Phylogeny And Genetic Resources Of European Abalone (Haliotis Tuberculata)

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Introduction

The geographic distribution of the European abalone (*Haliotis tuberculata*) spreads in the North Atlantic area (from Canary islands to the English Channel) and in Mediterranean Sea (Lee and Vacquier, 1995, Coleman and Vacquier 2002). As this species is becoming of economical importance for aquaculture in Europe, some hatcheries were created these last years and improvement programs started in the same time. In this context, it is important to study the genetic resources of this species to (i) be able to preserve them efficiency, and (ii) to use them in the breeding programs for a genetic basis increase or a foreign contribution to a trait of interest. For this reason, it is important to have an information as complete as possible concerning the phylogeny, the genetic structure and the diversity of the wild populations of the species. To obtain this information, molecular markers are powerful tools and are widely used for such purposes (Wan et al, 2004)

Material and methods

Sampling of the populations. 550 individuals from 20 populations were sampled in North Atlantic and Mediterranean Sea (figure 1). The number of individuals sampled varied from 7 to 53.

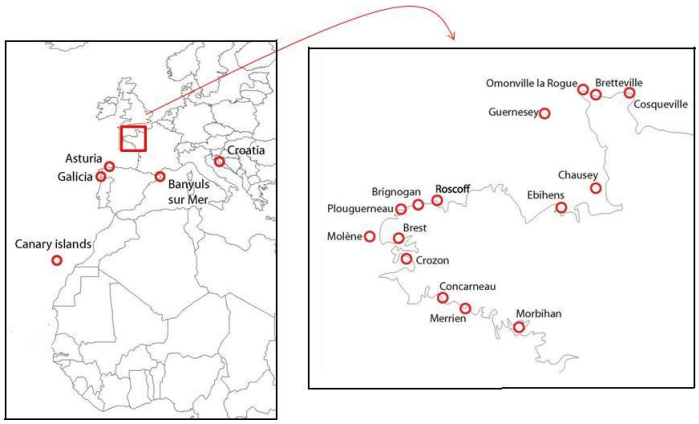

Figure 1: Geographical location of the 20 sampled populations

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Genetic analysis. DNA was extracted with the CTAB method (Doyle and Doyle 1987).
For mt COI gene, polymerase chain reactions (PCR) were performed on 0.1µg of DNA and we used the specific primers: COIfw: 5' - CCAGCTGGAGGAGGAGAYCC-3'
and COIrev: 5' - GCGTCTGGGTAGTCTGARTAKCG-3'.
For microsatellites, we used the eight specific markers described in Roussel et al (in revision). Amplified products were diluted in formamide containing GENESCAN-350(ROX) (Applied Biosystem) size standard, and size polymorphisms were screened using an ABI Prism 3130 DNA sequencer (Applied Biosystem). DNA fragments were analyzed using Genemapper software version 4.0 (Applied biosystem).

Data analysis. mt DNA sequences were aligned using the Clustal W accessory application of Bioedit (Hall TA, 1999) and treated with Mega 4.02 (Kumar et al. 2004; Tamura et al. 2007). Minimum evolution trees were determined and the average distance between the different clades calculated according to the Kimura 2-parameter model. Haplotypes were also studied by using the Fluxus Network Software (http://www.fluxus-engineering.com/sharenet.htm). Multivariate analysis was performed by using R software.
Microsatellite data were analyzed by using the R software for genetic distances calculations and representation. Bayesian analysis was performed with the TESS software (François et al. 2006). Runs were based on a burn-in period of 10,000 followed by 10,000 iterations. Five replicates were performed for a K value of 3 determined by using the DIC. All runs used the admixture model and a spatial dependence of 0.7.

Results and discussion

Phylogeny of European abalone. A number of 18 major haplotypes was evidenced in the 20 populations, and the first step of the work was to study the distribution of these haplotypes, population by population, (figure 2).

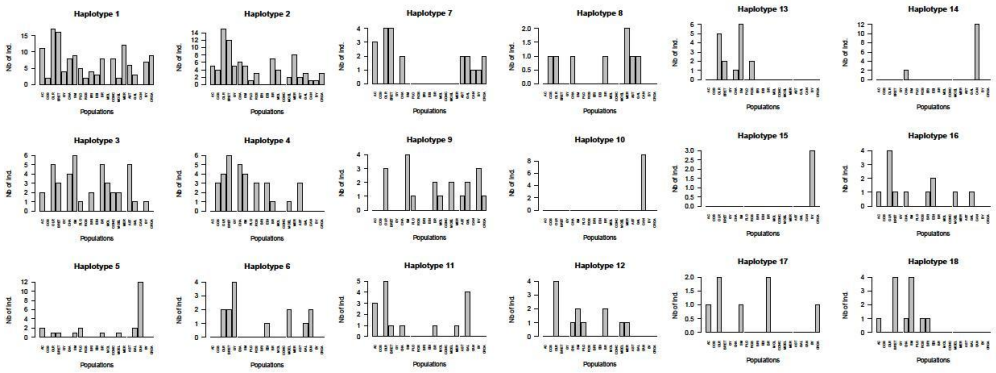

Figure 2: COI haplotypic distribution for the 20 populations.

