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Low genetic differentiation between two morphotypes of the gastropod *Nacella concinna* from Admiralty Bay, Antarctica

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Abstract: During laboratory and field experiments on *Nacella concinna* on the west coast of Admiralty Bay, King George Island (Antarctica) clear morphological and behavioural differences between two limpet forms (*N. concinna polaris* and *N. concinna concinna*) were found. They suggested presence of genetic divergence. AFLP (amplified fragment length polymorphism) profiling of *N. concinna* individuals representing the two forms revealed nearly 32% of polymorphic bands; only 2% of them differed between the forms. Our results suggest that the observed phenotypic variation seems to be a result of adaptation to environmental conditions and not of any genetic divergence.

Key words: Antarctica, *Nacella concinna*, genetic diversity, AFLP.

Introduction

Herbivorous gastropod *Nacella concinna* is a characteristic representative of the Maritime Antarctic benthos. It occurs in two forms: *N. concinna polaris* and *N. concinna concinna* (Berry and Rugge 1973; Branch and Branch 1981). Morphological and behavioral differences between these two forms were observed by many authors (Nolan 1991; Markowska and Kidawa 2007; de Aranzamendi *et al.* 2008). *N. concinna polaris* occurs on stony bed rock in the littoral zone, and *N. concinna concinna* is present in the sublittoral and in the intertidal zones below 4 m down to 110 m depth (Walker 1972; Berry and Rugge 1973; Castilla and Rozbaczylo 1985; Nolan 1991) along the Antarctic Peninsula and adjacent islands (Picken 1980). The intertidal morphotype has a taller and heavier shell as compared to the rock pool morphotype having a lighter and flatter shell (Powell 1951; Nolan 1991). These two morphotypes also differ in important physiological traits (Waller *et al*. 2006; Weihe and Abele 2008).

In previous studies limpets *Nacella concinna* were gathered from rock pools during the low tide and by divers from the subtidal area at the depths of 5–8 m. Limpets collected from rock pools were significantly smaller than the subtidal individuals (*t* test, $P < 0.005$), ranging from 19.8 to 43.3 mm shell length (mean = 21.2, SE = 0.32 mm, $n = 222$), in comparison to 23.7–47.0 mm (mean = 37.4, SE = 0.49, $n = 191$) for the subtidal ones. Visible difference in reaction to predators was also noted between the two described limpet populations in laboratory and field experiments (Markowska and Kidawa 2007; Markowska 2008).

The aim of this study was to explore the genetic relationships between the two forms described above: the rock pool *N. concinna polaris* and the subtidal *N. concinna concinna* form from the Admiralty Bay.

Material and methods

DNA extraction. — Samples were collected during the previous experiment run by Markowska (2008). Forty eight individuals from both forms were taken for the AFLP analysis. DNA was extracted from about 20 mg of tissue, stored in 70% ethanol. Samples were dried on Watman paper, then followed overnight proteinase K digestion. Next steps of extraction followed the manufacturer's protocol recommendation (NucleoSpin®Tissue, Macherzy-Nagel, AQUA LAB). Purity and quantity of the samples were determined spectrophotometrically. DNA integrity and lack of RNA impurities were tested by agarose gels electrophoresis (1 × TBE buffer with 0.5 µg/ml ethidium bromide at 20 V/cm).

AFLP analysis. — The AFLP technique was performed according to the previously described procedures with minor modifications (Chwedorzewska *et al.* 2006). Briefly, 250 ng of genomic DNA was digested simultaneously with two restrictive enzymes *Eco*RI and *Mse*I. This was followed by ligation of the appropriate adaptors, preselective and, finally, selective amplification steps (core sequence Table 1). For

Table 1

Adapter and primer sequences

Adapter/Primer code	Sequence (5'->3')
Adapter *Eco*RI	CTCGTAGACTGCGTACC
	CATCTGACGCATGGTTAA
Adapter *Mse*I	TACTCAGGACTCATA
	GAGTCCTGAGTAGCAG
*Eco*RI preselective primer	GACTGCGTACCAATTCA
*Mse*I preselective primer	GATGAGTCCTGAGTAAC
E-Azz - selective primer	GACTGCGTACCAATTCAzz
M-Cyy - selective primer	GATGAGTCCTGAGTAACyy

