

Evidence for natural hybridisation between the two oyster sub-species *Crassostrea gigas* and *Crassostrea angulata* in southern Europe

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The logo for Ifremer, featuring a stylized grey fish shape composed of a grid of dots, positioned above a yellow horizontal bar.

Ifremer

Hybridisation between closely related marine bivalve taxa

- ☞ Taxa distributed along geographic clines
- ☞ Restricted gene flow between populations of closely related taxa
 - ☞ Local genetic discontinuities, maintained by the lower fitness of hybrids

☞ **Hybrid zones**

e.g. Mytilus, Mercenaria...

C. angulata and *C. gigas* two closely related oyster taxa

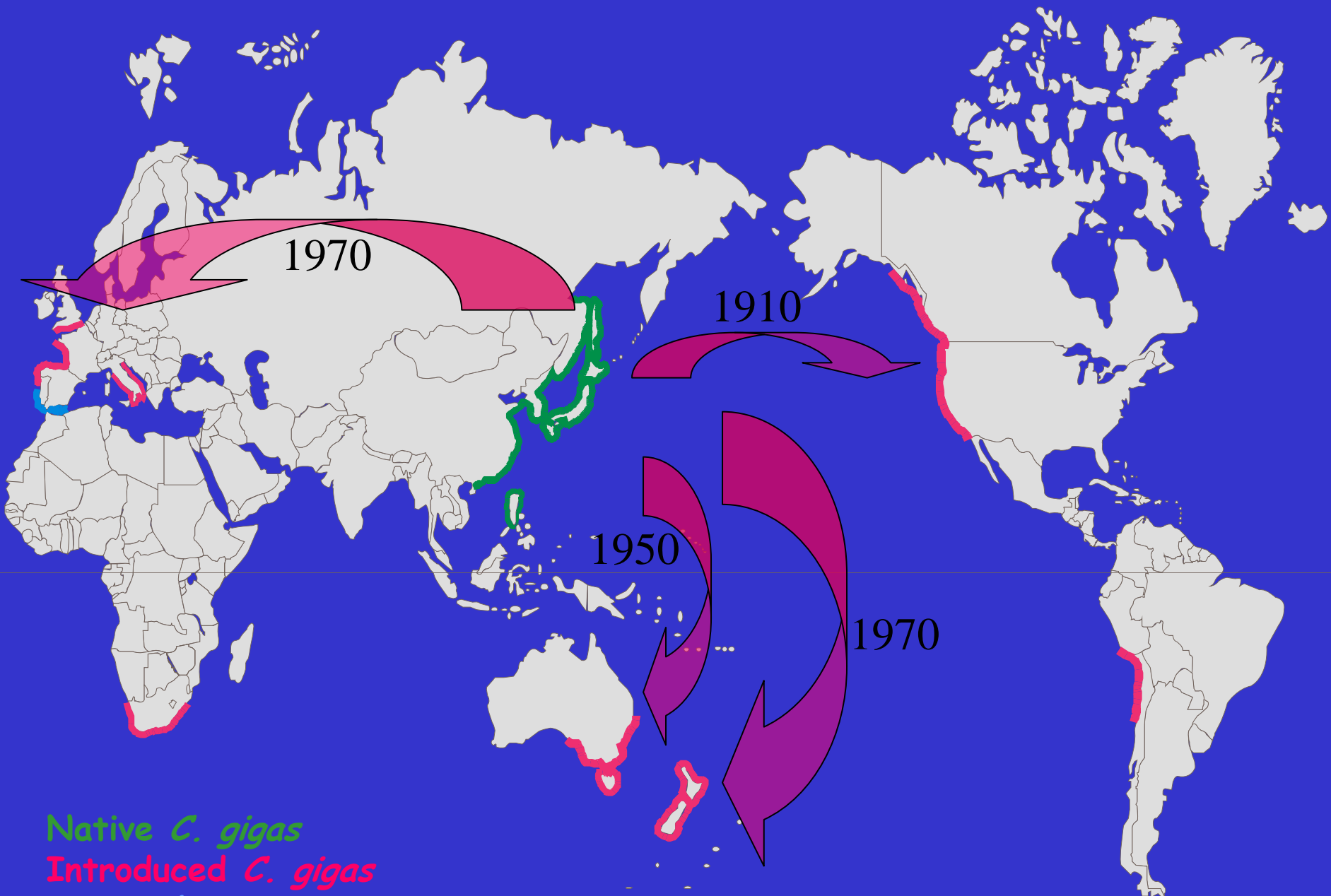
- Pacific oyster
Crassostrea gigas
Thunberg (1793)



- Portuguese oyster
Crassostrea angulata
Lamarck (1819)



