MITOCHONDRIAL AND MICROSATELLITE GENETIC DIFFERENTIATION OF THE FLAT OYSTER *OSTREA EDULIS* ALONG THE EUROPEAN COASTS, AND COMPARISON WITH ALLOZYME DATA.

S. Lapègue[1], E. Diaz Almela[2], S. Launey[1], C. Ledu[1], P. Boudry[1], Y. Naciri-Graven[1], F. Bonhomme[2]

[1]IFREMER, Laboratoire de Génétique et Pathologie, B.P. 133, 17390 La Tremblade, France.
[2]CNRS, Laboratoire Génome, Populations, Interactions. 1 quai de la daurade 34200 Sète, France.

Introduction

Natural populations of the European flat oyster, *Ostrea edulis*, have been harvested since Roman times. This species is distributed along the European Atlantic coasts, from Norway to Morocco, as well as the Mediterranean Sea and Black sea coasts. Previous studies, based on allozymes, have concluded that isolation by distance is taking place in the Mediterranean sea and the Atlantic ocean. Because of the presumed non neutrality of these markers, another approach was made to examine the genetic differentiation of this species using nuclear microsatellite and mitochondrial markers, both considered as neutral, and to compare the results to those observed with allozymes.

Materials and Methods

A total of 507 individuals from 13 populations sampled in the repartition area were analysed with 5 microsatellite markers and single strand conformation polymorphism (SSCP) of a 300 bp fragment of the mitochondrial 12S rRNA gene.

Results

The average intra-population gene diversity of the 12S rRNA gene was twice smaller than the average for microsatellite markers. The genetic differentiation between populations revealed using the mitochondrial marker was seven fold greater than the one obtained with microsatellite markers, and three fold greater than the one previously obtained with allozymes. The Neighbor-joining dendrograms also showed slight differences, but genetic and geographic distances were significantly correlated for all markers. We discuss the possible reasons for the differences observed between mtDNA and nuclear markers (effective population size, mutation rates, …). Two additional samples from the Black Sea showed large differences with the other populations, and haplotype diversity was very low. This suggests that gene flow from the Mediterranean Sea to the Black Sea is very small and that a recent bottleneck has occurred.

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Mitochondrial and microsatellite genetic differentiation of the flat oyster *Ostrea edulis* along the european coasts, and comparison with allozyme data

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Ostrea edulis L. (Linné, 1758)

- ✓ Native European species
- ✓ Harvested since Roman times
- ✓ Sensitive to *Bonamia ostreae* and *Martelia refringens*
- ✓ brooding species
- ✓ alternative hermaphrodite

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Ostrea edulis L.

(Jaziri, 1985)

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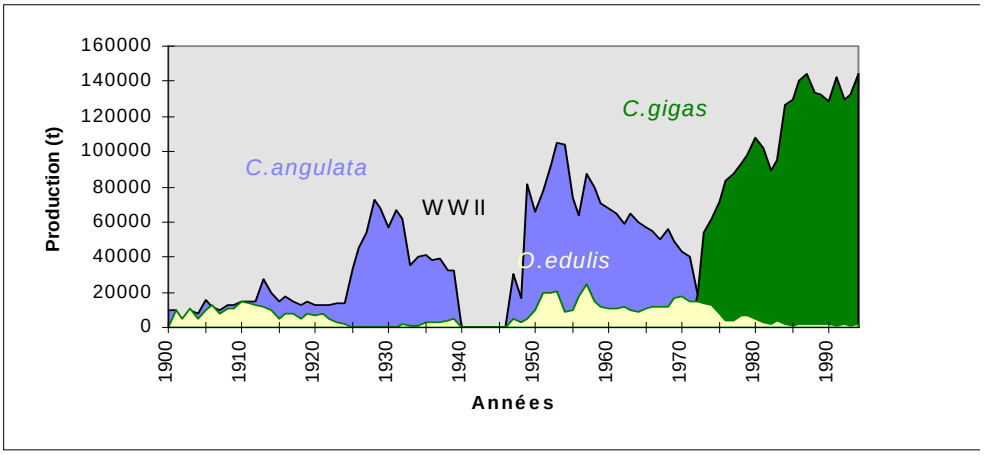
From hand gathering...