E and M are for any selective primer complementary to *Eco*RI and *Mse*I adaptors respectively; -zz, -yy - any combination of the nucleotides at the primers 3'ends.

the selective amplification seven primer pair combinations (*Eco*RI – AGG/*Mse*I – CAC, *Eco*RI – ACG/*Mse*I – CAC, *Eco*RI – ACT/*Mse*I – CGC, *Eco*RI – ACC/*Mse*I – CAC, *Eco*RI – AAA/*Mse*I – CAG, *Eco*RI – AGT/*Mse*I – CAA, *Eco*RI – AAA/*Mse*I – CGG) were used (Table 1). The *Eco*RI compatible primers were labelled at their 5'-ends with gamma – ^{32}P ATP. PCR products were separated on 5% PAGE and X-ray films were exposed to the gels at -70°C overnight.

Data analysis and statistics. — Reproducible (experiment was run twice), individual AFLP fragments were scored as either present (1) or absent (0). Their frequencies were calculated for all the markers. Xlstat v.7.5.2 excel add-in software (www.xlstat.com, Addinsoft) was used to perform clustral analysis (Aggregation criterion: unweighted pair-group average UPGMA, Jaccard coefficient of dissimilarity) and construct the dendrogram. Principal Component Analysis (PCA) and Analysis of Molecular Variance (AMOVA) was carried out using GeneAlex5.1 excel add-in software (Peakall and Smouse 2001).

Results and discussion

DNA profiling of the samples representing animals collected from rock pools and from subtidal zone revealed 374 scoreable fragments amplified by seven primer pair combinations. The number of the DNA fragments generated by an individual primer pair varied from 31 to 72 with an average of 53. In total all primer pairs generated 121 (32% of all signals) polymorphic fragments with an average of 17 per primer combination (Table 1).

Clustral and PCA analyses using all the polymorphic bands failed to differentiate among all samples collected from rock pools and from subtidal. Nevertheless, the first main axis of the PCA explained 88.00% and the second 4.34% of the identified variability (Fig. 1). Based on the mentioned methods samples were randomly distributed and formed no separate groups. The similarity matrix was analysed using UPGMA. Dendrogram was based on all DNA fragments obtained with seven selective primer pairs for all individuals. Cluster analysis did not reveal any groups (the results of both, cluster and PCA analysis were similar, thus only PCA diagram is presented).

Although molecular profiling of *N. concinna* individuals revealed nearly 32% of the polymorphic bands, few (2%) were discriminated between the analyzed populations as demonstrated by AMOVA (Table 2).

Table 2

Results of AMOVA of *N. concinna* samples from all analysed populations. Significance tested by a permutation analysis against alternative random partitioning of individuals (999) across populations.

Source of variation	*df*	*SS*	Estimate variance	Total variance (%)	P-value
Among populations	1.0	5.312	0.066	1.7	<0.001
Within populations	46.0	172.001	3.739	98.3	<0.001

