Proceedings of the Fifth International Coral Reef Congress, Tahiti, 1985, Vol. 4

GENETIC CONSEQUENCES OF MASS MORTALITY IN THE CARIBBEAN SEA URCHIN *DIADEMA ANTILLARUM*

LES CONSEQUENCES GENETIQUES DE LA MORTALITE EN MASSE DE L'OURSIN *DIADEMA ANTILLARUM* AUX CARAIBES

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ABSTRACT

Diadema antillarum, an abundant and ecologically important echinoid in Caribbean reefs, has suffered the most extensive (entire Caribbean, plus Bermuda) and most severe (94-100%) mass mortality documented to date for any coral reef organisms. Such severe population restrictions can cause changes in the genetic structure of populations, changes with long-term evolutionary implications.

Percent heterozygosity in fourteen loci coding for enzymatic proteins had been documented in two Panamanian populations of this species in 1977. It was found to be high. The same populations were assayed for the same loci in 1984, a year after the mass mortality event, in order to see whether genetic variability had decreased. It had not. Some changes in gene frequencies were noted, but they were similar to changes in two other echinoid species, which had not undergone any mass mortality. D. antillarum retained a good deal of genetic variability despite severe mortality, probably because the die-offs did not occur simultaneously in all populations, and larval exchange allowed the re-seeding of affected areas with recruits that had not been exposed to the bottleneck.

The results from D. antillarum suggest that coral reef organisms with planktonic larvae are likely to recover from local extinction events without significant losses of their genetic variability. Mass mortality due to disease or to short-lived physical fluctuations, even when spread over the entire species range, is unlikely to result in major upheavals of the genome.

RESUME

Diadema antillarum, un échinoderme abondant et écologiquement important dans les récifs des Caraïbes, a connu une mortalité massive (toute la région des Caraïbes, ainsi que les Bermudes), la plus importante (94-100%) mise en évidence dans les récifs coralliens. Les généticiens des peuplements soutiennent que des restrictions assez sévères de peuplements peuvent produire des changements dans la structure génétique des populations, ce qui pourrait avoir des implications dans l'évolution à long-terme.

On a mesuré le pourcentage d'hétérozygotie dans 14 loci qui contrôlent les protéines enzymatiques, dans des peuplements panaméens de ces espèces en 1977; le pourcentage obtenu est élevé. En 1984, un an aprés la mortalité massive, on a cherché les mêmes loci pour déterminer si la variabilité génétique avait diminué. Elle n'avait pas diminué. Quelques variations dans la fréquence génique ont été observées, mais elles sont identiques à celles observées chez deux autres espèces d'échinodermes qui n'avaient pas subi la mortalité en masse. D. antillarum maintient une grande partie de sa variabilité génétique, malgré la mortalité sévère, probablement parce que la mort n'a pas été simultanée chez toutes les populations, et les échanges larvaires autorisent une recolonisation des zones affectées, grâce à des recrues qui n'ont pas subi ces évènements.

Les résultats obtenus pour D. antillarum suggèrent que les organismes à larves planctoniques peuvent échapper à une extinction locale, sans perte significative de leur variabilité génétique. Il est peu probable que la mortalité massive due à une maladie ou à des fluctuations physiques de courte durée, même lorsque cette mortalité s'étend à tout le territoire de l'espèce, puisse conduire à un bouleversement majeur du génome.

Table 2. Gene frequencies, percent of observed heterozygotes (Het), goodness of fit to heterozygosity expected from Hardy-Weinberg equilibrium and comparisons between gene frequencies and observed heterozygosities between years in Diadema antillarum from Bocas del Toro. See Table 1 for explanation of symbols. Five loci, Est-1, Est-2, G6PDH, TO and XDH were monomorphic in both years.

