ON THE GENETIC STRUCTURE OF *RUDITAPES DECUSSATUS* (MOLLUSCA, BIVALVIA) INFERRED BY NUCLEAR AND MITOCHONDRIAL GENETIC MARKERS.

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Abstract

Analyses of 478 polymorphic AFLP loci and about 540 base pairs COI provided an account of genetic variation among *Ruditapes decussatus* populations from both the French Atlantic coast and the Mediterranean Sea. AFLP analyses of 357 specimens from 17 locations revealed evidence of significant genetic differentiation among populations (Fst = 0.16; P<0.001). Data on COI sequences from Mediterranean and Atlantic populations showed a lower genetic diversity among populations. Mismatch analysis found signatures of past genetic bottlenecks in *R. decussatus* populations. These findings confirmed the utility of AFLP for population genetic study while indicated that COI sequences, characterized by a lower intraspecific variability, could be more suitable markers for evolutionary studies

Keywords: Western Mediterranean, Genetics, Bivalves, Aquaculture, Lagoons

Introduction

The grooved carpet shell clam *Ruditapes decussatus* is a bivalve well appreciated for human consumption and one of the most expensive and commercially important clam species in fishery and aquaculture for the Mediterranean countries. Since wild stocks in most of the *R. decussatus* native range have been in decline over the last decades, to ensure a successful species management, it is extremely important to monitor the genetic variability both natural and hatchery populations. In the present study we examined both nuclear (AFLP) and mitochondrial DNA (COI) variation among samples of the Mediterranean basin and Atlantic Ocean with the goal of characterize its populations.

Material and methods

The majority of samples were collected in lagoons of the western Mediterranean basin: along the Sardinian coasts (n=10), in the Adriatic (Italy and Albania, n=4), in the Thau Lagoon (France, n=1), in the Ebro Delta (Spain, n=1). One sample came from the Arcachon lagoon (Atlantic coast, France). AFLP marker profiles were generated using the procedures described in [1] with minor modifications. Allele frequencies were estimated with AFLP-SURV v. 1.1. Genetic diversity within and among populations were estimated with ARLEQUIN 3.1 [2], and COA analysis using Adegenet [3]. Partial sequences of the mitochondrial COI gene were obtained using novel internal primers designed from the partial mitochondrial sequence of *Ruditapes decussatus* (GenBank Accession DQ184830). All sequences were aligned with MEGA 4 [4] and analysed with DnaSP 5.1 [5], and ARLEQUIN 3.11.

Results

AFLP analysis was successfully performed on all 357 individual samples and the two selected primer combinations produced a total of 478 polymorphic markers. Among populations, the percentage of polymorphic loci ranged from 41.6 % to 60.9%, the expected heterozygosity values were generally low (from 0.14 to 0.17). AMOVA showed a high genetic differentiation among populations (Fst = 0.16, P<0.001), with most of the total variation (83.7%) due to intra-population differences. Among all hypothetical groupings of populations examined, several were found to have significant percentage of among-groups variation but the higher differentiation were measured when populations were clustered according to their genetic distances and not to their geographic origin (Tab. 1: Structure A - 4 groups= Atlantic/ Adriatic/ Sardinia/ WMediterranean; Structure B - 3 groups= WMediterranean+Albania/ SGilla (3sites)/ all others). In particular, COA analyses confirmed these results (Fig. 1). Nucleotide sequences of about 540 bp in length were obtained from the mitochondrial cytochrome C oxidase subunit I (COI) gene. Analysis of sequence variation revealed the occurrence of 19 variable sites, a low nucleotide diversity (π: 0,00203) and haplotype diversity (Hd: 0.565). The most common haplotype was found in about 60% of the specimens and in all populations; other two less frequent haplotypes were present. Contrary to what is shown by the AFLP data, COI sequences seems to indicate that populations are not significantly differentiated. Mean overall Kimura genetic distance was K2P=0.2%, and pairwise inter-population distances ranged from 0.1% to 0.4%. The mismatch analysis and the star-like maximum parsimony network of COI sequences indicate the occurrence of past bottleneck events followed by recent population expansions of *R. decussates* populations. Our study confirm the utility of amplified fragment length polymorphism (AFLP) markers for population genetic studies; they permitted to retrieve a higher genetic diversity, and were developed at a relatively low cost and in a short period of time, which can make them ideal tools for generating large data sets for species that need a continuous monitoring. On the contrary, mitochondrial sequences resulted to be far less variable, and could be more suitable for evolutionary studies.

Tab. 1. AMOVA results of *Ruditapes decussatus* from AFLP data

Source of variation	%variation	F index
Structure A° (geographic clusters)		
Within populations	5.97	0.06**
Among groups	12.16	0.13***
Among populations within groups	81.87	0.18***
Structure B° (genetic clusters)		
Within populations	12.95	0.13***
Among groups	7.67	0.09***
Among populations within groups	79.37	0.21***

= Pvalue <0.01,*= value<0.001, ° see text for Structure details

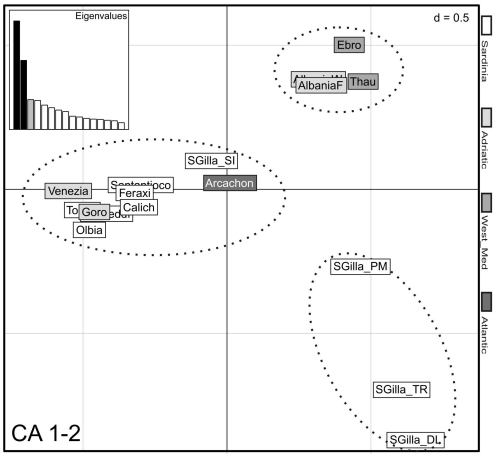

Fig. 1. COA (COrrespondance Analysis) of *Ruditapes decussatus* from AFLP data. Eigenvalues corresponding to the represented components are filled in black

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