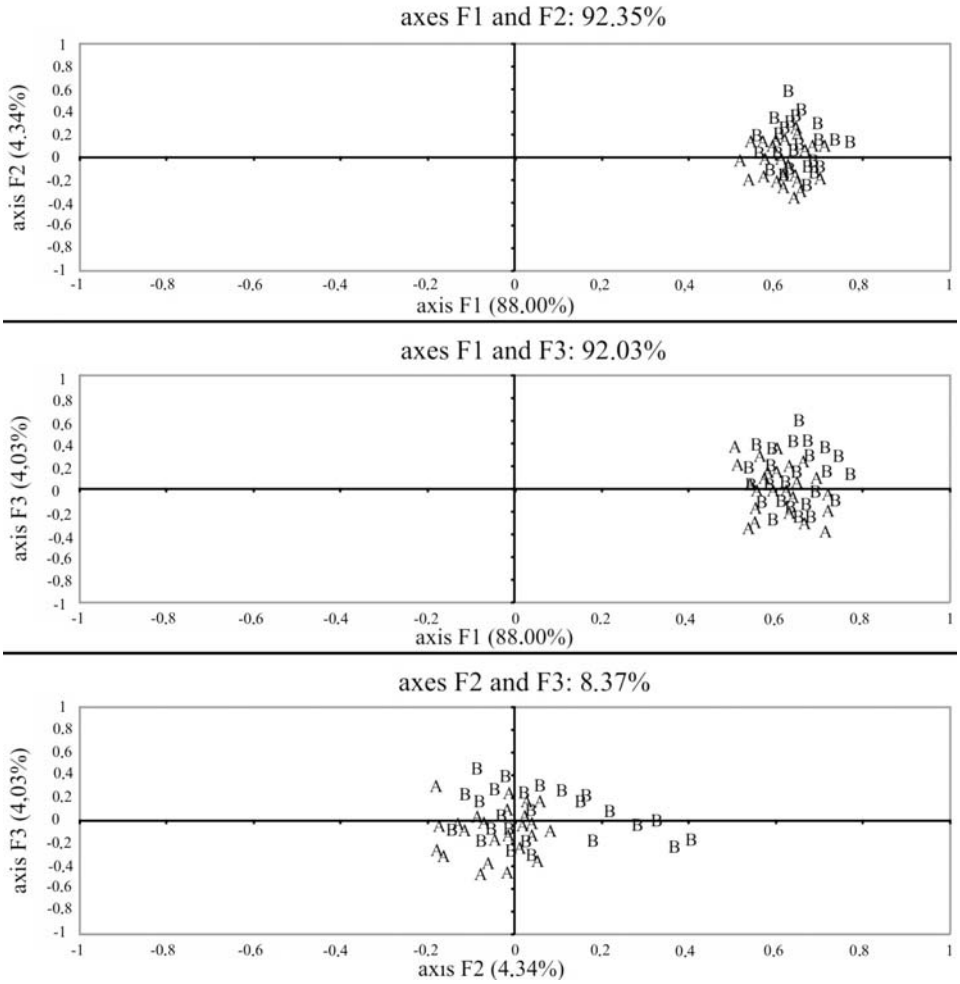

Fig. 1. Principal Component Analysis (PCA) of two population of *Nacella concinna* collected from rockpools (**A**) and from subtidal zones (**B**), based on AFLP data.

Genetic differentiation of the two forms was studied by de Aranzamendi *et al.* (2008) with use of ISSR-PCR markers and indicated that the two analyzed forms can be considered as genetically distinct populations maintaining low levels of gene flow. Our results, obtained by a different genetic method focused on total genomic DNA, showed much smaller differences between the analyzed forms (according to AMOVA less than 2%) and did not confirm results of de Aranzamendi *et al.* (2008) study. These differences may be linked to too small sample size taken for analysis by de Aranzamendi (only eleven individuals per population).

Thus, our results may point to specificity of this species reproduction physiology. *Nacella concinna* produces and disperses long-lived planktonic larvae, which can survive for two months in the water column (Bowden *et al.* 2006). The pelagic

long-lived larvae are able to colonise new sites rapidly, thus recruitment occurs within a common gene pool and there is little opportunity for local genetic divergence. In *N. concinna* the reproduction takes place about 3 weeks after the water temperature exceeds -1.4°C (Picken 1980, Picken and Allan 1983). Thus spawning appears to be synchronized with the increasing temperature and probably the availability of food (Brathes *et al.* 1994). However, it does not seem to be a sufficient barrier to avoid gene exchange in form of partial reproductive isolation. Moreover, the intertidal morphotype (equivalent of rock pool in presented study) is also migratory, moving seasonally between the intertidal and subtidal zones (Walker 1972). Probably these phenotypic and behavioural variations (Markowska 2008), specific to these populations, might be the result of phenotypic plasticity expressed in particular environmental conditions, rather than it reflects genetic differences. The heterogeneous environment that characterises intertidal region (different wave exposures, temperature, ultraviolet irradiation and variable chemical-physic parameters over a short vertical distance, different predators) could be responsible for such a significant morphological variability.

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