These results evidence that Canary Islands population presents differences by report to the other populations: one haplotype (14) is only present in this population, another one (10) is present in this population and in another one but with a lower representativity, and a last one is find in numerous populations in low frequence, but in high frequence in Canary Islands. Two other haplotypes are present in all populations but not in Canary Islands.

A phylogeny study (data not shown) evidenced the presence of two clades inside the populations, with a 3.3% divergence. The individuals belonging to these clades live in sympatry, but with a different ratio of each ones in the populations. These two clades do not exactly fit with the classical separation of the two sub-species based on morphological traits: *Haliotis tuberculata tuberculata* and *Haliotis tuberculata coccinea*. A principle component analysis was performed to observe the populations differenciation on the basis of COI haplotypes (figure 3).

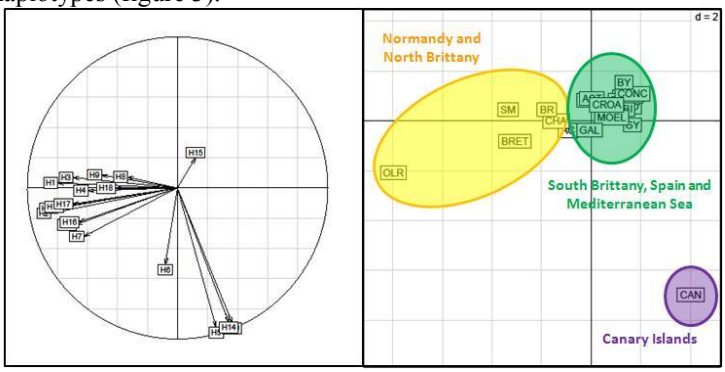

Figure 3: Principle Component Analysis for the 20 populations, based on the results of the COI gene data (plan 1).

Here, the populations can be separated according to the sub-species ratio: population from Canary Islands presents a high proportions (90%) of clade 2 halpotypes whereas the other populations presents highest proportions of clade 1 haplotypes with a less important value (75%) for Normandy and North Brittany populations than for south Brittany and Spain (85%), and 100% value for Mediterranean sea. And when individuals from the two clades live in the same area, they can cross themselves without any problem.

Population genetics of European abalone. Contrarily to the mt COI gene of maternal inheritance, microsatellite markers used for population genetics are nuclear and of biparental inheritance. A multiple component analysis evidenced the presence of 2 groups of populations (figure 4) Mediterranean Sea populations and Atlantic populations.

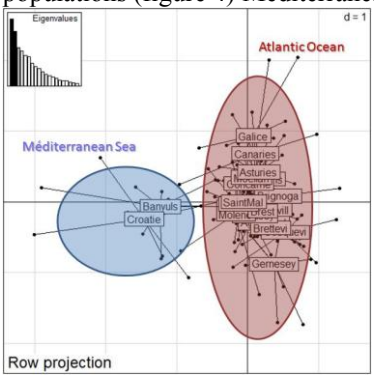

Figure 4: Multiple Component Analysis for the 20 populations, based on the results of the microsatellite data.

A more precise analysis with a genetic distance calculation (Nei's distance) was performed (data not shown), and evidence the presence of 3 groups: Mediterranean group, Canary Island and Spain group, and Brittany and Normandy group (with a subdivision between Normandy and North Brittany, and West and South Brittany). A bayesian analysis was also performed to complete these results (data not shown), and we obtained a confirmation of the previous results, with some precisions. With an hypothesis of 3 genetic clusters, Brittany and Normandy populations belongs to the first one, Canary Islands belongs to the second one with some genetic traces of the first one, Spain populations belongs to the first one with traces of the second one, and Mediterranean populations belongs to the third one with traces of the first cluster for the population closest to Gibraltar strait. This last results seems to show that Mediterranean populations were genetically different from Atlantic populations, but that some individuals from North Atlantic are colonizing this area.

Conclusion

These results bring some useful information concerning the phylogeny and population genetics of European abalone. The existence of two sub-species defined by morphometry did not bring information concerning the history of the species contrarily to molecular analysis. Different scenario of evolution are proposed to explain these discrepancies. Moreover, the results concerning population genetics brings some useful information concerning the genetic pools of European abalone species. For instance Mediterranean species are not used in selection because of their small size. But as they belongs to another genetic cluster, we can suppose that they possess original genes which could be used in selection programs, for example for diseases resistance.

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