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# Mitochondrial and nuclear DNA phylogeography of two cupped oysters *Crassostrea gigas* and *Crassostrea angulata*

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#### Introduction

- 1793 *Crassostrea gigas* (Thunberg)
- 1819 *Crassostrea angulata* (Lamarck)
- 1868 Accidental introduction of *C. angulata* from Portugal into France
- 1950's Up to 100,000 tons per year of *C. angulata* produced in France
- 1966 First case of introduction of *C. gigas* into France
- 1970 Major mortality leading to disappearance of *C. angulata* by 1973
- 1971 Massive introduction of *C. gigas* from Canada and Japan into Europe
- 1974 "Portuguese and Japanese oysters are the same species" (Menzel, 1974): they are inter-fertile and morphologically identical.
- 1980's Allozyme-based studies showed no genetic differentiation between *C. angulata* and *C. gigas* (Buroker et al. 1979, Mattuicci & Villani, 1983).

## How do we explain the presence of these two conspecific taxa on opposite sides of the world ?

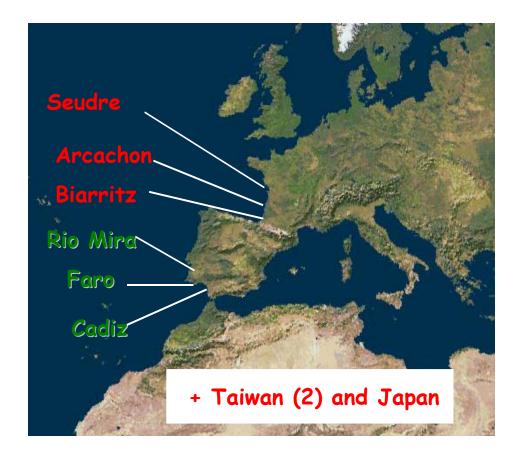
2 hypotheses:

- (1) Common ancestry: C. gryphoides (Stenzel, 1971; Durve, 1986).
- (2) Introduction: From Japan to Portugal or reverse (Buroker *et al.*, 1971).

This study:

Genetic differentiation among *C. angulata* and *C. gigas* populations using mitochondrial and nuclear DNA markers.

## Sampling of *C. gigas & C. angulata*



9 populations 383 individuals

Samples were first classified as *C. angulata* or *C. gigas* according to the information provided by the suppliers.

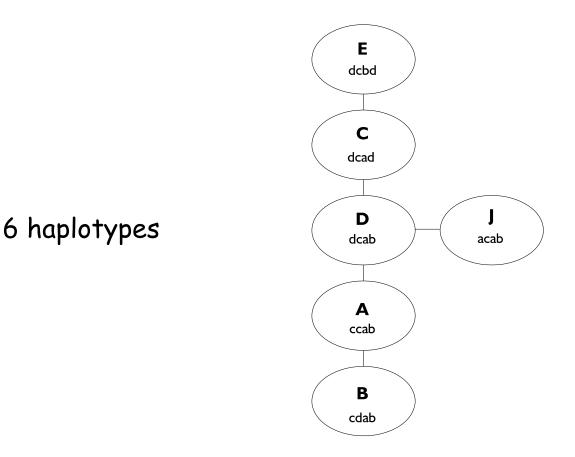
## Mitochondrial markers

(Boudry et al. 1998)

PCR amplification of a 710nt mitochondrial fragment (Cytochrome Oxydase C subunit I)

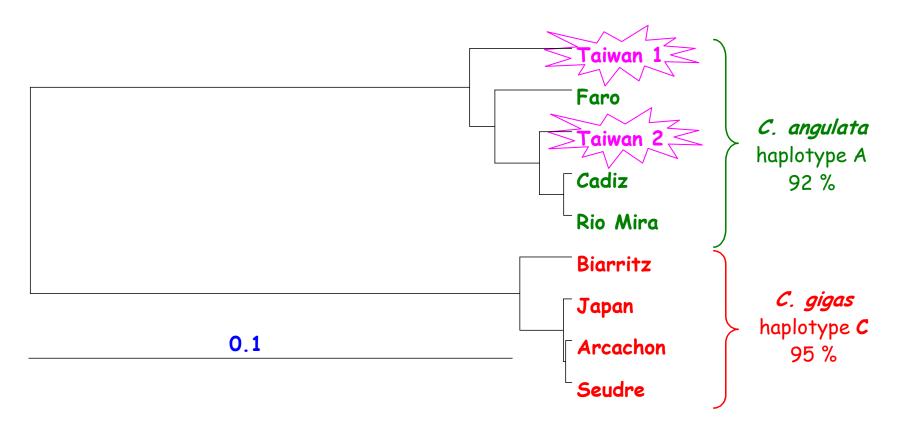
9 restriction enzymes tested

4 restriction enzymes showed polymorphism



#### **Mitochondrial DNA**

Neighbor-joining tree based on Reynolds' genetic distance (Reynolds, 1983)

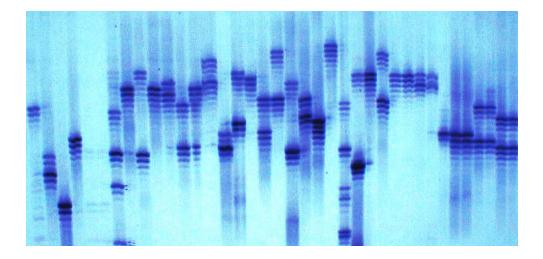


### Microsatellite markers

(Huvet, 1998)

