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NOTE

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Genetic similarity of outbreak populations of crown-of-thorns starfish (*Acanthaster planci*) that were 15 years apart in Okinawa, Japan

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Introduction

Outbreak populations of crown-of-thorn starfish (*Acanthaster planci*) have periodically been reported in the Ryukyu Islands and on the Great Barrier Reef (GBR) (Yamaguchi 1986; Sapp 1999). However, the population densities of *A. planci* between outbreaks are very low in Yaeyama, islands in the southern part of the Ryukyu archipelago (Kotera and Kimura 2002; M. Katoh and K. Hashimoto, personal observations). How starfish populations are maintained during non-outbreak periods is not clear. Previous studies of allozyme variation revealed low differentiation among outbreak populations sampled in 1982–83 in the western Pacific including the Ryukyus (Nishida and Lucas 1988) and among outbreak populations sampled in 1981 on the GBR (Nash et al. 1988). Those results indicated that the outbreak populations shared a gene pool. Another allozyme study indicated that genetic differentiation in non-outbreak populations was greater than that in outbreak populations sampled in 1986 and 1987 on the GBR (Benzie and Stoddart 1992). The finding was consistent with a common gene pool for outbreak populations. Moreover, non-outbreak populations might change allele frequencies through genetic drift. Benzie and Wakeford (1997) compared outbreak populations occurring 10 years apart. The 1986 and 1996 outbreak populations on the GBR had few genetic differences, suggesting that the results of genetic drift had been small. Major outbreak populations of crown-of-thorn starfish were recorded throughout the Ryukyus from 1969–83 (Yamaguchi 1986). A major infestation of *A. planci* that started from reefs at Onna village on Okinawa Island in 1996 provides an opportunity to compare outbreak populations that were 15 years apart. In this note we use allozyme data to compare the population genetic structure of *A. planci* on Okinawa from the 1996 outbreak to two populations from the 1982–83 outbreak.

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Methods

Forty crown-of-thorn starfish were collected at Onna village on Okinawa Island (Fig. 1) on 24 June 1997 by members of the Onna Fishermen's Association. Specimens (disc diameter, 9–23 cm; whole diameter, 17–39 cm) were transported by air to the laboratory at Ishigaki Tropical Station, Seikai National Fisheries Research Institute, and dissected on the next day. Small portions of the pyloric caeca were stored in tubes individually at −85 °C for later allozyme analysis. Extracts of pyloric caecal tissue were prepared and analyzed by allozyme analysis as described by Nishida and Lucas (1988). The same combinations of buffers and enzymes (Nishida and Lucas 1988) were used, and seven enzyme systems were analyzed in this study. They included glucosephosphate isomerase (GPI; EC number 3.1.3.1), malate dehydrogenase (MDH; 1.1.1.37), mannose-6-phosphate isomerase (MPI; 5.3.1.8), leucyl-glycine peptidase [PEP(Lg); 3.4.13.–], leucyl-proline peptidase [PEP(Lp); 3.4.13.–], phosphoglucomutase (PGM; 2.7.5.1), and superoxide dismutase (SOD; 1.15.1.1). Nine enzyme loci were resolved in this study. Multiple loci encoding the same enzyme (isozymes) were designated by consecutive numbers, with '1' denoting the fastest migrating isozyme in the anodal direction. Alleles were assigned based on their relative mobility as described by Nishida and Lucas (1988).

Unbiased genetic identity and distance (Nei 1978) were calculated for pairwise combinations of the Onna sample and the previously studied populations (Nishida and Lucas 1988). Allelic frequencies of the Onna sample at each locus were tested with χ^2 against frequencies expected from the Chatan and Sesoko populations in the 1982–83 data (Nishida and Lucas 1988). *PEP(Lg)* and *PEP(Lp)* in the Sesoko population were excluded from the tests because of the small sample sizes. Alleles that had low expected numbers were pooled before the analyses. A total of 12 tests were conducted and a Bonferroni adjustment was made for tests of

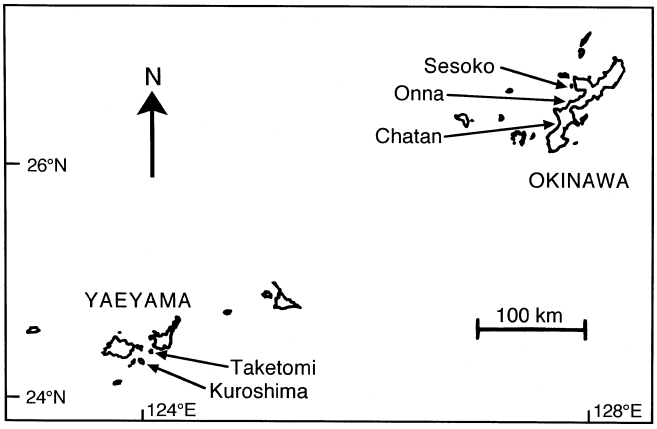

Fig. 1 Location of the Okinawan sampling sites in this study and in Nishida and Lucas (1988)

significance (significant: $p < 0.004$). Standard statistics for population genetics were calculated using the computer software POPGENE version 1.32 (Yeh and Boyle 1997).