Locus	N		Allele	Frequency		Het (%)		Hardy-Weinberg (chi sq.)		Comparisons (chi sq.)	
	1977	1984		1977	1984	1977	1984	1977	1984	Frequency	Het
Am-2	47	33	a	0.000	0.000	8.5	0.0	0.03 NS	-	1.40 NS	1.44 NS
			b	0.957	1.000						
			c	0.043	0.000						
HK	37	49	a	0.000	0.041	8.3	10.2	0.05 NS	0.02 NS	2.12 NS	<0.01 NS
			b	0.958	0.949						
			c	0.042	0.010						
MDH-1	41	25	a	0.976	1.000	4.9	0.0	0.11 NS	-	0.14 NS	0.14 NS
			b	0.024	0.000						
MDH-2	50	25	a	0.970	0.960	6.0	8.0	0.06 NS	0.10 NS	0.03 NS	0.03 NS
			b	0.030	0.040						
M6PI	32	25	a	0.000	0.020	34.4	56.0	20.8***	1.30 NS	28.46***	1.86 NS
			b	0.281	0.220						
			c	0.000	0.000						
			d	0.125	0.440						
			e	0.359	0.260						
			f	0.234	0.060						
PGI	48	50	a	0.000	0.010	10.4	4.0	0.01 NS	0.12 NS	1.65 NS	0.71 NS
			b	0.948	0.980						
			c	0.052	0.010						
PGM-1	19	50	a	0.026	0.120	26.3	48.0	0.56 NS	5.56***	13.06**	1.84 NS
			b	0.000	0.230						
			c	0.763	0.540						
			d	0.211	0.100						
			e	0.000	0.010						
PGM-2	28	48	a	0.000	0.104	10.7	20.8	11.8***	8.84***	35.66***	0.66 NS
			b	0.661	0.167						
			c	0.339	0.698						
			d	0.000	0.031						
TPI	21	50	a	1.000	0.970	0.0	6.0	-	0.06 NS	0.24 NS	0.25 NS
			b	0.000	0.030						

than average heterozygosity. In D. antillarum the number of alleles averaged over all polymorphic loci has changed little from 1976 to 1984. At Isla Margarita there were 2.5 alleles per polymorphic locus before the mortality and 2.6 after it (Table 1). At Bocas del Toro the corresponding values were 2.22 and 2.89 (Table 2). Thus the number of alleles per locus also show no effects of a genetic bottleneck.

The comparisons of the observed genotype frequencies in each sample to those expected from Hardy-Weinberg equilibrium also support the conclusion that genetic variability in D. antillarum has not been affected by the mass mortality. If the surviving population were a biased sample of the original one, genotype proportions which were previously at equilibrium would be expected to have shifted away from it. In fact the trend, if one can be said to exist, is in the reverse direction. Loci with no significant departures from equilibrium remained in conformity with Hardy-Weinberg. One locus with heterozygote deficiency before the mass mortality, PGM-2 at Bocas del Toro (Table 2), remained deficient afterwards, but the proportion of its heterozygotes increased. Three loci, M6PI at Bocas del Toro (Table 2) and PGM-1, PGM-2 at Isla Margarita (Table 1) had significant heterozygote deficiencies before the mass mortality but came sufficiently close to equilibrium in 1984 to produce non-significant goodness of fit chi square values. The only loci that was not significantly different from Hardy-Weinberg in 1976-1977 but showed significant heterozygote deficiency in 1984 are M6PI at Isla Margarita and PGM-1 at Bocas del Toro (Table 2). The latter is clearly an artifact of the small size of the 1977 sample, because the percent of observed heterozygotes is higher in 1984. In Echinometra viridis (Table 3) and Echinometra lucunter (Table 4) the same trends were evident as in D. antillarum, namely a shift through time towards equilibrium in the loci that in 1977 had fewer heterozygotes than expected. Thus, the analysis relative to Hardy-Weinberg equilibrium, like the comparison between years, supports the conclusion that the mass mortality did not affect the genetic variability of D. antillarum.

There were significant changes in gene frequencies of D. antillarum at three loci at Bocas del Toro (Table 2), but not at Isla Margarita (Table 1). They occurred in M6PI, PGM-1 and PGM-2,

Table 3. Gene frequencies, percent of observed heterozygotes (Het), goodness of fit to heterozygosity expected from Hardy-Weinberg equilibrium and comparisons between gene frequencies and observed heterozygosities between years in Echinometra viridis from Bocas del Toro. See Table 1 for expanation of symbols. Three loci, Am-1, G6PDH, and XDH, were monomorphic in both years.

