
Pgdh	0.149***	0.010	0.009	0.000
Pgm	0.138***	0.069***	0.081***	0.090***
Sod-1	0.110***	0.110***	0.090***	0.000
Tpi	0.005	0.000	0.000	0.000
Mean ±SE	0.072*** ±0.015	0.037*** ±0.013	0.023*** ±0.010	0.011 ±0.008

* $0.01 < P < 0.05$, ** $0.001 < P < 0.01$, *** $P < 0.001$

Table 3 summarizes estimates of genetic variability within populations of *Acanthaster* in terms of average number of alleles per locus (A), proportion of loci polymorphic ($P_{0.99}$), and average heterozygosity (H). The *A. planci* populations showed high genetic variability, with the exception of HAW, which was markedly less variable. The mean value of H, 0.200, for these populations of *A. planci* is a high level of genetic variability compared to most variable values for marine animals, e.g. Nevo (1978), Valentine and Ayala (1978), and Ritte and Pashtan (1982).

Genetic variability estimates for *Acanthaster brevispinus* were somewhat lower (A and H) than, or comparable ($P_{0.99}$) to, those for an average population of *A. planci* (Table 3).

Population differentiation

Table 4 summarizes the standardized variance of allele frequency (F_{ST}) for each of the 11 polymorphic loci in *Acanthaster planci* at four different geographic scales: over the Pacific, in the western Pacific, in the northwestern Pacific, and in the Ryukyus. The average genetic differentiation between populations over the Pacific was only about 7% ($F_{ST} = 0.072$), although statistically significant ($P < 0.01$, χ^2-test). The mean F_{ST} for western Pacific populations was nearly half that for the entire Pacific. The F_{ST} within the Ryukyus was very low, 0.011, which was not significant ($P > 0.05$, χ^2-test). However, the Ryukyu populations were not completely homogeneous. There

Table 5. *Acanthaster planci* and *A. brevispinus.* Unbiased genetic identities (left diagonal) and distances (right diagonal) of Nei (1978) between pairs of populations. Sample abbreviations as in Fig. 1 and Table 2

	SSK	CTN	TKT	KRS	SPN	GUA	GBR	FIJ	HAW	CLF	BR
SSK		0.004	0.013	0.003	0.023	0.004	0.005	0.022	0.063	0.048	0.200
CTN	0.996		0.000	0.001	0.020	0.008	0.009	0.014	0.058	0.028	0.172
TKT	0.987	1.000		0.004	0.019	0.014	0.016	0.020	0.073	0.037	0.154
KRS	0.997	0.999	0.996		0.016	0.001	0.003	0.014	0.044	0.040	0.181
SPN	0.977	0.980	0.981	0.984		0.008	0.023	0.036	0.098	0.063	0.182
GUA	0.996	0.992	0.986	0.999	0.992		0.004	0.026	0.050	0.050	0.194
GBR	0.995	0.991	0.985	0.997	0.978	0.996		0.017	0.048	0.044	0.203
FIJ	0.978	0.986	0.981	0.986	0.965	0.974	0.983		0.070	0.050	0.187
HAW	0.939	0.943	0.930	0.957	0.907	0.951	0.953	0.933		0.095	0.336
CLF	0.954	0.972	0.964	0.961	0.939	0.951	0.957	0.951	0.909		0.165
BR	0.819	0.842	0.857	0.835	0.833	0.824	0.816	0.829	0.715	0.848	

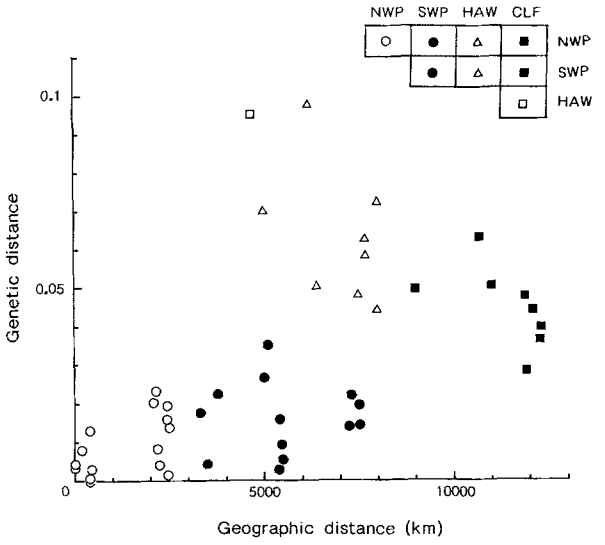

Fig. 2. *Acanthaster planci.* Unbiased genetic distance plotted against geographic distance between populations. The classes of pairwise combinations of populations are indicated in inset. CLF: Gulf of California; HAW: Hawaii, NWP: northwestern Pacific; SWP: southwestern Pacific

