ALLOZYME GENETIC VARIABILITY IN THE SEA CUCUMBER (*HOLOTHURIA POLII)* AROUND THE MALTESE ISLANDS

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Abstract

Allozyme electrophoresis was carried out over 7 loci that were selected from a maximum of fourteen enzymes. These 7 loci showed an average polymorphism, over the entire population, of 23%. Genetic index and distance showed that a genetic variation is present in the sub-populations surveyed, with a significant difference within the sub-populations of Delimara and Anchor Bay. 54% of the specimens sampled show that they are coming from the same population. Another 12%, mainly specimens collected from Anchor Bay, show that they are genetically different. In both these sites, different sub-populations may have been in contact with different environmental conditions allowing for changes to occur. Differences in allelic frequencies between *Holothuria polii* from different sites were also detected.

Keywords: Mediterranean Ridge, Malta Channel, Coastal waters

Introduction

Information on the biology and ecology of the sea cucumber *Holothuria polii* is sparse, since unlike most of its close relatives, it does not hold any economic value. However, being a relatively abundant species in the Mediterranean Sea, genetic studies on this species would have been expected, especially due to its unique characteristic organs' evisceration reflex on predatory-like stimulation. This allows for easy tissue collection, preventing any harm to the local population, as the organism can be put back in its natural habitat. Being filter-feeders, and relatively sedentary, holothurians are highly dependent on their environment (Ocana *et al.* 2005, Purwati & Luong-van 2003), allowing for behavioural changes to occur if any slight change in the environment occurs.

Materials and Methods

Allozyme electrophoresis

Approximately 300mg of frozen (-80^{o}C) gut tissue was mixed with the same amount of volume of extraction buffer (100mM Tris adjusted to pH 8.0 with HCl). Electrophoresis of all enzymes was carried out on 12% horizontal starch gels. In an initial screening process, 14 enzyme systems were used in order to select the best enzyme loci. The seven enzyme loci surveyed were: Phosphoglucomutase (E.C. 2.7.5.1, PGM*), Malate Dehydrogenase (E.C. 1.1.1.37, MDH*), Malate Dehydrogenase Phosphate (E.C. 1.1.1.40, MDHP*), Superoxide Dismutase (E.C. 1.15.1.1, SOD*) and Alcohol Dehydrogenase (E.C. 1.1.1.1, ADH*) as described in May 1992 These were analysed on R-Buffer (Gel Buffer: 0.03M Tris, 0.005M Citric acid, 2.5% Tray Buffer, pH 8.5; Tray Buffer: 0.06M Lithium Hydroxide, 0.3M Boric Acid, pH 8.1; electrophoresis for 7 hours at 200V and 38±2mA). Isocitrate Dehydrogenase (E.C. 1.1.1.42, IDH*) and Glucose-Phosphate Isomerase (E.C. 5.3.1.9, GPI*) as described in Murphy *et al.* 1996, were analysed on Tris-Citrate Buffer (Tray: 0.687M Tris, 0.157M Citric, pH 8.0; Gel: 1:25 Tray buffer diluted with water; electrophoresis for 5 hours at 100V and 34±4mA).

Results

Polymorphism in this investigation was found to be 23%. When compared to the value of 44% given by Bader (1998), it can be interpreted that polymorphism was low in the individuals sampled in the Maltese Islands. On the other hand in this study the mean heterozygozity was 0.13, while that reported in Bader (1998) was 0.124. These results agree with the statement that Bader made, "that the calculation of polymorphism is much more prone to statistical tests due to small sample sizes". The expected heterozygozity is not affected by this.

A statistical difference that was observed in this study was on the locus of PGM. There was no significant difference between the F_{IS} index of the other loci, and also in the mean F_{IS}. McDonald (1991) mentioned that there is evidence for temperature selection to occur at the PGM locus in certain organisms. In fact one should note that 1.3km north from Kalanka (sampling site), Hofra IZ-Zghira, the Enemalta power station is located, which releases hot water into the surrounding environment. This electricity generating plant is thus allowing for higher than average sea temperatures along the Delimara creek (Axiak and Sammut.2002; Borg, 2002). This is because the power plant, discharges its coolant water directly into the marine environment (Marsaxlokk and Delimara), at a temperature of 120°C (Borg, 2002).

Another prominent observation was at Anchor Bay sub-populations, were values for the genetic distance were found to be above 0.2. These indicate that a divergent sub-population is present. This can be related due to the fact that, one of the major outfalls is located in this area.

However to further critically assess the genetic variation around the Maltese islands, the sampling sites were grouped according to their vicinity and aspect.

The results show that although, the values, including the site for Anchor Bay, do not fit in the index range for sub-population divergence, they do not fit in the values for closely related sub-populations either. Thus the sub-populations found in the region, consisting of Anchor Bay, Ghajn Tuffieha and Ghar Lapsi (West) were still indicating divergence from the rest of the entire population.

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