Locus	N		Allele	Frequency		Het (%)		Hardy-Weinberg (chi sq.)		Comparisons (chi sq.)	
	1977	1984		1977	1984	1977	1984	1977	1984	Frequency	Het
Am-2	50	32	a	0.970	1.000	6.0	0.0	0.06 NS	-	0.64 NS	0.65 NS
			b	0.030	0.000						
HK	29	24	a	0.017	0.000	17.2	4.2	0.12 NS	0.25 NS	1.80 NS	1.12 NS
			b	0.879	0.979						
			c	0.103	0.021						
MDH-1	50	22	a	0.990	0.878	2.0	4.5	0.24 NS	2.97 NS	0.03 NS	0.03 NS
			b	0.010	0.041						
			c	0.000	0.082						
MDH-2	50	18	a	0.000	0.026	6.0	5.6	0.06 NS	0.53 NS	0.26 NS	0.27 NS
			b	0.030	0.056						
			c	0.970	0.923						
			d	0.000	0.026						
			e	0.000	0.026						
M6PI	40	23	a	0.037	0.050	22.5	60.9	16.19***	<0.01 NS	6.73 NS	7.69**
			b	0.375	0.083						
			c	0.550	0.483						
			d	0.037	0.350						
			e	0.000	0.033						
PGI	43	25	a	0.221	0.040	60.5	72.0	5.91*	0.26 NS	26.45***	0.49 NS
			b	0.326	0.160						
			c	0.058	0.000						
			d	0.244	0.380						
			e	0.035	0.300						
			f	0.093	0.100						
			g	0.023	0.020						
PGM-1	44	25	a	0.023	0.000	50.0	60.0	13.63**	<0.01 NS	8.63 NS	0.30 NS
			b	0.079	0.026						
			c	0.352	0.078						
			d	0.284	0.169						
			e	0.182	0.571						
			f	0.068	0.091						
			g	0.011	0.065						
PGM-2	54	23	a	0.046	0.141	5.6	30.4	0.36 NS	<0.01 NS	3.32 NS	6.77**
			b	0.954	0.808						
			c	0.000	0.051						
TO	60	50	a	0.992	1.000	1.77	0.0	0.22 NS	-	0.21 NS	<0.01 NS
			b	0.008	0.000						

multi-allelic systems with no clear numeric predominance of one allele, and they were mostly due to the appearance of new alleles, not detected in the 1977 sample. These changes could be the result of mass mortality, but it seems more prudent to assume that they are due to other causes, such as heterogeneity in the genotype frequency of larvae settling at any given time (Johnson and Black, 1984). Loci with multiple alleles, such as HK, PGM-1, and PGM-2 in Echinometra lucunter (Table 4) and PGI in E. viridis (Table 3) also exhibited significant shifts in the relative proportions of the alleles through time, even though these species did not go through any known bottleneck between the two times they were sampled. Thus, none of the D. antillarum genotypes sampled here showed any clear evidence of differential survivorship through the mass mortality event.

DISCUSSION

None of the possible changes of genetic structure that could have been caused by catastrophic mortality seems to have occurred in D. antillarum. Genetic variability remained high, and no previously highly polymorphic locus seems to have been driven closer to fixation, although some gene frequencies changed significantly from 1977 to 1984. It seems clear that, despite the low number of individuals that remained on each reef, the populations did not go through a genetic bottleneck. Nor was there any differential survivorship of any of the genotypes detected by electrophoresis. To understand the possible reasons for these results it is necessary to understand what the 1984 samples represented.

Mortality in Isla Margarita probably occurred in January 1983, at the same time that it was

Table 4. Gene frequencies, percent of observed heterozygotes (Het), goodness of fit to heterozygosity expected from Hardy-Weinberg equilibrium and comparisons between gene frequencies and observed heterozygosities between years in Echinometra lucunter from Maria Chiquita (1977) and Isla Margarita (1985). See Table 1 for explanations of symbols. Three loci, TO, G6PDH, and XDH were monomorphic in both years.

Locus	N		Allele	Frequency		Het (%)		Hardy-Weinberg (chi sq.)		Comparisons (chi sq.)	
	1977	1985		1977	1985	1977	1985	1977	1985	Frequency	Het
Am-1	48	33	a	0.010	0.015	12.5	3.0	0.07 NS	0.19 NS	0.70 NS	0.011 NS
			b	0.073	0.030						
			c	0.917	0.955						
Am-2	48	35	a	0.042	0.057	6.2	11.4	0.29 NS	0.03 NS	0.04 NS	0.19 NS
			b	0.948	0.943						
			c	0.010	0.000						
Am-3	47	22	a	0.925	1.000	10.6	0.0	0.07 NS	-	2.08 NS	1.19 NS
			b	0.074	0.000						
HK	28	44	a	0.000	0.023	17.9	38.6	10.27**	0.22 NS	15.20***	2.57 NS
			b	0.482	0.159						
			c	0.518	0.795						
			d	0.000	0.023						
MDH-1	32	44	a	0.953	0.932	6.2	13.6	0.05 NS	0.01 NS	0.34 NS	2.93 NS
			b	0.031	0.023						
			c	0.016	0.045						
MDH-2	53	27	a	0.009	0.019	28.3	11.1	0.11 NS	0.06 NS	7.16 NS	2.13 NS
			b	0.802	0.944						
			c	0.179	0.019						
			d	0.009	0.019						
M6PI	42	42	a	0.048	0.036	45.2	45.2	1.30 NS	0.82 NS	4.77 NS	0.05 NS
			b	0.190	0.059						
			c	0.631	0.631						
			d	0.107	0.250						
			e	0.024	0.024						
PGI	50	46	a	0.000	0.012	68.0	56.5	0.93 NS	0.01 NS	1.71 NS	0.90 NS
			b	0.260	0.196						
			c	0.030	0.043						
			d	0.550	0.609						
			e	0.160	0.022						
			f	0.000	0.119						
PGM-1	56	43	a	0.045	0.023	57.1	48.8	3.98*	5.96 *	13.78 *	0.63 NS
			b	0.062	0.035						
			c	0.179	0.047						
			d	0.152	0.163						
			e	0.455	0.523						
			f	0.098	0.105						
			g	0.009	0.105						
PGM-2	35	39	a	0.014	0.141	25.7	30.8	2.39 NS	0.01 NS	15.21***	0.05 NS
			b	0.729	0.808						
			c	0.257	0.051						