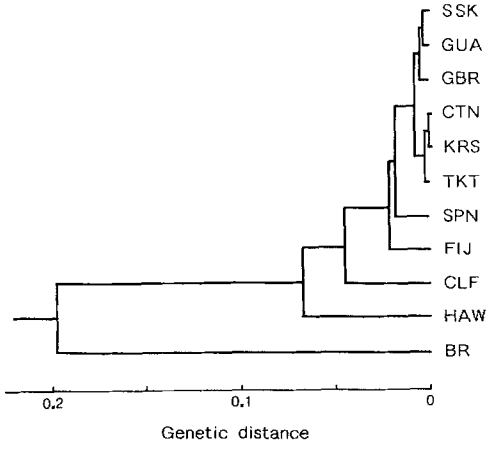

Fig. 3. *Acanthaster planci* and *A. brevispinus.* UPGMA dendrogram of populations derived from unbiased genetic distances based on 14 loci. BR: *A. brevispinus;* other abbreviations of collection sites as in Fig. 1

were significant allele frequency differences even between adjacent populations. Allele frequencies differed significantly at *Mdh-2* ($0.005 < P < 0.01$, χ^2-test) and *Pgm* ($0.001 < P < 0.005$, χ^2-test) between the TKT and KRS populations, which are separated by only 10 km. A significant allele frequency difference was also found at *Pgm* ($P < 0.001$, χ^2-test) between SSK and CTN, separated by 40 km.

Genetic distance

A matrix of unbiased genetic identity (I) and distance (D) values for all pairwise comparisons between *Acanthaster* populations is given in Table 5. The genetic distance values between populations of *A. planci* and *A. brevispinus* ranged from 0.154 to 0.336 with a mean of 0.197 ± 0.016. In contrast, the D values between conspecific populations of *A. planci* varied at a very low level from 0.000 to 0.098, with a mean of 0.029 ± 0.004.

HAW showed the largest differences from the other *Acanthaster planci* populations ($D = 0.067 \pm 0.007$). The D values between HAW and others were significantly higher than those between CLF and others ($D = 0.050 \pm 0.007$; $0.01 < P < 0.05$, two-tailed Mann-Whitney U-test). CLF and HAW were not genetically most similar ($D = 0.095$), in spite of the fact that they are most proximate to each other.

The relation between genetic and geographic distance was analysed (Fig. 2). Because of the lack of independence among values of each distance matrix, the degrees of freedom term in regressions was reduced to the number of populations minus one. This procedure provides a rather conservative test. Correlation between the distances was positive, but not significant ($r = 0.552$, $P > 0.05$), when all populations were included. Elimination of the HAW population, however, resulted in a highly significant correlation ($r = 0.788$, $0.005 < P < 0.01$).

A UPGMA dendrogram summarizing the genetic relationships among *Acanthaster* populations based on the D values is shown in Fig. 3. Most of the trends described above are reflected in the dendrogram. The first dicho-

tomy separated *A. brevispinus* from all *A. planci* populations at a level of $D=0.197$. The second dichotomy separated HAW from the other *A. planci* populations at a much lower level of $D=0.067$, and then the third separated CLF. The remaining populations in the western Pacific were rather genetically similar to one another.

Discussion

Population differentiation in the Pacific

These electrophoretic data indicate that *Acanthaster planci* populations throughout the Pacific are on the whole, genetically very similar in spite of the great distances separating them. The average D value for *A. planci* populations throughout the Pacific ($D=0.03$) lies near the median of D values observed for other coastal marine animal populations widely separated by an ocean basin (Campbell et al. 1975; Buroker et al. 1979, 1983, Vawter et al. 1980, Winans 1980, Rosenblatt and Waples 1986), which are all remarkably low in view of the great geographic distances separating the populations. F_{ST} value for the *A. planci* populations is also very low ($F_{ST}=0.07$), being comparable with that for milkfish from the Philippines to Hawaii (Winans 1980). The low values of both D and F_{ST} clearly indicate high genetic homogeneity among *A. planci* populations throughout the Pacific, and corroborate, on an even broader scale, the previous findings of high genetic similarity among far-distant populations in other marine organisms.

It is notable that this genetic homogeneity includes populations on either side of the Eastern Pacific Barrier (Briggs 1974): the 5 000+ km expanse of ocean without land or coral reefs between the offshore islands of the eastern Pacific and the Hawaiian and Line Islands in the west. Thus, since the major source of this high degree of genetic similarity among *A. planci* populations is most likely to be ongoing (or recent) gene flow by planktonic larvae, it appears that their larvae can at least occasionally travel over vast ocean distances. The very unlikely alternative is that very similar selective pressures are acting throughout the species' range, preventing genetic divergence (see Rosenblatt and Waples 1986).

