

RAPD ANALYSIS OF THE GENETIC VARIABILITY IN THE POPULATION OF THE SEA CUCUMBER *HOLOTHURIA POLII* AROUND THE MALTESE ISLANDS, CENTRAL MEDITERRANEAN

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Abstract

Randomly amplified polymorphic DNA (RAPD) analysis was used to assess the genetic variation of the sea cucumber *Holothuria polii* population. A total of 33 loci were chosen from the 7 primers used. The study revealed that there is divergence between sub-populations found on the east coast and the west coast of Malta, especially at the Anchor Bay site ($I = 0.7$; $D = 0.3$). On the other hand, Delimara's sub-population showed a high homozygosity and less effective alleles ($H = 0.214$, $N_e = 1.295$), possibly due to a decline in numbers, as a result to new selection pressures. The UPGMA dendrogram obtained of the entire Maltese population shows that *H. polii* is sub-divided into two distinct populations, the north-westerly sub-populations and the south-eastern sub-populations.

Keywords: *Mediterranean Ridge, Malta Trough, Malta Channel, Coastal waters*

Introduction

Studies carried out on species similar to *H. polii* were carried out in different environments, some consisting of large geographical areas, and in protected reefs (Uthicke & Benzie, 2001). Here, "no apparent restrictions to gene flow" (Uthicke & Benzie, 2001) were noticed, meaning that no genetic differences were present. The Maltese Islands are small and situated in the middle of the Mediterranean, making the sea currents in the area highly erratic. In fact, due to the shallow bathymetry and the proximity of the two major islands in the Maltese archipelago, a complex system of mesoscale eddies and gyres forms close to the islands (Drago et al., 2003).

Material and Methods

In this study, the RAPD primers, shown in table 1 below, were selected at random from other studies carried out on other echinoderm species.

Tab. 1. RAPD primers utilised in this study

Name	Sequences 5'-3'	TA	Reference
Primer 104	GGG CAA TGA T	36°C	Karako et al., 2002
Primer 148	TGT CCA CCA G	36°C	Karako et al., 2002
Primer 159	GAG CCC GTA G	36°C	Karako et al., 2002
Primer 2	GTT TCG CTC C	36°C	Chenuil et al., 2004
Primer OPI-07	CAG CGA CAA G	41°C	Dupont et al., 2000
Primer OPF-13	GGC TGC AGA A	41°C	Dupont et al., 2000
Primer OPA-11	CAA TCG CCG T	41°C	Dupont et al., 2000

A total of 15 primers were screened, and 7 were selected to produce 33 loci. A statistical correction in accordance with Lynch and Milligan (1994) was applied, in order to result in 6 highly polymorphic loci. Gene diversity within the three islands was found to be at an average of 43%. Overall, the Maltese Island sub-populations were found to be closely related except for the locations of Anchor Bay and Delimara.

Results

The average heterozygosity obtained in this study is higher than that found in a previous study carried out by Spiteri (2007). In this previous study the average heterozygosity was found to be 0.13. The higher value of expected heterozygosity should not be at all surprising since in this present study the population had a greater amount of alleles and a much larger distribution, than the previous study, due to the use of more loci. In fact once the grouping of closely related sites was carried out, the expected heterozygosity increased drastically, as the possibility of a small sample size was removed.

The Delimara sub-population revealed a lower effective number of alleles and therefore lower heterozygosity, which could be due to biological factors such as inbreeding, possibly due to a decline in numbers, and the Wahlund effect. The sub-population could also have passed through a recent bottleneck. In fact Freeland, 2008 describes that "allelic diversity usually decreases after a bottleneck because rare alleles are lost". A study on the same population (Spiteri, 2007) using allozymes, also resulted in an observation of a high level

of inbreeding within the same site. Inbreeding can also lead to homogeneity, the effect of which can be made worse in a small population due to breeding with closely related individuals. This inbreeding can eventually lead to a reduction in genetic diversity. Although inbreeding may cause problems in future generations because of the existence of deleterious recessive alleles, there might still be a genetic advantage by preventing recombination between adaptive loci (Primack, 1998).

Once the analysis was carried out, in order for the Maltese islands to be studied as an entire population it was observed that the sub-populations at Anchor Bay (pop. 6) and Delimara Creek (pop. 1) were the most divergent from the rest of the sub-populations. A test was carried out using UPGMA (Unweighted Pair-Group Method using an Arithmetic average), which defines the "intercluster distance as the average of all the pair-wise distances for members of two clusters" (Weir, 1996). The estimated distances of the branch length refer to the likelihood ratio, meaning that the further away the branching is, the more divergent the sub-population is.

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