... to modern oyster farming...

... and decline...



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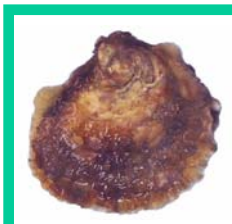
Research program initiated in 1985
to select flat oysters resistant to bonamiosis
first by pathologists then with geneticists

Ifremer

in the Genetic and Pathology Laboratory
of IFREMER, La Tremblade, France



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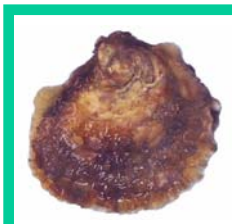
Ostrea edulis population genetic studies:

✓ to evaluate genetic diversity,

✓ to establish a selection program based on a large genetic basis

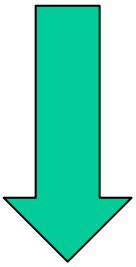
Which genetic markers ???

- ✓ **Nuclear markers:**
  - **allozymes**
  - **microsatellites**
- ✓ **Mitochondrial markers**



ALLOZYME STUDIES:

- ✓ Numerous studies
- ✓ **Saavedra *et al.*, 1987, 1993, 1995:**
  - ✓ 3 alleles by locus
  - ✓ Low variability in Atlantic and also in Mediterranean Sea
  - ✓ **clinal pattern of differentiation around Gibraltar**



- ✓ secondary contact between two earlier (last Quaternary glaciations) differentiated stocks (Atlantic and Mediterranean)

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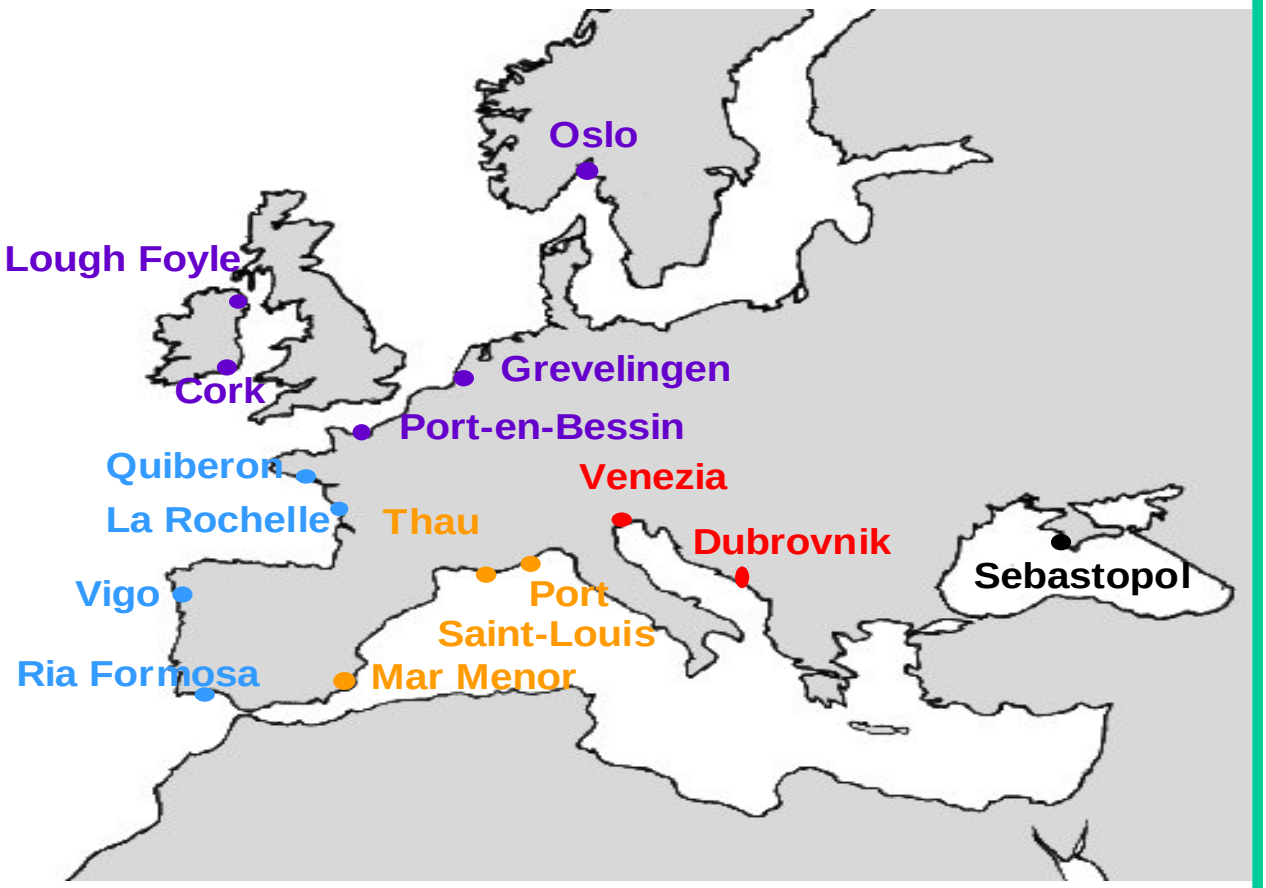
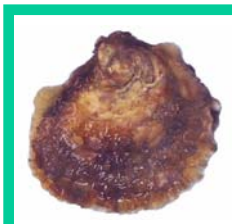
Sampling