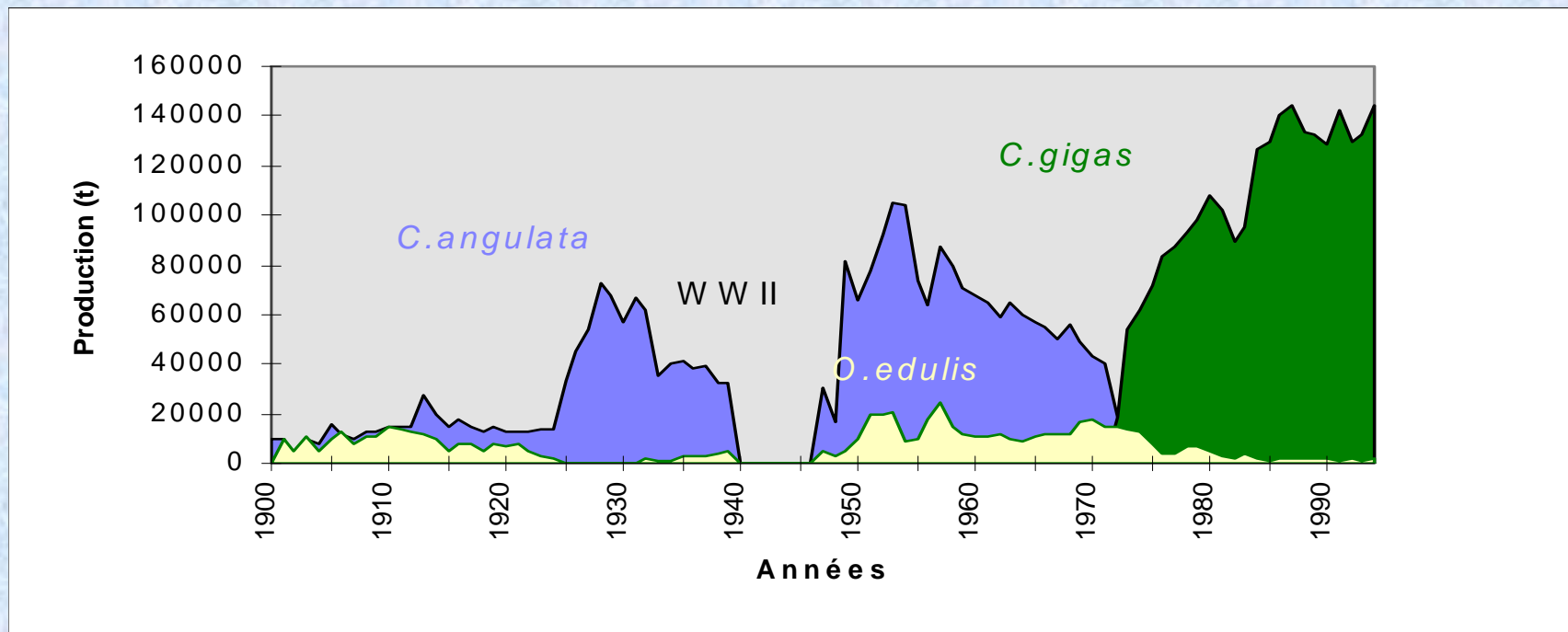
- ↙ Initially distinguished according to their geographic origin (*i.e.* Japan *versus* Portugal)



Native *C. gigas*
Introduced *C. gigas*
C. angulata

History of oyster farming in France

- Introduction of *C. angulata* from Portugal into France in 1868
- Introduction of *C. gigas* from Japan into Europe in the early 1970s



↙ *C. gigas* was introduced into Europe due to the disappearance of *C. angulata*

C. angulata and *C. gigas*: a single species

- Absence of reproductive barriers
- Morphologically indistinguishable
- Highly similar karyotypes
- No significant genetic differentiation based on allozymes
- Limited genetic differentiation based on microsatellites (F_{st} between taxa = 0.02)

but...

- Higher growth rate in *C. gigas*
- Differential susceptibility to the "gill disease" (iridovirus)
- Diagnostic mitochondrial haplotypes

The Asian origin of *C. angulata*

Recent studies, based on mitochondrial DNA (O'Foighil et al., 1998; Boudry et al., 1998) and microsatellite markers (Huvet et al., in press) revealed the Asian origin of *C. angulata*