This conclusion is important because there have been no observations on the morphology or development of *Acanthaster planci* larvae to suggest that they are especially adapted to teleplanic life. There are some other observations, however, that may help to explain the ability for long range dispersal. Although the minimum period of larval development of *A. planci* is about 10 d at optimum environmental conditions, it extends to 6 wk or more on marginal diets (Lucas 1982). The ability of these larvae to suspend metamorphosis in the absence of settlement substrates is not known. *Acanthaster planci* larvae show geonegative swimming behaviour and Yamaguchi (1977) has suggested that this is important for the long-distance dispersal. Production of vast numbers of eggs during the characteristic population explosions also may increase the frequency of successful long-distance dispersal.

Circumstantial evidence, relating to changing current systems and times of population outbreaks, has been provided by Yamaguchi (1986) for larval transport over at least hundreds of kilometers by offshore oceanic currents in the northwestern Pacific.

The effects of gene flow are reflected in the observed correlation between degree of genetic differentiation and geographic scale (Table 4, Fig. 2). If there is significant gene flow among populations by planktonic larval dispersal, spatially proximate populations should be more genetically similar than far-distant populations, because they should exchange genes more frequently. The data obtained in this study are consistent with this prediction: the F_{ST} value increased with geographic scale, and the genetic distance was positively correlated with geographic distance, especially in the analysis without the HAW population.

The finding that the HAW population is not genetically intermediate between the western Pacific populations and the CLF population (Table 5, Fig. 3), as its geographical position would suggest, is compatible with our understanding of the eastern Pacific ocean current systems. There are no eastward currents at higher latitudes capable of transporting larvae between the Hawaiian Islands and the eastern Pacific (Rosenblatt and Waples 1986). Near the equator, the North Equatorial Countercurrent could carry larvae eastward from the Line Islands to the eastern Pacific. Thus, gene flow between the western and eastern Pacific populations is not through the Hawaiian Islands, but probably through the Line Islands. Furthermore, gene flow between the Hawaiian Islands and the eastern Pacific populations must be indirect, involving much longer distances than the direct route between them. This relative isolation of the Hawaiian Islands, as well as their limited number and size, may be a factor responsible for the observed low genetic variability of the HAW population.

D and F_{ST} values indicate very high genetic homogeneity among populations in the Ryukyus and also in the northwestern Pacific. This conclusion also applies to the southwestern Pacific as shown by another study (Nash 1983, Nash et al. In press). D and F_{ST} values for 7 populations along the Great Barrier Reef, 1 200 km apart, based on 10 polymorphic loci were 0.009 and 0.019, respectively. These values for the GBR are comparable to the data of this study for 6 populations in the northeastern Pacific, i.e., D and F_{ST} values of 0.009 ± 0.002 and 0.023 ± 0.010, respectively.

Micro-geographic variation

In view of the possibility of long-distance gene flow in *Acanthaster planci*, the findings between adjacent populations of significant allele frequency differences at some loci is unexpected. The most remarkable instance is that of the

TKT and KRS populations: separated by only 10 km, yet showing highly significant allele frequency differences at the *Mdh-2* and *Pgm* loci. We sometimes found that additional bands developed on the PGM zymograms and incorrect scoring due to the occasional appearance of additional bands on the PGM gels might contribute in part to the allele frequency differences at this locus (although this was not indicated by significant departures from Hardy-Weinberg equilibrium). However, there was no ambiguity in scoring of MDH-2.

The low $Mdh\text{-}2^b$ frequency in KRS might be expected if this population had received more recruits than TKT from the Mariana populations (SPN, GUA), which have relatively lower frequencies of this allele (Table 2). No evidence for such differential immigration of recruits was, however, found at other loci in these populations and allele frequency differences in odd loci, such as here, must be presumed to result from selection.

There is evidence for differential selective mortality in a planktonic stage or just after settlement in the American eel *Anguilla rostrata* (Williams et al. 1973) and the limpet *Siphonaria jeanae* (Johnson and Black 1984). For *Acanthaster planci,* however, it is most plausible that there was selective mortality associated with population explosions. It is probable that mortality patterns change under specific conditions, such as scarcity of food and shelter, caused by unusually high population densities. Both TKT and KRS were high density outbreak populations and, from observations during sampling, the TKT population seemed to be declining after an outbreak. This is in agreement with results of a contemporary survey of *A. planci* and living corals in the area, including Taketomi and Kuroshima Islands (Fukuda and Miyawaki 1982). Allele frequency change at the *Mdh-2* locus may be caused by selective mortality associated with unusually high density. Nash (1983) found that a remnant population of *A. planci,* after a population explosion on Green Island differed from other "normal" populations in allele frequency at the *Mdh-1* locus: the only significant interpopulation difference found in that study.

