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High levels of gene variation and the population structure of *Bunodosoma caissarum* (Cnidaria: Actiniidae)

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Abstract: High levels of gene variation have been recently reported for coelenterates and sponges from temperate waters. These levels are supposedly related to their particular life histories, ecological conditions and population sizes. However, few studies have been made so far on tropical sea anemone populations. Here we report an analysis of 20 putative isozyme loci in three populations of *Bunodosoma caissarum*, a Brazilian actiniid anemone extremely abundant in the intertidal zone of rocky shores along Rio de Janeiro. Heterozygosity levels were high (between 0.284 and 0.299), as in the anemones from the temperate zone. This suggests that latitude-related variables are not necessary conditions for high heterozygosity levels. A low level of gene differentiation (I = 0.954) was observed between populations up to 200 km apart, indicating a high homogeneity for the population. The evolutionary age of the group and the high population sizes present in these organisms may be responsible for the high levels of gene polymorphism observed in their populations.

Key words: Actiniaria, heterozygosity, *Bunodosoma*, genetics, population

The Brazilian endemic sea-anemone species *Bunodosoma caissarum* Correa, 1964 is widely distributed along the Sao Paulo and Rio de Janeiro coasts (Correa 1976, Maggs et al. 1979, Gouvea et al. 1985, 1989). *B. caissarum* is an exclusively sexually reproducing actiniid anemone (M.J. Belem, personal communication, 1989) which is particularly abundant in polluted waters where it can reach population densities of up to 150 individuals/square meter (unpublished results). Because of its abundance, this anemone has been used for the extraction of biologically active compounds (Kelecom et al. 1982), and as a radioactivity biomonitor (Gouvea et al. 1985, 1989). However, the population dynamics and genetics of this ecologically important sea-anemone are still unknown.

The genetic study of coelenterate populations has given a better insight into their ecology, systematics, and reproductive biology. In *Actinia equina*, an European species ecologically similar to *Bunodosoma caissarum*, for example, population genetics has been used to help to clarifying problems in taxonomy (Carter & Thorpe 1981, Haylor et al. 19840, Solé-Cava & Thorpe 1987) and reproductive biology (Orr et al. 1982). Populations of *Actinia* show a moderately high level of gene variation (Solé-Cava & Thorpe 1987, 1989), and a high differentiation between allopatric populations (Solé-Cava 1986). Similar results were obtained for anemones of the genus *Sargatia* (Shaw et al. 1987). On the other hand, low levels of gene differentiation were observed within sub-tropical *Bunodosoma* (McCommas & Lester 1980), and temperate *Metridium* (Bucklin & Hedgecock 1982), *Anthopleura* (Smith & Potts 1987), and *Urticina* (Solé-Cava et al. 1985) species. The contrast between the population structures in those anemone species could be related to dispersal capabilities and the balance between sexual and asexual reproduction.

The aim of this work was to estimate the basic genetic parameters of three populations of *Bunodosoma caissarum* and relate them to those

reported for coelenterates from temperate waters.

MATERIAL AND METHODS

Samples (approx. 30 in each site) of *Bunodosoma caissarum* were collected in the intertidal zone of two sites in Guanabara Bay (Urca 22°47'S; 43°10'W, and Bananal 22°57'S, 43°10'W, State of Rio de Janeiro) and one site outside the bay (Forno Beach, 22°58'S; 42°01'W). The anemones were transported in ice to the laboratory, where they were kept at -20 °C until electrophoresis. All samples were analyzed within one month after the collection.

The samples were homogenized using a glass rod on an acrylic plate, with a maximum dilution of 1:1 of distiled water. Horizontal, 12.5% starch gel electrophoresis was carried out as previously described (Solé-Cava et al. 1985, Solé-Cava 1986). The buffer system used was the Tris-Citrate pH 8.0 (Ward & Beardmore 1977). Other buffer systems such as the discontinuous Tris-Citrate-Borate, pH 8.3 (Poulik 1957) and the Tris-Citrate, pH 7.0 (Brewer 1970) were also tried but gave at best similar results to those obtained with this buffer system. Enzyme nomenclature and staining recipes follow Brewer (1970) and Harris & Hopkinson (1978).

Voucher specimens of the studied species were deposited in the Cnidaria collection of the Zoology department from the Federal University of Rio de Janeiro, under the number DZIBUFRJ 2-867. This was done in order to allow the correct specific assignment of the populations studied if the systematics of the genus is changed in the future.