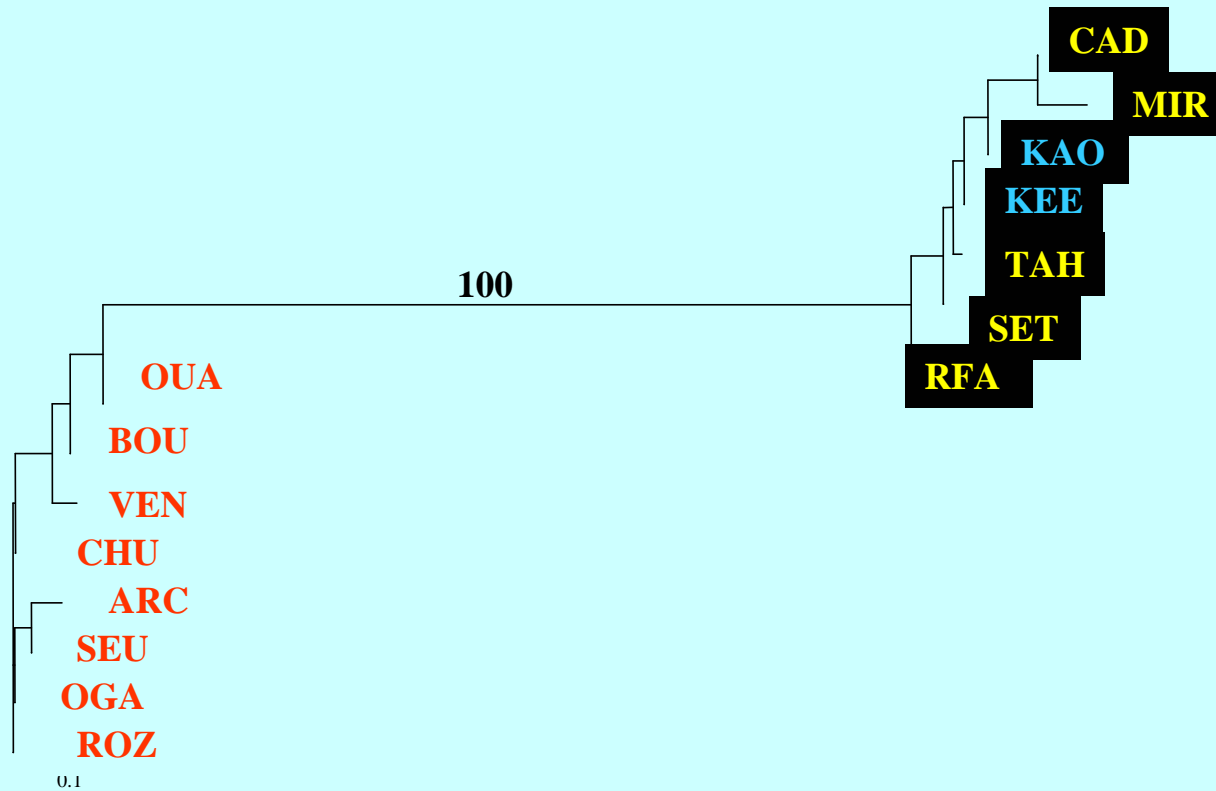
Hypothesis : *C. angulata* was transported from Asia to Portugal by merchant ships during the XVIIth century. They naturalised in Southern Europe where they were then described by Lamarck.