observed at Punta Galeta, less than a kilometer away (Lessios et al. 1984 b). Mortality at Bocas del Toro was not observed, but given that the mortality agent seemed to be dispersed by currents, it probably reached this area between January 1983, the date it was observed at Galeta, and June 1983, the date it appeared at Costa Rica (Lessios et al., 1984 a). Thus the 1984 samples were taken about a year and a half after the populations had experienced mass mortality. They consisted of adult animals, 3 to 6 cm in diameter. The growth rate of D. antillarum is more than 2 cm per year (Randall et al., 1964; Lewis, 1966). The samples, therefore, like the populations they represent, were probably composed of both survivors of the mortality, and individuals that recruited after the event from larvae that were either in the water column when the adults were dying, or came from upstream populations that had not yet been affected by the die-offs. In Panama there was considerable D. antillarum recruitment immediately after the mortality, which declined after the mortality reached upstream populations (Lessios, unpublished data). Larvae released by as yet unaffected populations could obviously not bear the mark of mass mortality on their genetic constitutions. Thus, if the mass mortality had an effect on the genetic structure of the local populations, this effect was immediately diluted by the incoming

recruits. If so, the brief episode of change, if it occurred at all, had no evolutionary significance.

Changes due to random genetic drift could hardly be expected in the first generation after the bottleneck, particularly if, as postulated, this generation contained offspring that by means of alternate life stages bypassed the bottleneck entirely. However, if the population sizes remain low for many generations, they will result in an increase of the importance of stochastic processes in the evolution of this species (Wright, 1948). Whether this increase is substantial enough to be detected in a human lifetime will depend on parameters that cannot be measured. In addition to effective population size, these parameters include mutation rate, selection, and migration between local populations. The problem can only be approached empirically through repeated sampling of the same populations over time. In this sense the present paper represents the first two points in a time series.

That the extensive and severe mass mortality of D. antillarum had no detectable genetic consequences is important for our view of evolutionary processes that affect coral reef organisms. It indicates that catastrophic mortality due to sea level changes, climatic upheavals, disease, predator plagues or other such events that paleontologists often invoke as responsible for saltational evolutionary change, may not always result in the expected drastic consequences. The entire range of the species is rarely affected simultaneously by any agent of catastrophic mortality. If this agent is a disease, a predator, or a reversible climatic change, larvae can act as a refuge for genetic variability. Larval immigration from unaffected populations can replenish the genetic constitution of affected ones, which can then reciprocate after the mortality has reached the previous donors. Clearly such genetic replenishment will only occur in organisms with larvae that can stay in the water column for substantial periods of time and are capable of long-distance dispersal; it is also limited to situations where the source of catastrophic mortality ceases to operate soon after it has killed off a local population. Limiting as these conditions may be, they still encompass a large number of coral reef organisms and a substantial portion of the potential sources of catastrophic mortality.

ACKNOWLEDGMENTS

F. Riviera, V. Starczak, O. Vallarino, A. Velarde, and J. Weinberg helped with the collections. Y. Cerrud, M.L. Martinez, and M. Purmalis helped in the processing and assaying of the samples. J. Christy, J.B.C. Jackson, N. Knowlton, D. Potts, D.R. Robertson, V. Starczak, J. Weinberg, and M-J. West-Eberhard commented on the manuscript. Supported by a grant from the Scholarly Studies Program of the Smithsonian Institution and by research funds from the Smithsonian Tropical Research Institute.

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