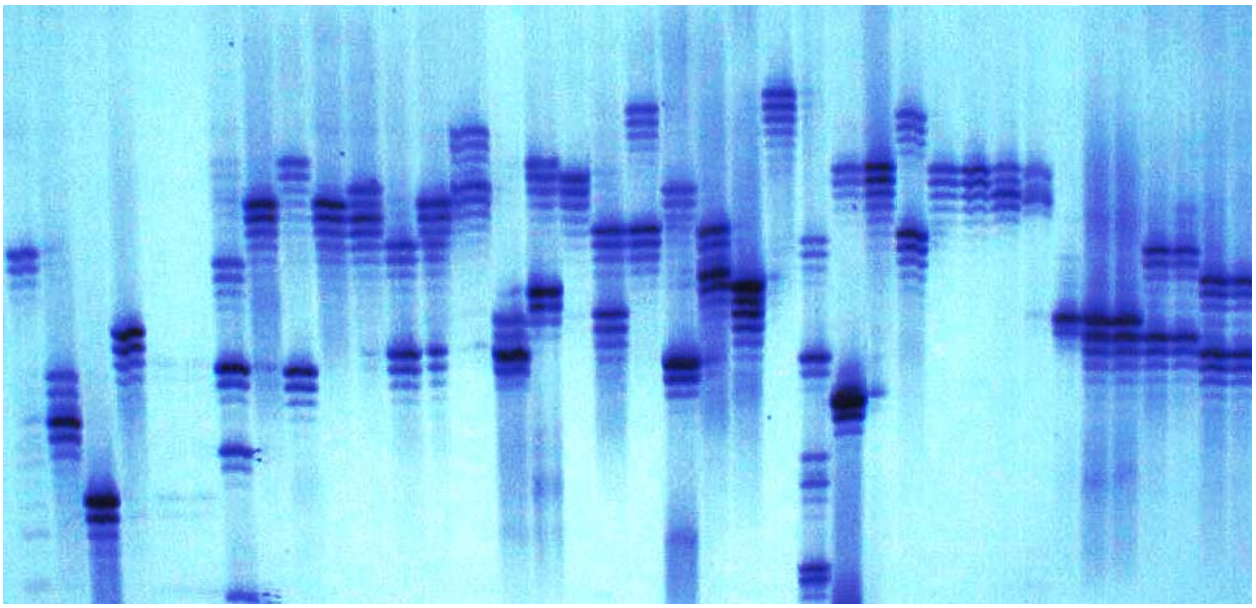
15 populations :

- Atlantic Ocean
  - north (5)
  - south (4)
- Mediterranean Sea
  - West (3)
  - East (2)
- Black Sea (1)