C. angulata populations

Taiwan populations

C. gigas populations

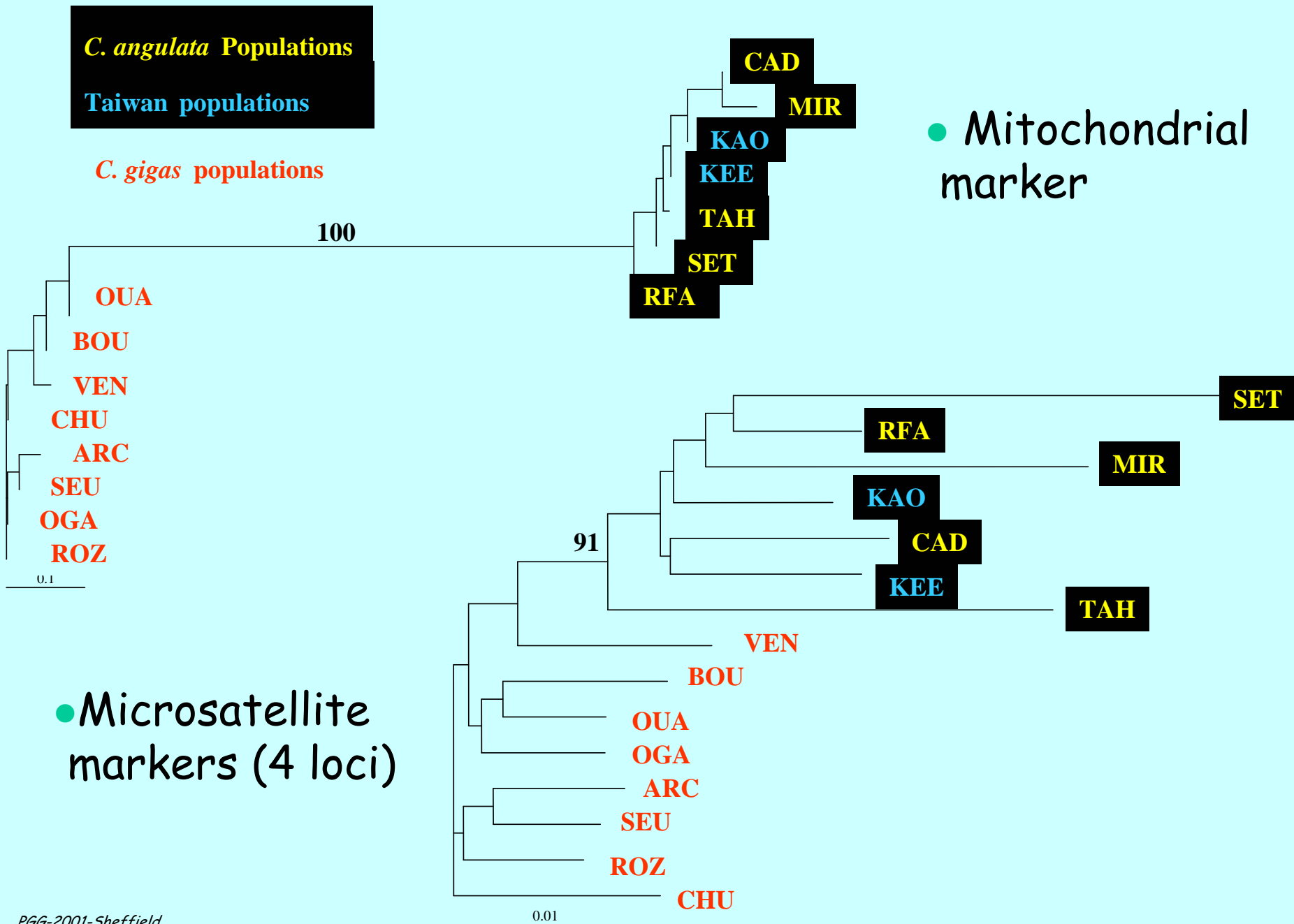
- Mitochondrial marker



***C. angulata* Populations**

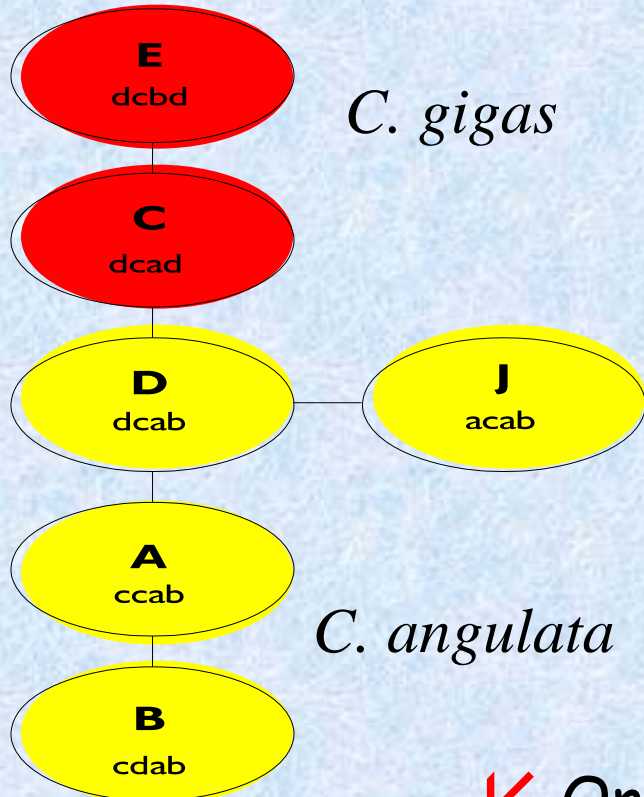
Taiwan populations

C. gigas populations



Mitochondrial diagnostic DNA marker (COI)