RESULTS

Fifteen enzymes produced useful results. The isozyme patterns observed could be conservatively interpreted as the result of 19 gene loci. Furthermore, a cathodic region of blue bands was observed, some individuals showing one and others two bands. These patterns were electrophoretically similar to those described for carotenoproteins in *Urticina felina* (Solé-Cava et al. 1985), which also belongs to the Actiniidae. Carotenoproteins are common in marine invertebrates (Cheesman et al. 1967) and carotenoids have been described for *Bunodosoma granulifera* (Leboef et al. 1981a,b). The polymorphic cathodic bands observed in *B. caissarum* were thus interpreted as the expression of a di-allelic locus codifying for a monomeric carotenoprotein. Further studies on this putative carotenoprotein are necessary.

The gene frequencies of the 20 loci analyzed are presented in Table 1. From the total of 47 loci studied, three (Pgm-Arraial, Pgd-Urca and Sod.1-Arraial) exhibited significant (chi-squared test, $0.01<P<0.05$) deviations from Hardy-Weinberg equilibrium. The levels of genetic variation in each of the populations analyzed are presented in Table 1.

DISCUSSION

The deviations observed from the expected for populations in Hardy-Weinberg equilibrium could be tentatively explained as resulting from selective or stochastic phenomena. However, in 47 tests one should normally expect to find 2.4 significant results (at the level of 5%) by chance alone. Since chance is a sufficient explanation for the observed deviations, other, more complex explanations are unnecessary.

The pairwise levels of gene identity (Nei 1972) and similarity (Thorpe 1979) for the three populations were high (Table 2). These values are similar to those observed between populations of *B. cavernata* and *B. granulifera* from the Gulf of Mexico (McCommas & Lester 1980, McCommas 1982), but are much higher than those found between conspecific populations of *Actinia equina* in the Irish Sea (Solé-Cava 1986). The reason for a higher differentiation between *Actinia* populations is still not clear, but it might be related to the predominance of asexual reproduction in those organisms (Brace & Quicke 1985, Quicke et al. 1985). Sea anemone populations which reproduce asexually usually present a higher structuring of

TABLE 1

Gene frequencies for 20 protein loci of Bunodosoma caissarum *from 3 locations in Rio de Janeiro. n - number of alleles sampled for each locus.* Ho *and* He *- Observed and Hardy-Weinberg expected (unbiased estimate, Nei 1978) heterozygosity levels for each sample*

LOCUS	ALLELES	URCA	n	ILHA	n	ARRAIAL	n
Acp	1	1.000	10	0.870	46	0.857	56
	2	0.000		0.130		0.148	
BluPtn	1	0.464	56	0.389	36	-	
	2	0.536		0.611			
Cat	1	0.063	16	0.361	36	0.167	12
	2	0.625		0.444		0.500	
	3	0.313		0.194		0.333	
D-Est	1	0.976	42	0.971	34	1.000	46
	2	0.024		0.029		0.000	
Gdh	1	0.300	40	0.139	36	0.167	12
	2	0.700		0.806		0.750	
	3	0.000		0.056		0.083	
Got	1	0.083	24	0.000	28	0.083	12
	2	0.667		0.429		0.250	
	3	0.250		0.571		0.667	
Hbdh	1	-		1.000	26	-	
Hk-1	1	-		0.071	28	0.000	10
	2			0.357		0.300	
	3			0.571		0.700	
Hk-2	1	-		0.857	28	1.000	10
	2			0.143		0.000	
Lap	1	0.130	46	-		0.022	46
	2	0.696				0.848	
	3	0.087				0.022	
	4	0.087				0.109	
Mdh-1	1	0.889	36	1.000	36	1.000	10
	2	0.111		0.000		0.000	
Mdh-2	1	-		0.000	36	0.083	12
	2			0.944		0.833	
	3			0.056		0.083	
Me	1	0.933	30	-		-	
	2	0.067					
Mpi	1	-		0.071	28	0.200	10
	2			0.321		0.300	
	3			0.000		0.100	
	4			0.357		0.400	
	5			0.214		0.000	
	6			0.036		0.000	
Pep	1	-		0.200	30	-	
	2			0.800			
Pgd	1	0.237	38	0.417	36	0.000	10
	2	0.579		0.528		0.900	
	3	0.184		0.056		0.100	
Pgi-1	1	0.425	40	0.200	10	-	
	2	0.575		0.800			
Pgm	1	0.200	10	0.000	36	0.054	56
	2	0.800		0.694		0.714	
	3	0.000		0.083		0.125	
	4	0.000		0.222		0.127	
Sod-1	1	1.000	10	1.000	38	0.929	56
	2	0.000		0.000		0.071	
Sod-2	1	0.020	50	0.000	38	0.036	56
	2	0.980		1.000		0.964	
Ho		0.290		0.299		0.284	
He		0.289		0.294		0.266	