MICROSATELLITES STUDY (S. Launey, 1998)

✓ 5 microsatellites, anonymous and hypervariable



• Mean number of alleles by locus and by region: 24

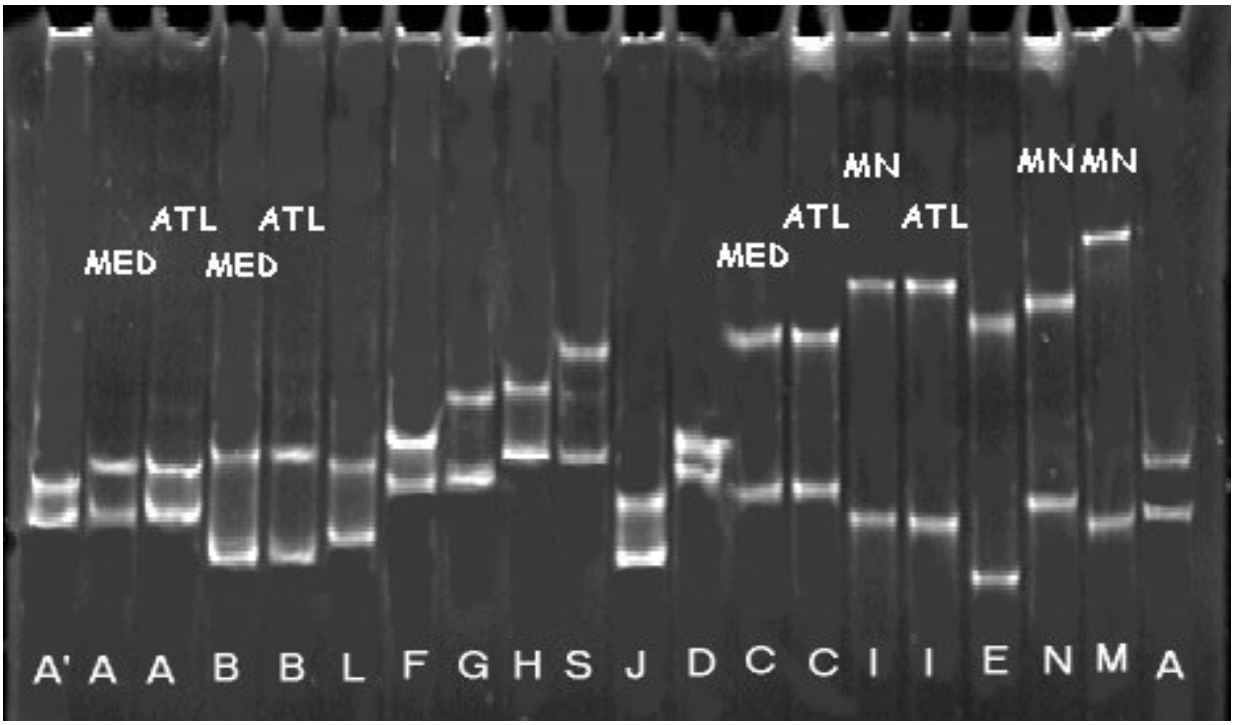
• Up to 38 alleles for one locus

MITOCHONDRIAL STUDY (Diaz Almela, 1999)

- ✓ 1 locus: a 12S gene fragment
- ✓ studied by SSCP

(Single Strand Conformation Polymorphism)