- 6 haplotypes



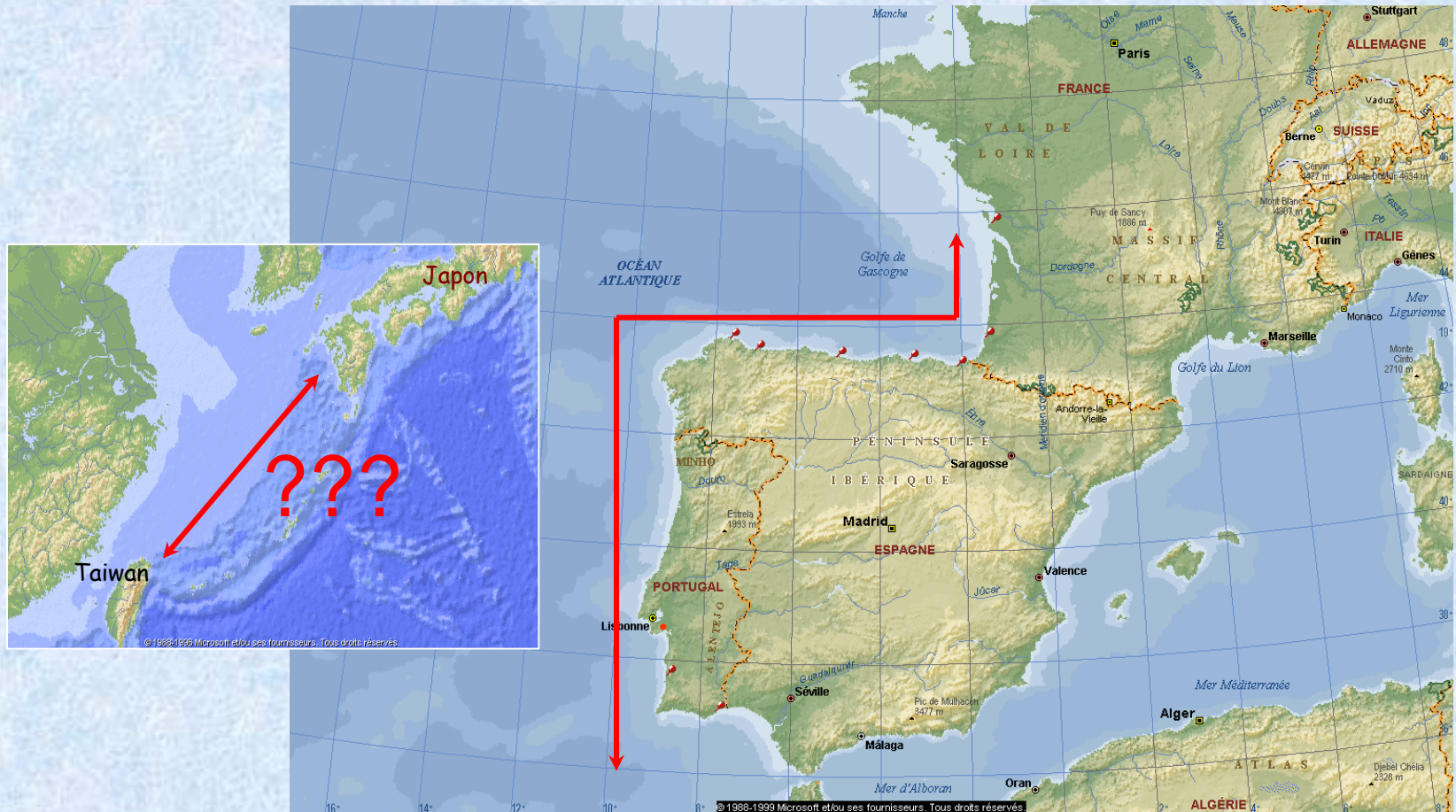
Pays	Population	N	Haplotypes					
			A	B	C	D	E	J
France	SEU	47			0.98		0.02	
	ARC	42			0.98		0.02	
	BOU	49	0.08		0.90		0.02	
	ROZ	48			0.96		0.04	
Japan	OGA	49			0.96		0.04	
South Korea	CHU	45			0.96		0.04	
Italy	VEN	24			0.92			0.08
Morocco	OUA	21	0.1		0.85		0.05	
	TAH	16	0.87	0.13				
Taiwan	KEE	23	0.88	0.04				0.08
	KAO	40	0.90	0.08				0.02
Portugal	RFA	35	0.86	0.03	0.11			
	MIR	29	1					
	SET	13	0.87	0.13				
	CAD	44	0.96	0.02		0.02		

⚡ Only 4 slightly "mixed" populations (<11%)

Two potential hybrid zones

↙ Taiwan - Japan ???

