Demographic history of Diadema antillarum, a keystone herbivore on Caribbean reefs

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The sea urchin Diadema antillarum was the most important herbivore on Caribbean reefs until 1983, when mass mortality reduced its populations by more than 97%. Knowledge of its past demography is essential to reconstruct reef ecology as it was before human impact, which has been implicated as having caused high pre-mortality Diadema abundance. To determine the history of its population size, we sequenced the ATPase 6 and 8 region of mitochondrial DNA from populations in the Caribbean and in the eastern Atlantic (which was not affected by the mass mortality), as well as from the eastern Pacific D. mexicanum. The Caribbean population harbours an order of magnitude more molecular diversity than those of the eastern Pacific or the eastern Atlantic and, despite the recent mass mortality, its DNA sequences bear the genetic signature of a previous population expansion. By estimating mutation rates from divergence between D. antillarum and D. mexicanum, that were separated at a known time by the Isthmus of Panama, and by using estimates of effective population size derived from mismatch distributions and a maximum likelihood coalescence algorithm, we date the expansion as having occurred no more recently than 100,000 years before the present. Thus, Diadema was abundant in the Caribbean long before humans could have affected ecological processes; the genetic data contain no evidence of a recent, anthropogenically caused, population increase.

Keywords: mitochondrial DNA; mismatch distribution; coalescence; human impact

1. INTRODUCTION

The long-spined black sea urchin Diadema antillarum was, until 1983, the most important herbivore on Caribbean reefs affecting plant (Ogden et al. 1973; Carpenter 1981, 1986; Sammarco 1982a, b) and coral (Bak & van Eys 1975; Sammarco 1980, 1982) cover, competing with other herbivores (Williams 1980, 1981; Sammarco & Williams 1982; Hay & Taylor 1985), and removing more calcium carbonate from reef framework than any other organism (Ogden 1977; Scoffin et al. 1980). In 1983, D. antillarum suffered mass mortality due to an unidentified pathogen that reduced its densities throughout the tropical western Atlantic by more than 97%, the most extensive and most severe mass mortality recorded in a marine animal (Lessios et al. 1984a; Lessios 1988). A decade after the mass mortality, populations had not recovered (Hughes 1994; Lessios 1995a). The demise of D. antillarum in the Caribbean accelerated the degradation of coral reefs through shifts from coral- to algal-dominated communities (Hughes et al. 1987; Lessios 1988; Levitan 1988; Carpenter 1990a; Hughes 1994; Ostrander et al. 2000). In Jamaica, recent local recovery of Diadema was accompanied by reduction of algal cover and increase in coral recruitment (Aronson & Precht 2001; Edmunds & Carpenter 2001). These correlated changes confirmed the importance of the ecological role of this species, and indicated that the magnitude of its previous impact was due to its high abundance.

How long ago Diadema reached high population densities is an important question that remains unanswered. Hay (1984), through measurements of the intensity of herbivory in areas of high and low fishing pressure, came to the conclusion that the high abundance of Diadema was a recent phenomenon due to the removal of its fish predators and competitors by humans. Post-mortality increases in herbivorous fish abundance in areas of low fishing pressure (Robertson 1991) and extensive overgrowth of corals by algal mats in areas of high fishing pressure (Lessios 1988; Levitan 1988; Hughes 1994) provided support for the hypothesis that Diadema competed with fish for plant resources (Carpenter 1990b). Levitan (1992) studied the relation between the size of D. antillarum jaw apparatus and the diameter of the body in museum specimens collected between 1881 and 1986. He found that in areas of increasing human population density there were signs of increasing D. antillarum food limitation, registering as larger relative tooth size (Ebert 1980; Levitan 1991). He reasoned that such food limitation was the result of higher intraspecific competition, and thus that there was a positive correlation between human and Diadema population density. However, variation through time was smaller than variation between localities, indicating that human influence played only a small role in regulating the abundance of Diadema. Jackson (1997), based on Levitan’s conclusion, along with the presence of 125,000 year old diadematoid skeletal remains in the Falmouth Formation of Jamaica (Donovan & Gordon 1993) and on anecdotal accounts of Diadema abundance in the writings of early naturalists going back to 1725, argued that D. antillarum was abundant long before humans began to remove fish from the Caribbean. Resolving the question...
of when population abundance of *Diadema* attained its pre-1983 levels is important, not just for a successful reconstruction of the ecology of Caribbean palaeo-reefs, but also for deciding the issue, important for modern reef management, of whether such high abundances were ‘natural’.