15 mitochondrial haplotypes



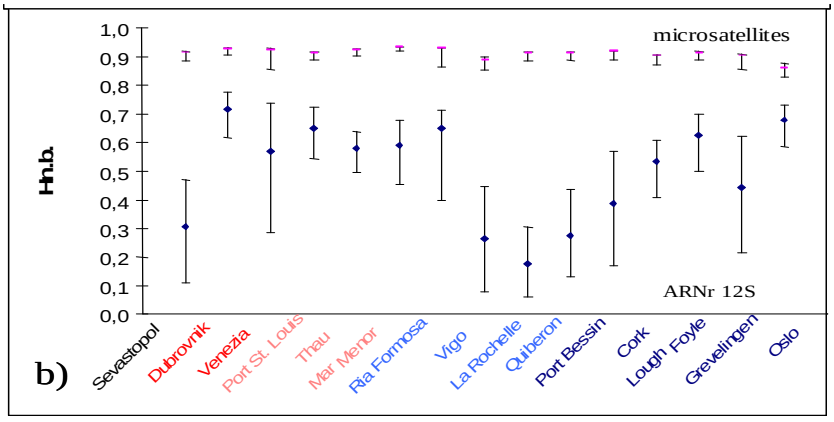
2 frequent haplotypes (A and B)

Within population variability:

✓ depends on the marker studied

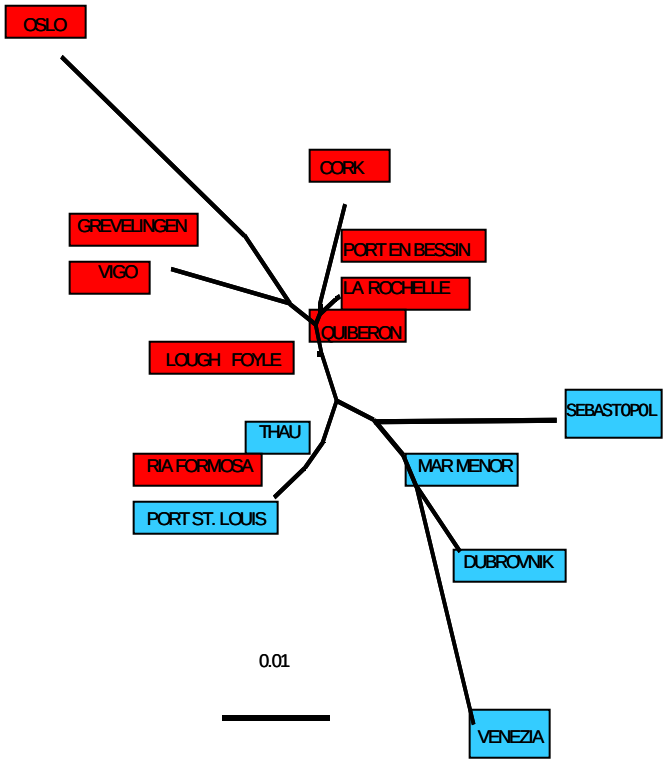
✓ nuclear versus mitochondrial markers:
Atlantic < Mediterranean