Locally differentiated populations are unlikely to function as evolutionary units, however. Few larvae originating in a subpopulation are likely to return to the parental subpopulation after several weeks of planktonic dispersal. Thus, localized differences may be ephemeral, as has been indicated for the limpet *Siphomaria jeanae* (Johnson and Black 1984). The large scale homogeneity accompanied by small scale heterogeneity found in the Crown-of-thorns starfish may be a common feature of the genetic population structuring in widely distributed, sedentary marine animals with planktonic larvae and population fluctuations.

Systematic and evolutionary implications

The genetic differences between populations of *Acanthaster planci* and *A. brevispinus,* including those in sympatry in the Great Barrier Reef region, were much greater than between populations of *A. planci* on either side of the Pacific. Further genetic evidence for reproductive isolation between these two species (an allele substitution at an *Est* locus) was obtained by Lucas et al. (1985). Previously, in vitro fertilization and rearing studies had shown that fertile F_1 hybrids could be produced in the laboratory (Lucas and Jones 1976). But a subsequent study showed that F_2 hybrids and back-crosses were of poor viability and some were morphologically abnormal, suggesting that there are barriers to introgression of genes between *A. planci* and *A. brevispinus* (Lucas et al. 1985). These previous studies and this study show that reproductive isolation exists between the two species in their natural state.

Very short spines is one characteristic of *Acanthaster brevispinus* and the short spines of the eastern Pacific *Acanthaster* populations might reflect genetic similarity of these populations to *A. brevispinus* (Lucas et al. 1985). However, in these results, the degree of genetic differences between the eastern Pacific *A. planci* and *A. brevispinus* was basically the same as that between the western Pacific *A. planci* and *A. brevispinus.*

Specific status for the eastern Pacific *Acanthaster* is not supported by the genetic data of this study. The mean of genetic distance values between populations of the eastern and western Pacific ($D = 0.050 \pm 0.007$) was about a quarter of that between *A. brevispinus* and *A. planci.* Instances have been found where reproductively isolated populations do not necessarily show high genetic distance (Avise et al. 1975, Kirkpatrick and Selander 1979, Phelps and Allendorf 1983), and thus genetic distance alone cannot be definitive in solving taxonomic problems. However, in this case, there is the added difficulty of giving the eastern Pacific *Acanthaster* specific status when the degree of genetic difference between a Hawaiian population, which has not been recognised as morphologically distinctive, and other populations is greater (Fig. 3). We conclude that *A. ellisii* (Gray) should be treated as a junior synonym of *A. planci* (L.).

The CLF population, with the HAW population, has fewer alleles than the western populations (Table 3) and these two populations have no unique alleles (Table 2). This suggests that gene flow across the Pacific is from west to east and that probably this was the original direction of invasion across the eastern Pacific. Briggs (1974), reviewing the distributions of transpacific marine species concluded that "for the tropical marine shore fauna in general, including both fishes and invertebrates, it seems likely that successful migration across the East Pacific Barrier takes place in one direction only – from west to east". Briggs found no example of a typical New World genus invading the central Pacific.

Lucas and Jones (1976) hypothesized that *Acanthaster planci* evolved from an *A. brevispinus*-like ancestor, since it is more probable that a specialized coral predator evolved from an unspecialized sand inhabitant than *vice versa.* The indication from this study of a western Pacific origin of *A. planci* accords with the hypothesis of Lucas and Jones,

because *A. brevispinus* is known only from the shelf-waters of the western Pacific.

When did *Acanthaster planci* speciate? Its wide geographic distribution and high genetic variability suggest that *A. planci* has been evolving for some period of time. On the other hand, absence of *Acanthaster* from the Caribbean suggests that *A. planci* arose after the rise of the Panama landbridge, at about 3 m.yr BP (Keigwin 1978). Thus, it is most plausible that *A. planci* speciated some time ago during the Pleistocene period.

On the assumption that the rate of protein evolution is approximately proportional to time, Nei's genetic distance can be used for crude estimates of the time since divergence (Nei 1975). Estimate of a divergence time may be calculated either from theoretical equations (Nei 1975), or from correlation of genetic distance with albumin immunological distance (Sarich 1977, Carlson et al. 1978 for the calibration of AID). The estimates for the mean genetic distance between *Acanthaster planci* and *A. brevispinus* range from 1 m.yr BP by the former method to 3.7 m.yr BP by the latter. Because a genetic distance value is subject to various errors and calibration is not strict, caution is needed in evaluating a divergence time estimate. Nevertheless, it is notable that these estimates are in agreement with the above hypothesis, nearly corresponding to an early or middle Pleistocene speciation. If the hypothesis that *A. planci* evolved as a specialized coral predator from an unspecialized *A. brevispinus*-like ancestor is correct, the evolution of the feeding and habitat specialization and morphological change must have proceeded rapidly within the Pleistocene period.

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