Amore likely explanation for thenegative values of $D$ and $F_s$ isthat they are theresult of population expansion. Thus, both a comparison ofmolecular diversity in Caribbean $Diadema$ to that in other populations thatdid not suffer recent mass mortality and an analysis of the inherentpattern of variation of its own mtDNA indicatethat the 1983 massmortality in the western Atlantic did not erase the genetic signature ofpast population history. It may have removed 97% of individuals in theCaribbean but, as might beexpected, in the 4–16 generations ($D.antillarum$ reachessexual maturity in one year (Carpenter 1997)) that elapsed between the mass mortality event and the time of

![Figure 1. Molecular diversity ($\pi$) + 95% confidence intervals in populations of $Diadema$ in the Caribbean, the eastern Atlantic, and the eastern Pacific: (a) samples from each region pooled; (b) individual localities in which $N > 7$. Molecular diversity incorporates the correction of Tamura and Nei (1993), based on a gamma distribution of substitutions, with $\alpha = 0.164$. Sample size for each population is indicated above the bars.](image1)

![Figure 2. Mismatch distributions (Rogers & Harpending 1992; Harpending 1994; Rogers 1995) of haplotypes of $Diadema$ in (a) the Caribbean, (b) the eastern Atlantic, and (c) the eastern Pacific. The continuous line represents the mismatch distribution expected from sudden expansion. Probability values ($p$) for rejection of the sudden expansion model are based on a comparison of the sums of squares of expected and observed mismatch distributions, using parametric bootstrap with 10,000 iterations (Schneider & Excoffier 1999).](image2)
Figure 3. Time since the beginning of the expansion of modern day populations, as estimated from the observed distribution of haplotype differences (Rogers & Harpending 1992; Harpending 1994; Rogers 1995). Parameters were estimated from the mismatch distribution by generalized nonlinear least squares, allowing for variable mutation rates and multiple hits per site; 95% confidence intervals were estimated from parametric bootstrap (Schneider & Excoffier 1999) with 10 000 iterations. Mutation rates $\mu$ are expressed as substitutions per site per millennium.

sampling for this study, there were no obvious bottleneck effects on its genetic variability.

How recently did the expansion of effective population size occur in the Caribbean? If the mutation rate $\mu$ is known, changes in effective population size $N_e$ can be calculated from estimates of $\theta$, where $\theta = 2N_e\mu$. One means of estimating the trajectory of $\theta$ through time is the ‘mismatch distribution’, i.e. the distribution of pairwise differences between haplotypes in a sample (Rogers & Harpending 1992; Harpending 1994; Rogers 1995). For populations that have undergone a sudden expansion (and thus display a unimodal distribution of haplotype differences), the mismatch distribution permits the calculation of initial and final values of $\theta$, and also of $\tau$, the product of $2\mu$ multiplied by the time since expansion started. All three populations of Diadema fit the sudden expansion model (figure 2). However, the mean number of sites with nucleotide differences between haplotypes in the Caribbean is 2.99, whereas the mean number in the eastern Atlantic is 0.19 and in the eastern Pacific 0.14, indicating an earlier expansion and/or a larger $N_e$ in the Caribbean.