✓ variable among populations with the mitochondrial marker

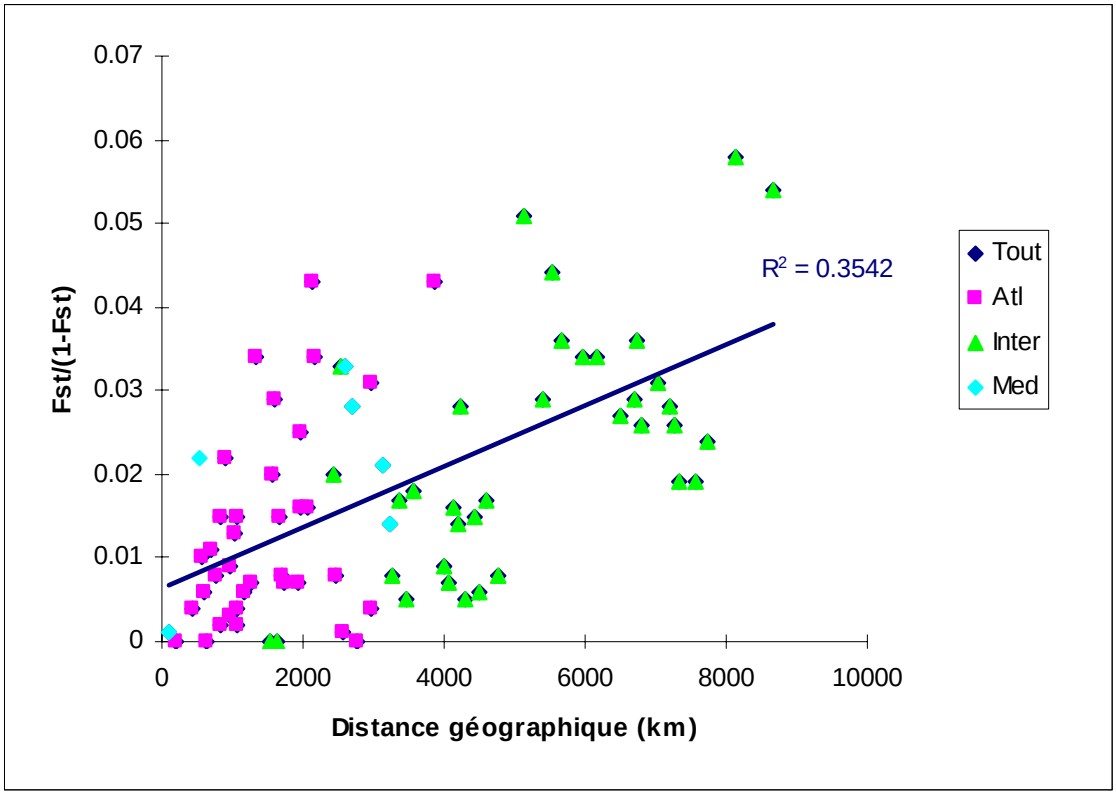


Differentiation

✓ very congruent for the nuclear markers:
Atlantic / Mediterranean structure



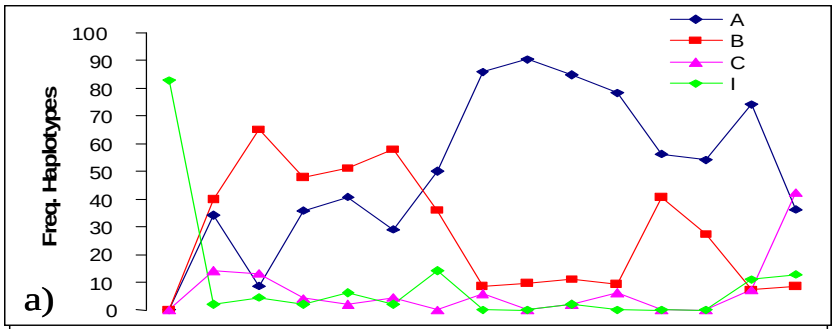
Isolation by distance



✓ there is a correlation between genetic and geographic distances for the microsatellite markers

Mitochondrial differentiation

✓ haplotypes A et B respectively more frequent in Atlantic and Mediterranean populations



✓ Black Sea population particular status

Mitochondrial versus nuclear differentiation

✓ global Atlantic / Mediterranean Sea structure but:

✓ no intra Mediterranean structure

✓ and a high quantitative difference:

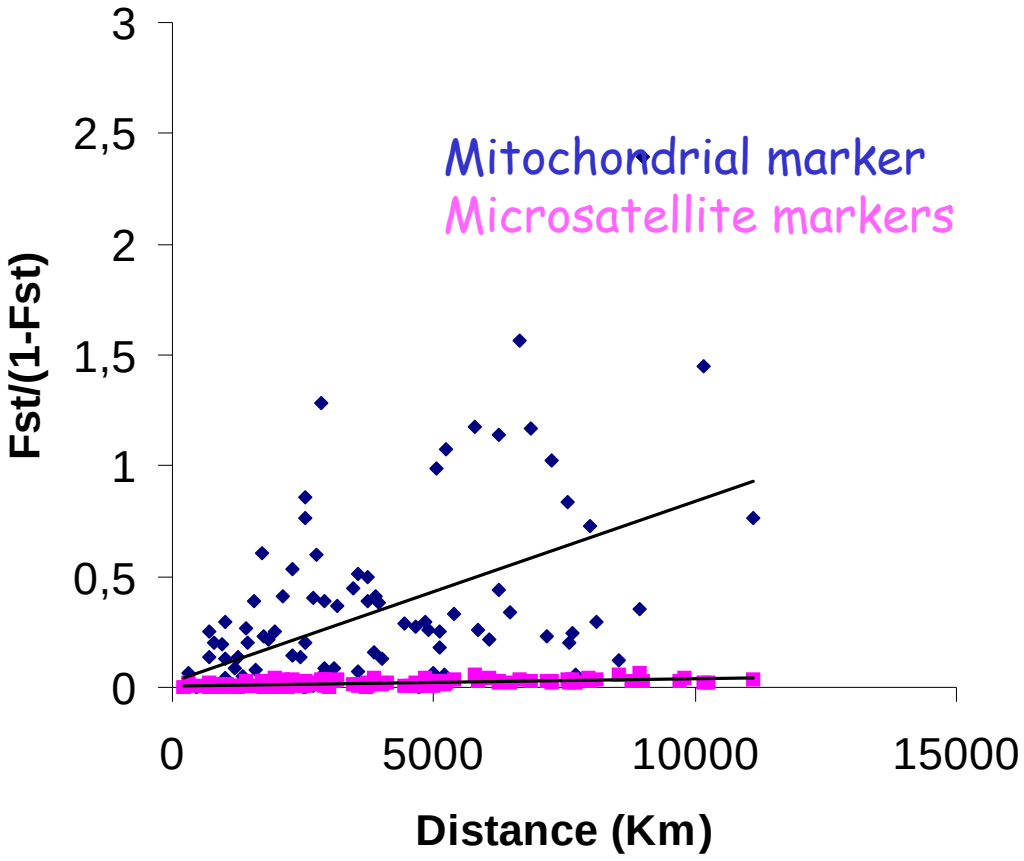
F_{ST} microsatellites = 0.019

10 fold

F_{ST} mitochondrial = 0.244

✓ Causes:
- ✓ biology
- ✓ markers bias

Isolation by distance



CONCLUSIONS

- ✓ confirmation of the genetic structure
- ✓ different levels of variability

- ✓ a strong mitochondrial structure: causes and consequences

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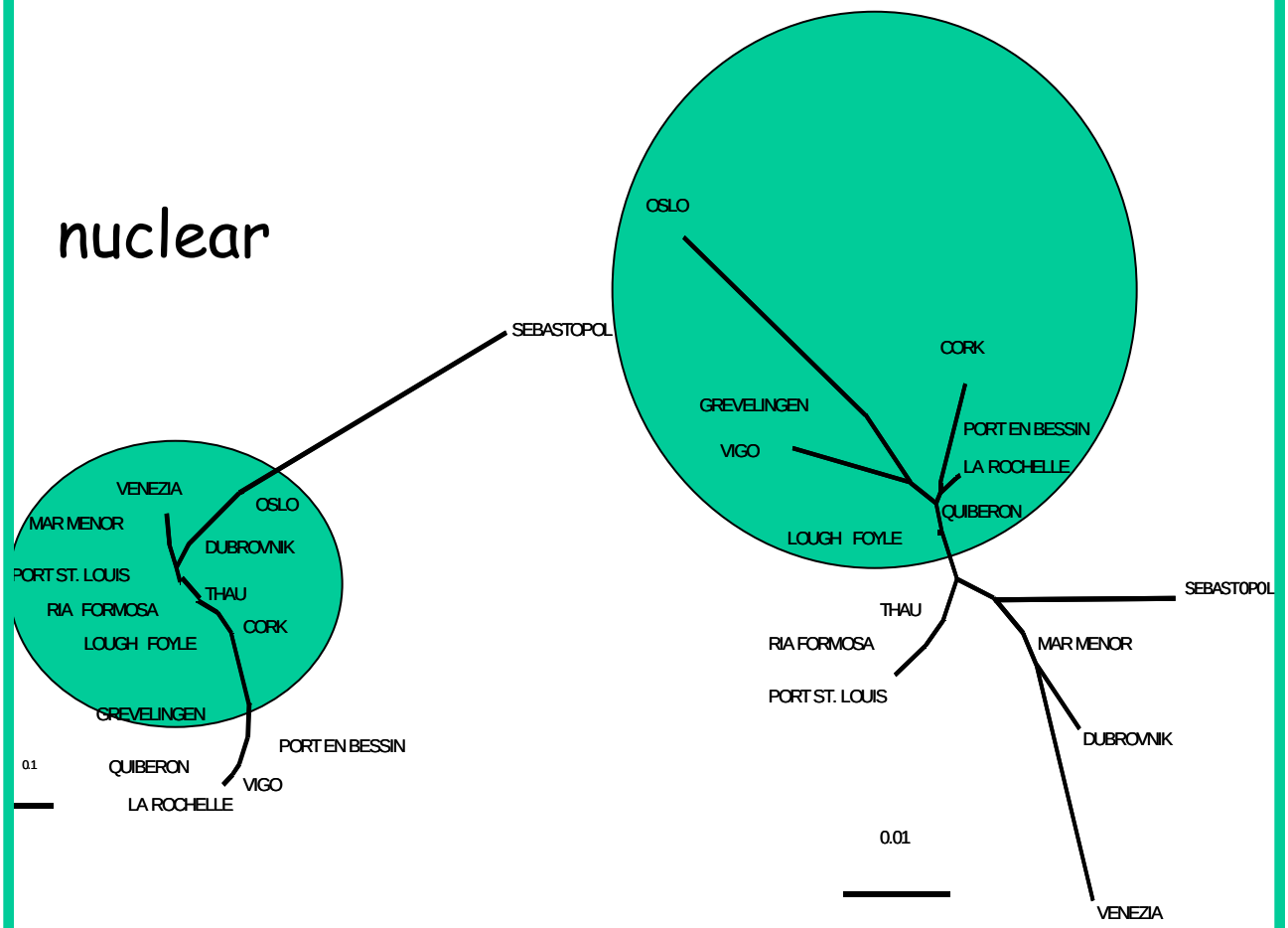
mitochondrial