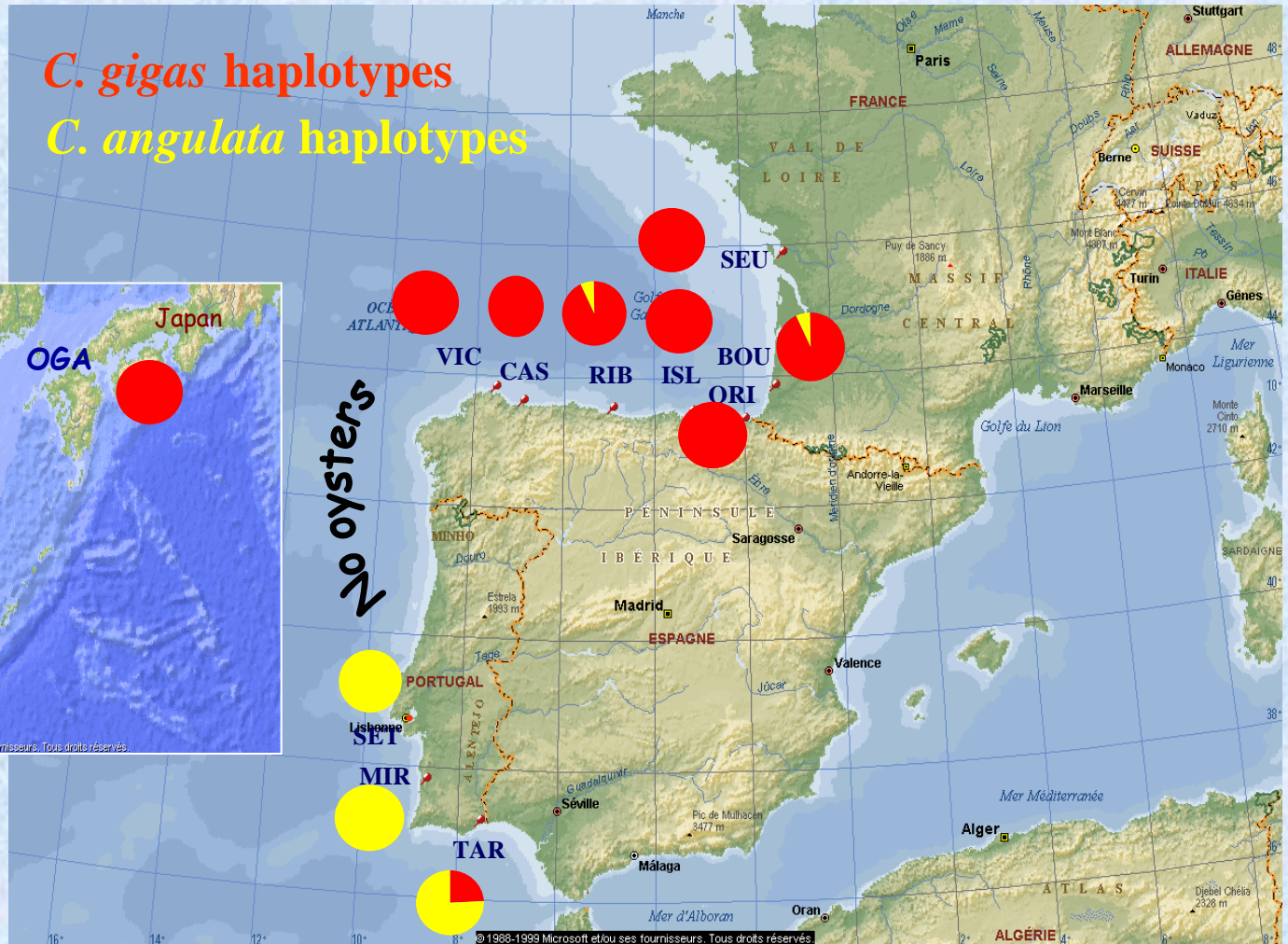
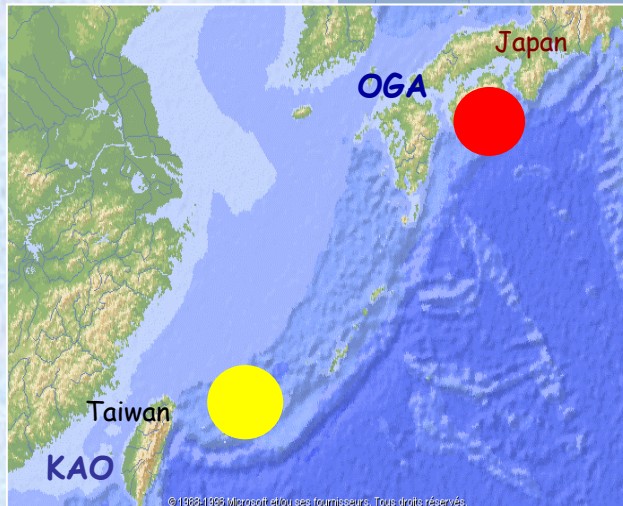
↙ France - Portugal ? → new sampling



Mitochondrial DNA (COI)

C. gigas haplotypes

C. angulata haplotypes



↙ Hybridisation or mixture ?