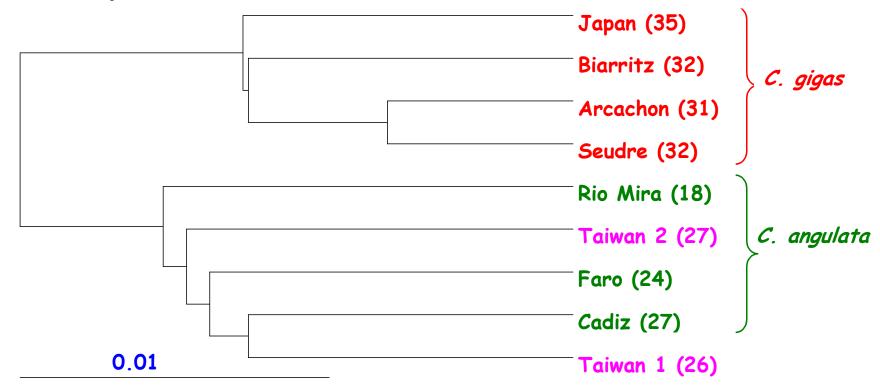
PCR amplification of 3 polymorphic loci (Magoulas *et al.*, 1998)

	CG44	CG49	<i>CG</i> 108
Total number of alleles	52	52	54
Alleles/population	18-29	17-38	20-40
Observed heterozygosity (Ho)	0.5-0.9	0.4-1	0.8-1
Gene diversity ( <i>He</i> )	0.9	0.9	0.9



#### Microsatellite markers

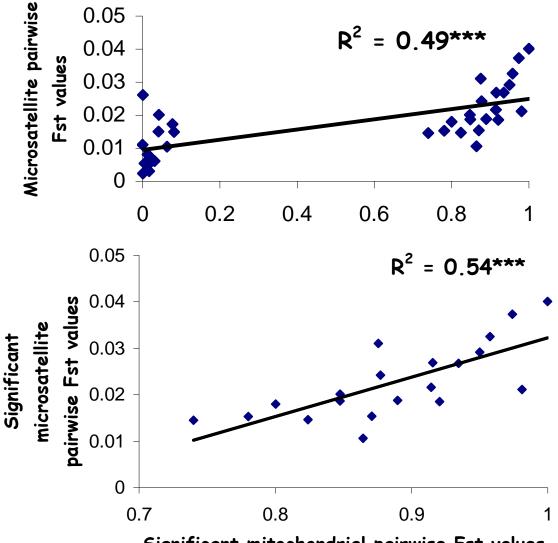
Neighbor-joining tree based on Reynolds' genetic distance (Reynolds, 1983)



Population (mean number of alleles)

## Comparison between mitochondrial and microsatellite data

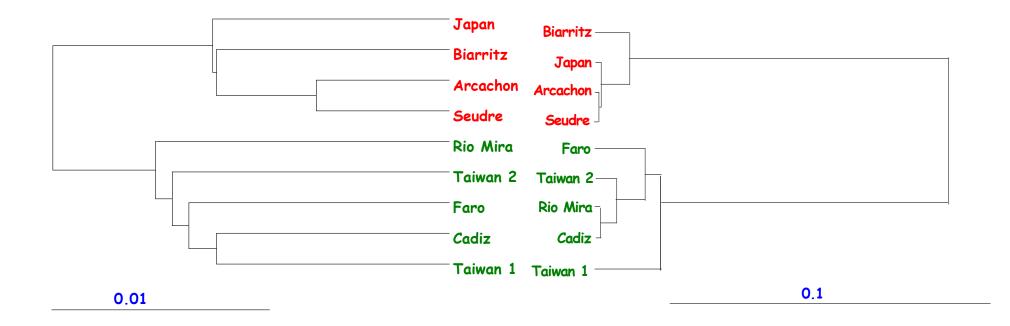
	Global Fst	
Mitochondrial DNA	0.81	
Microsatellites	0.017	



Significant mitochondrial pairwise Fst values

#### Comparison between mitochondrial and microsatellite data

Neighbor-joining tree based on Reynolds' genetic distance (Reynolds, 1983)



### Conclusion

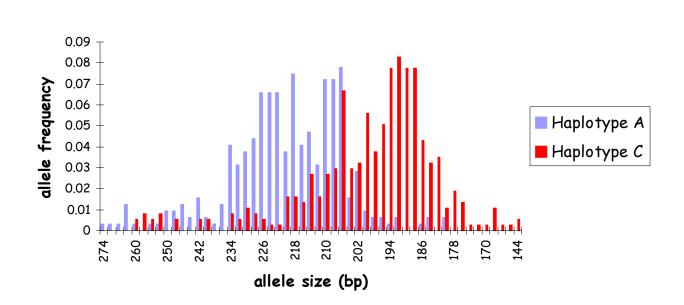
Portuguese oysters originated from Asia, probably from Taiwan.

The Asian origin of *C. angulata* was supported by *mt*DNA sequence data and the divergence date between *C. angulata* and *C. gigas* was estimated at 1 to 2 million years (O'Foighil *et al.* 1998).

Portuguese oysters were probably transported by ships from "Formosa", reached by European seafarers during the XVII<sup>th</sup> century. These naturalised in Southern Europe and then were described by Lamarck.

Microsatellite markers show no evidence of drift following this introduction.

Mitochondrial and markers give qualitatively but not quantitatively congruent results, probably due to different evolutionary forces on these markers (drift, mutation).



Distribution of allele frequencies in terms of allele size for the locus CG44