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

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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*
**Introduction of**

*C. angulata* from Portugal into France in 1868

*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 (Fst between taxa = 0.02)

but...

- Higher growth rate in C. gigas
- Differential susceptibility to the “gill disease” (iridovirus)
- Diagnostic mitochondrial haplotypes
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.
Mitochondrial marker

C. angulata populations
Taiwan populations

C. gigas populations

PGG-2001-Sheffield
C. angulata Populations
Taiwan populations

C. gigas populations

Microsatellite markers (4 loci)

Mitochondrial marker

PGG-2001-Sheffield
Mitochondrial diagnostic DNA marker (COI)

- 6 haplotypes

C. gigas

C. angulata

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:**

<table>
<thead>
<tr>
<th></th>
<th>CG44</th>
<th>CG49</th>
<th>CG108</th>
<th>L8</th>
<th>L10</th>
<th>L16</th>
<th>L48</th>
<th>µsatAMY</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Size (C. gigas) (bp)</strong></td>
<td>256.5</td>
<td>151.1</td>
<td>143.4</td>
<td>229.8</td>
<td>150.8</td>
<td>165.6</td>
<td>130.3</td>
<td>233.4</td>
</tr>
<tr>
<td><strong>Size (C. angulata) (bp)</strong></td>
<td>277.5</td>
<td>162.6</td>
<td>151.3</td>
<td>225.4</td>
<td>149.2</td>
<td>163.6</td>
<td>126</td>
<td>236.1</td>
</tr>
<tr>
<td>(Ta - Tg) (bp)</td>
<td>21</td>
<td>11.5</td>
<td>7.9</td>
<td>-4.4</td>
<td>-1.6</td>
<td>-2</td>
<td>-4.3</td>
<td>2.7</td>
</tr>
<tr>
<td><em>P</em></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.03</td>
<td>0.45</td>
<td>0.28</td>
<td>0.008</td>
<td>0.06</td>
</tr>
</tbody>
</table>

- ***
- **
- *
- ns
Microsatellite allele size differences: locus CG44

Allele size differences:

locus CG44

C. angulata
C. gigas

P < 0.001
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*)

**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

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

- “Tavira” is clearly a mixed population

● mitochondrial marker COI
"Tavira": mixture or hybridisation?

- Intermediate allele frequencies at loci COI and CG44R
- Non significant heterozygote deficiencies at locus GR44R ($Fis = 0.11$) 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

4 The two taxa hybridise when they are brought together following the transportation of oysters due to aquaculture.

4 There is no evidence of natural gene flow between populations of the two taxa in Europe.

4 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...).