Search for a nuclear diagnostic marker

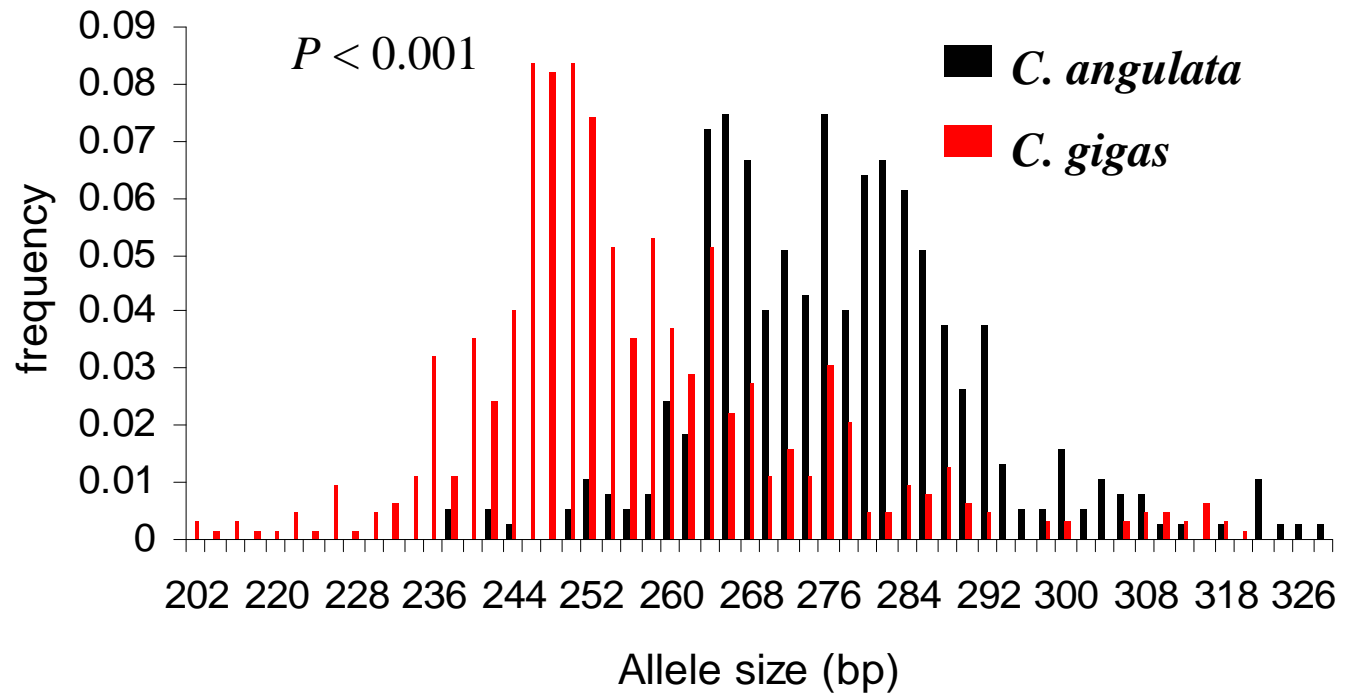
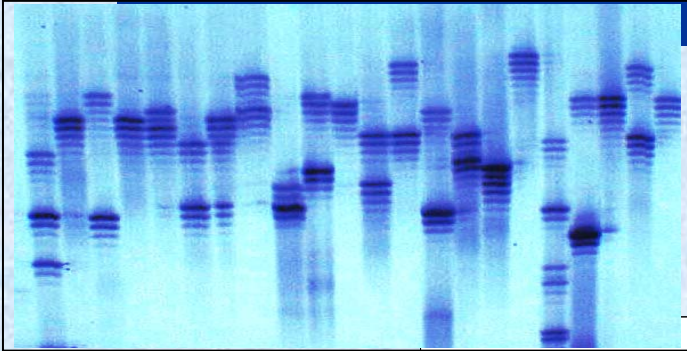
- ↙ Allozymes
- ↙ ITS DNA sequences
- ↙ intron length polymorphism
- ↙ DALPs



- ↙ Microsatellite allele size differences :

	CG44	CG49	CG108	L8	L10	L16	L48	μsatAMY
Size (<i>C. gigas</i>) (bp)	256.5	151.1	143.4	229.8	150.8	165.6	130.3	233.4
Size (<i>C. angulata</i>) (bp)	277.5	162.6	151.3	225.4	149.2	163.6	126	236.1
(Ta - Tg) (bp)	21	11.5	7.9	-4.4	-1.6	-2	-4.3	2.7
<i>P</i>	<0.001 ***	< 0.001 ***	< 0.001 ***	0.03 *	0.45 ns	0.28 ns	0.008 **	0.06 ns

Microsatellite allele size differences : locus CG44

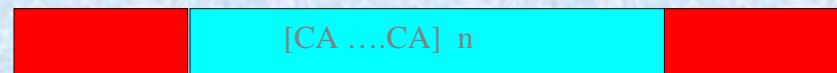


DNA sequencing of 2 alleles of the CG44 locus

- allele 266pb from a *C. angulata* individual
- allele 188 pb from a *C. gigas* individual

- 11 substitutions, 2 insertions (21 and 43 bp)
- allele 266 = 7 CA repeats (\neq 39 expected CA repeats)

allele 188
(*C. gigas*)

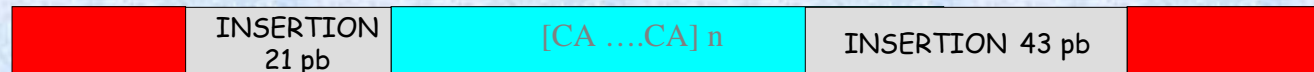


primer
CG44R S

MICROSATELLITE CG44

primer
CG44R AS

allele 266
(*C. angulata*)