TABLE 2

Gene Identity (above the diagonal - Nei 1972) and similarity (below the diagonal - Thorpe 1979) indices between the three populations of Bunodosoma caissarum *studied*

REGION	URCA	ILHA	ARRAIAL
URCA	-	0.960	0.954
ILHA	0.847	-	0.969
ARRAIAL	0.840	0.874	-

their populations (Francis 1979, Shick et al. 1979, Bucklin 1987, Smith & Potts 1987). Although the asexual mode of reproduction does not produce, per se, any change in the genotypic proportions of the population, it enhances any disturbances caused by selection (e.g. Shick et al. 1979) or by genetic drift (e.g. Black & Johnson 1979).

The very high levels of gene identity found between samples up to 180 km apart of *Bunodosoma caissarum* indicate that these anemones must have high dispersal (or low divergence) rates. Adult and gamete dispersal are unlikely to be important along large geographical distances in sea anemones. The larvae of *B. caissarum* are still unknown but they are possibly long-lived, since the suitable substrata for this species are not continuous along the Rio de Janeiro coast.

The high levels of gene variation found in *Bunodosoma caissarum* are comparable to those found in other sea-anemone species (McCommas 1982; Bucklin 1985; Solé-Cava & Thorpe 1987, 1989; Shaw et al. 1987; Smith & Potts 1987). The high heterozygosity levels generally observed in marine organisms have been often associated with ecological (Selander & Kaufman 1973, Karlin & Levikson 1974, Valentine & Ayala 1974, Solé-Cava 1986) or random stochastic phenomena (Johnson & Black 1982, 1984, Kimura 1983, Solé-Cava 1986). High levels of gene variation have also been related to the antiquity or conservativeness (Soule 1972, Gorman & Kim 1977) of organisms. Coelenterates are usually considered "primitive" due to their apparent structural simplicity. The reasons for the primitiveness/heterozygosity relationship, however, are far from clear, since it may be explained both by the stability of the effective population size through geological time, or from the lower level of homoeostatic capability that these organisms might present.

Bunodosoma caissarum exhibits large population sizes, being particularly abundant in polluted places (Gouvea et al. 1985, 1989, personal observations). This might be helped by the high resistance of these anemones to anoxia (Ellington 1982) and to osmotic stress (Howard et al. 1987). It has been suggested that, in marine polluted areas, high levels of genetic variation can be adaptive (Nevo et al. 1984). It would be tempting, thus, to try to relate the high abundance of *Bunodosoma* in polluted places to adaptation through high levels of heterozygosity in its populations. However, the relationship between levels of pollution and genetic variation has not been confirmed, at least in some benthic organisms (Fevolden & Garner 1986), and, thus, alternative explanations based solely on high population sizes (Kimura 1983) cannot be excluded. Interestingly, the two genetically most polymorphic sea-anemone genera, *Anthopleura* and *Urticina* (Smith & Potts 1987, Solé-Cava et al. 1985) are also very abundant where they occur. Selectionist and neutralist factors could thus be equally evoked to explain the results observed in *B. caissarum*. It is not unlikely that a bi-directional interaction exists between genome and environment, and that drift and selection probably played different roles during the evolution of each group of organisms (Wright 1978, Levins & Lewontin 1985).

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RESUMEN

Recientemente se ha informado sobre altos niveles de variación génetica en celenterados y esponjas de aguas templadas. Se presume que tales niveles se relacionan con sus modos de vida, condiciones ecológicas y tamaños de población. Sin embargo, se sabe muy poco sobre las poblaciones de anémonas marinas tropicales.

Aquí se analizan 20 supuestos loci isoenzimáticos en tres poblaciones de *Bunodosoma caissarum*, una anémona actinida del Brasil que es muy abundante en la franja intramareal de las costas rocosas de Río de Janeiro. Los niveles de heterocigosis son altos (0.284-0.299), al igual que ocurre en especies de zonas templadas. Esto sugiere que los factores dependientes de la latitud no son indispensables para los altos niveles de heterocigosis. Se observó un nivel bajo de diferenciación génica (I = 0.954) entre poblaciones separadas por hasta 200 km, lo que indica una alta homogeneidad poblacional. La edad evolutiva del grupo y las grandes poblaciones de esta especie son causas factibles para tan altos niveles de polimorfismo génico.

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