Results and discussion

Allele frequencies of the seven polymorphic loci are listed in Table 1. *MPI* and *SOD-2* were monomorphic. Nei's (1978) standard genetic distances between the Onna sample in 1997 and the 1982–83 data from Nishida and Lucas (1988) indicated that the Onna sample was most closely related to the Chatan sample ($D = 0.003$) on Okinawa Island (Fig. 1; ca. 40 km southwest of Onna village along the west coast). Nei's (1978) genetic distances were small between the Onna sample and the other 1982–83 Okinawan samples (to Kuroshima Island, ca. 450 km southwest of Onna village, $D = 0.012$; to Taketomi Island, ca. 450 km southwest and to Sesoko Island, ca. 20 km north, both $D = 0.015$) and larger between the Onna sample and the remaining Pacific samples (to the Great Barrier Reef and to Fiji, both $D = 0.023$; to Guam, $D = 0.024$; to Saipan, $D = 0.039$; to Hawaii, $D = 0.044$). The allelic frequencies of the Onna sample at each locus (Table 1) were further tested against frequencies expected from the genetically closest sample, Chatan, and the geographically closest sample, Sesoko. The allele frequencies of the Onna sample were significantly different from the Sesoko sample at *PEP(Lp)* ($p = 0.003$, $\chi^2 = 11.17$, $df = 2$) and at *GPI* ($p < 0.001$, $\chi^2 = 15.71$, $df = 1$) and from the Chatan sample at *GPI* ($p = 0.002$, $\chi^2 = 12.39$, $df = 2$). Because of the small sample size ($n = 40$), small differences in allele frequency might be undetected.

Genotypic frequencies observed at each locus in the Onna sample were within the expectation of the Hardy-Weinberg equilibrium, except at *PEP(Lg)*, which had a low expected number of rare homozygotes. This might have inflated the χ^2 value. Nei's (1978) unbiased heterozygosity was 0.246 ± 0.170 (mean $\pm$ standard deviation) over nine loci studied. This is within the range of

Table 1 Allele frequencies of *Acanthaster planci* at Onna village in 1997 and at Sesoko and Chatan in 1982–83 in Okinawa, Japan (Nishida and Lucas 1988). Number of individuals examined is in parentheses

Locus	Allele	Onna	Sesoko	Chatan
GPI	*113*	0.175	0.083	0.073
	100	0.738	0.881	0.841
	93	0.000	0.000	0.024
	87	0.038	0.012	0.061
	74	0.050	0.024	0.000
		(40)	(42)	(41)
MDH-1	*115*	0.100	0.057	0.012
	100	0.900	0.943	0.988
		(40)	(53)	(41)
MDH-2	*120*	0.125	0.130	0.159
	100	0.775	0.770	0.756
	70	0.100	0.100	0.085
		(40)	(50)	(41)
PEP(Lg)	*100*	0.788	0.700	0.667
	94	0.150	0.200	0.333
	89	0.050	0.100	0.000
	84	0.013	0.000	0.000
		(40)	(30)	(6)
PEP(Lp)	*130*	0.013	0.000	0.071
	115	0.064	0.071	0.000
	100	0.885	0.738	0.714
	85	0.038	0.190	0.214
		(39)	(41)	(7)
PGM	*120*	0.000	0.071	0.000
	100	0.650	0.898	0.646
	80	0.350	0.031	0.354
		(40)	(49)	(41)
SOD-1	*100*	0.875	0.837	0.866
	80	0.125	0.163	0.134
		(40)	(52)	(41)

Okinawan populations in 1982–83 [$D = 0.197$–0.251; Nishida and Lucas (1988)]. The observed heterozygosity of the Onna sample was 0.209. The lower value observed indicated that there was an excess of homozygotes, mainly at *MDH-1* and *PEP(Lg)*. A deficiency of heterozygotes was also observed at *MDH-1* in Sesoko and *PEP(Lg)* in Hawaii (Nishida and Lucas 1988).

The genetic similarity between the 1997 Onna and the 1982–83 Chatan samples suggests that the results of genetic drift had been small. Similarly, close clustering in genetic space was observed in two sets of outbreak populations occurring 10 years apart on the GBR (Benzie and Wakeford 1997). The result in this study may be explained by the large population size that *Acanthaster planci* maintained during non-outbreak periods. Since 1987, the Onna village and Onna Fishermen's Association have attempted to control populations by removing *A. planci*. Three to five or more tons of starfish (ca. 8,000 to 17,000 individuals or more) were captured and removed from the reefs of Onna village during non-outbreak years. Fifty-nine tons of *A. planci* (ca. 171,000 individuals) were caught in 1996 at Onna village. These indirectly indicate the presence of a large population of *A. planci* around Okinawa Island during both outbreak and non-outbreak periods. In fact, moderate control efforts may have prolonged the

Acanthaster infestations because the population size was kept optimal with plenty of coral food. Moreover, the heterozygosity of the Onna sample was large and did not show evidence of severe bottleneck events. Because the heterozygosities of outbreak and non-outbreak populations were similar (Benzie and Stoddart 1992), existing populations of *A. planci* may not experience severe population reduction. If populations of *A. planci* had experienced a bottleneck event either after an outbreak or in conjunction with a widespread bleaching event of corals, the genetic diversity of populations might have been depleted. For example, *A. planci* had hardly been seen in Yaeyama islands in the southern part of the Ryukyu archipelago from 1992 until recently (Kotera and Kimura 2002; M. Katoh and K. Hashimoto, personal observations since 1997). Major outbreaks occurred in Yaeyama between 1972 and 1983 (Yamaguchi 1986).

A small but significant genetic difference was observed between the Onna and Chatan samples. Two samples were separated by 15 years. During that period, *A. planci* has gone through seven generations because *A. planci* becomes sexually mature late in its second year of life (Lucas and Jones 1976). Small genetic changes can occur by genetic drift alone through several generations. Selection may maintain similar allele frequencies in Okinawa for a long time. However, this explanation is inconsistent with minor genetic differences found within Okinawan populations in this study and a previous one (Nishida and Lucas 1988). Therefore, we conclude that the population remained large over the 15 years, which kept their allele frequencies similar.

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