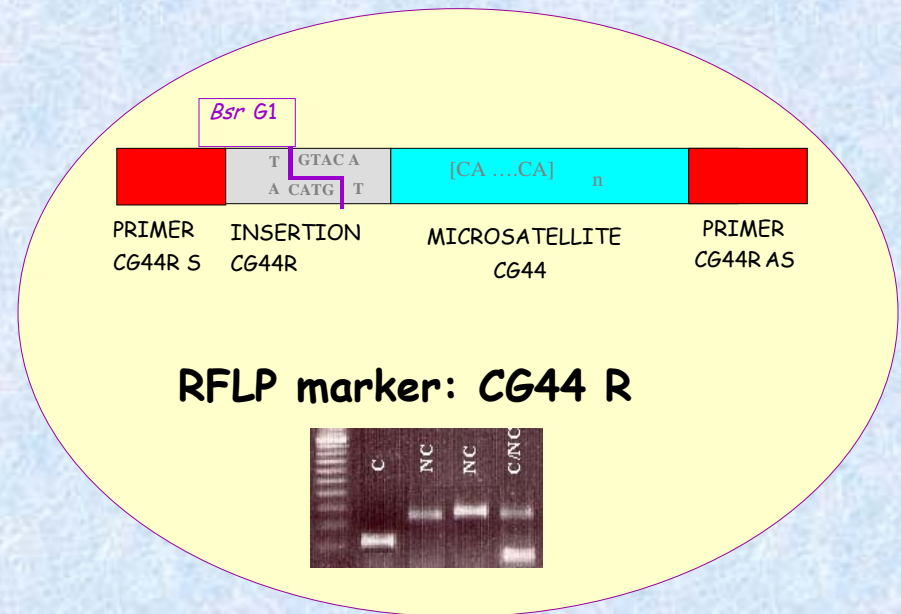


Frequencies of these 2 insertions ?

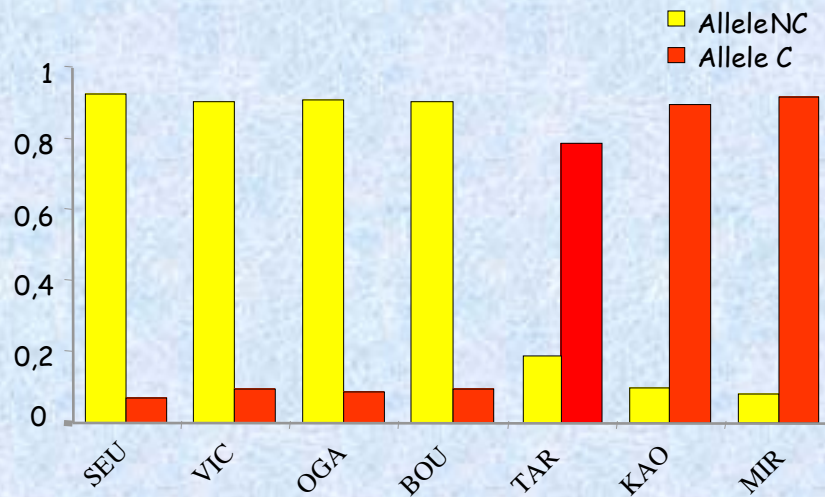
- 43bp Insertion : PCR amplified in 99% of the scored individuals 😞
- 21 bp Insertion : detected by the presence of a BsrGI restriction site:

Seudre = 90 %
(*C. gigas*)

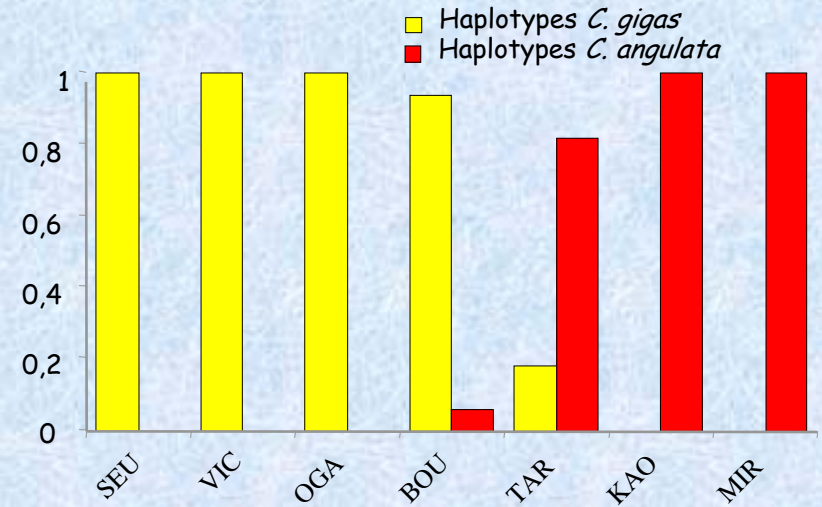
Rio Mira = 10 %
(*C. angulata*)



- nuclear marker CG44R



- mitochondrial marker COI



↙ CG44R is diagnostic at the population level
(Fst between taxa = 0.7)

↙ "Tavira" is clearly a mixed population

"Tavira" : mixture or hybridisation ?

- Intermediate allele frequencies at loci COI and CG44R
- Non significant heterozygote deficiencies at locus GR44R ($F_{is} = 0.11$) and a heterozygosity double that of the non mixed populations at locus CG44R (0.3 versus 0.16)
- Non significant linkage disequilibrium between the two loci ($d = 0.15$)
- ✚ Hybridisation between *C. angulata* and *C. gigas* in Southern Portugal

Conclusions

- ❏ The two taxa hybridise when they are brought together following the transportation of oysters due to aquaculture
- ❏ There is no evidence of natural gene flow between populations of the two taxa in Europe
- ❏ There is no hybrid zone between the two taxa in Europe

↙ Further studies are required to document the evolutionary history of both taxa in their natural habitats (drift, gene flow, selection...)