nuclear



Atlantic populations

Neighbor joining trees

MICROSATELLITES STUDY (S. Launey, 1998)

✓ 5 microsatellites, anonymous and hypervariable

A

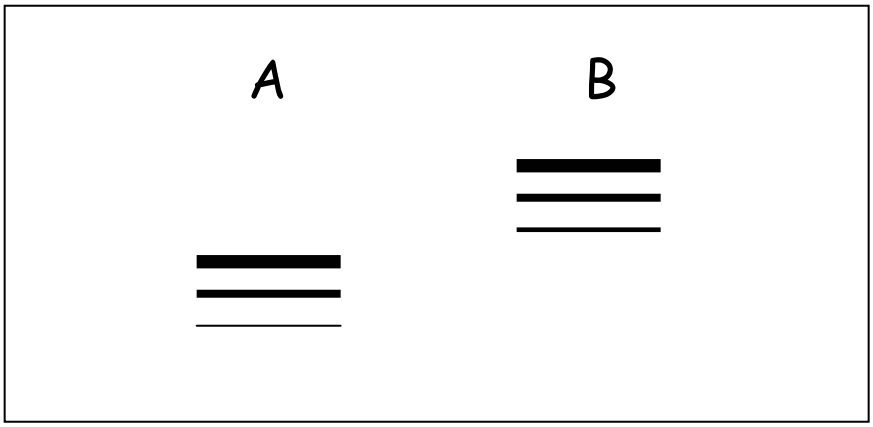
5'- AATCGCGCGCGCGCGCGCGTAC -3'

3'- TTAGCGCGCGCGCGCGCGCATG -5'

B

5'- AATCGCGCGC**CGCGCG**GCGCGCGCGTAC -3'

3'- TTAGCGCGCG**GCGCGC**CGCGCGCGCATG -5'



MITOCHONDRIAL STUDY (Diaz Almela, 1999)

✓ 1 locus: a 12S gene fragment

✓ studied by SSCP (Single Strand Conformation Polymorphism)

A
5'- AATCGTTGCAACTCGTAGT -3'
3'- TTAGCAACGTTGAGCATCA -5'

B
5'- AATCGTTGCCACTCGTAGT -3'
3'- TTAGCAACGGGTGAGCATCA -5'