To date the initiation of each expansion, it is necessary to estimate the rates at which mutations accumulate in Diadema. Such an estimate can be obtained by comparing sequences of Atlantic and eastern Pacific populations. The two oceans were separated by the emergence of the Isthmus of Panama. The Central American landbridge was completed 3.1 Myr BP (Coates & Obando 1996), but there is a possibility that it was breached by sea intrusions as recently as 2.0 Myr BP (Cronin & Dowsett 1996). Because Diadema shows less transisthmian divergence than six other genera of sea urchins in both mitochondrial and nuclear markers, the possibility exists that its populations on the two coasts of Central America may have re-established genetic contact during the breach (Lessios et al. 2001). The rate of mutation for the sequenced segment can, therefore, be either $1.2 \times 10^{-5}$ substitutions per site per millennium (if separation between D. antillarum and D. mexicanum is assumed to have lasted 3.1 Myr) or $1.9 \times 10^{-5}$ substitutions per site per millennium (if separation lasted 2 Myr). The slower rate of substitution dates the beginning of Diadema population expansion in the Caribbean at 330 000 yr BP, with a lower 95% confidence limit of 146 000 yr BP (figure 3). The faster rate produces an estimate of 213 000 yr BP with a lower 95% confidence limit of 94 000 yr BP. Thus even the 95% confidence limit of the most recent estimate of the initiation of population expansion pre-dates by far the onset of any possible fishing pressure in the Caribbean.

The mismatch approach has been criticized for not incorporating the genealogical information inherent in DNA sequences (Felsenstein 1992). An approach that takes this information into account is based on the distribution of the estimates of common ancestors between sequences. This distribution depends on mutation rate and effective population size. We used the procedure of Kuhner et al. (1998) to obtain maximum likelihood estimates of population growth rate $r$ and of $\mu$. This algorithm uses initial values of $\theta$ and $r$, and samples possible genealogies based on their posterior probability given the data; the sampled genealogies are then used to evaluate the likelihood of other values of $\theta$ and $r$. Using the program FLUCTUATE of Kuhner et al. (1998), we obtained the maximum likelihood estimates of the two parameters and then calculated effective population size through time, based on our estimates of mutation rates of Diadema. Based on these estimates, $N_e$ of Caribbean Diadema becomes zero 940 000 yr BP by the fast mutation rate and 1.52 Myr BP by the slow mutation rate. An effective population size of 1 million females is reached at 180 000 yr BP and 320 000 yr BP respectively (figure 4). Thus, the results from the maximum likelihood analysis of coalescence agree rather well with those obtained from mismatch distributions in dating the initiation of the population expansion of Diadema populations in the Caribbean at about 200 000 yr BP, a time which strongly suggests that there were large populations of D. antillarum on Caribbean reefs long before humans could remove their predators and competitors.

How accurate are these estimates of time since the expansion of Diadema population size? Many assumptions of this analysis can induce errors, but almost all such possible errors would cause underestimation, rather than overestimation, of the age of the Caribbean populations. Obviously, the actual number of individuals in the population at any given time would be at least double the effective number of females, estimated by mtDNA. Both of the genetic models assume that growth of the populations has been monotonic, whereas it is more likely to have been fluctuating. The model of Kuhner et al. (1998) also assumes exponential population growth, which Diadema is not likely to have attained for long periods of time. If selective sweeps, undetected by the McDonald–Kreitman (1991) test, have occurred, their
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Figure 4. Estimates of effective population size \( N_e \) through time in three clades of Diadema, based on two possible mutation rates \( \mu \) and maximum likelihood estimates of growth rate and of \( \theta = 2 \mu N_e \). (Kuhner et al. 1998): (a) \( \mu = 1.9 \times 10^{-5} \) substitutions per site per millennium; (b) \( \mu = 1.2 \times 10^{-5} \) substitutions per site per millennium.

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REFERENCES


Polanski, A., Kimmel, M. & Chakraborty, R. 1998 Application of a time-dependent coalescence process for inferring the
history of population size changes from DNA sequence data. Proc. Natl Acad. Sci. USA 95, 5436–5441.


Schneider, S., Roessli, D. & Excoffier, L. 2000 Arlequin ver. 2.000 A software for population genetics data analysis. Switzerland: Genetics and Biometry Laboratory, University of Geneva.


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