Characterization of the model brown alga *Ectocarpus siliculosus* sex locus

Master internship carried out at the Station Biologique of Roscoff, in the context of Marinexus WP4, using resources provided by WP2 (Akira Peters)

• Algal Genetics (UMR 7139) with Susana Coelho
• Evolutionary Biology and Marine Diversity (UMR 7144) with Myriam Valero

Marinexus meeting, Plymouth, 14-15 June 2011
• Sex chromosomes:
  – Independant evolution between the taxa but evolutive convergence
Sex chromosomes evolution

- Autosomal evolution of a master sex-determining gene
- Suppression of recombination
- Accumulation of transposons
- Acquisition of more genes involved in sex
- Ongoing gene conversion and expansion

Small non-recombining region

- Common evolutionary mechanisms
- Different stage depending on the species
- Experimental data validate this model initially proposed by Ohno (1967)

Heteromorphic sex chromosomes

From Fraser and Heitman, 2005
• Sex chromosomes:
  – Independant evolution between the taxa but evolutive convergence
  – Non recombining regions which should present a low rate of neutral polymorphism (Charlesworth et al., 1993)

Without recombination

- B2 remains linked to A2, both mutations are eliminated

With recombination

- B2 can be associated with A1 and thus be maintained
• Sex chromosomes:
  – Independant evolution between the taxa but evolutive convergence
  – Non recombining regions which should present a low rate of neutral polymorphism (Charlesworth *et al.*, 1993)
  – Evolution should differ between organisms with an haploid dioecy and organisms with a diploid dioecy (Bull, 1978)
Diploid dioecy vs haploid dioecy

Diploid dioecy and asymmetric evolution
Ex: Mammals

Haploid dioecy and symmetric evolution
Ex: Liverworts

Y is permanently heterozygous
Hidden recessive detrimental mutations + no recombination= accumulation of recessive detrimental mutations and Y degenerescence

X and Y are both permanently heterozygous
X and Y should exhibit the same degenerescence degree

Still few data on organisms with haploid dioecy
Introduction

• Sex chromosomes:
  – Independant evolution between the taxa but evolutive convergence
  – Non recombining regions which should present a low rate of neutral polymorphism (Charlesworth et al., 1993)
  – Evolution should differ between organisms with an haploid dioecy and organisms with a diploid dioecy (Bull, 1978)

• *Ectocarpus siliculosus*:
  – Model for the brown algae
Introduction

• Sex chromosomes:
  – Independant evolution between the taxa but evolutive convergence
  – Non recombining regions which should present a low rate of neutral polymorphism (Charlesworth *et al.*, 1993)
  – Evolution should differ between organisms with an haploid dioecy and organisms with a diploid dioecy (Bull, 1978)

• *Ectocarpus siliculosus:*
  – Model for the brown algae
  – Haploid-diploid organism with an haploid dioecy
Ectocarpus siliculosus sexual life cycle

- **Meiosis**
- **Sporophyte** 2N
- **Gametophyte** ♀ N
- **Gametophyte** ♂ N
- **Fertilization**
Why studying sex locus in *E. siliculosus*?

Broad diversity of sexual systems in brown algae

- isogamy
- oogamy

mating type

sex chromosomes
**Introduction**

- **Sex chromosomes:**
  - Independant evolution between the taxa but evolutive convergence
  - Non recombining regions which should present a low rate of neutral polymorphism (Charlesworth *et al.*, 1993)
  - Evolution should differ between organisms with an haploid dioecy and organisms with a diploid dioecy (Bull, 1978)

- **Ectocarpus siliculosus:**
  - Model for the brown algae
  - Haploid-diploid organism with an haploid dioecy
  - Genome of a male strain recently sequenced (Cock *et al.*, 2010)
Context and objectives

- Comparative genome hybridization (Dittami et al., 2010)

- cDNAs from a female strain
  - Cy3 labeled

- cDNAs from a male strain
  - Cy5 labeled

- Comparison of the two hybridization signals
- Comparison to a reference hybridization signal

- Microarray
  - cDNAs from the male sequenced strain
Comparative genome hybridization (Dittami et al., submitted)

- Development of markers for this zone and sex linkage test
- Localisation thanks to the genetic card developed for *E. siliculosus* (Heesch et al., 2010): within the linkage group 30 which presents emblematic characteristics of sex chromosomes

**Supercontig 68 is located in the male sex locus**
1. How large is the *E. siliculosus* male sex locus?
   ➔ **Development of sex linked markers**

2. Does the recombination is suppressed within the *E. siliculosus* male sex locus as in the other studied organisms sex loci?
   ➔ **Study of recombination**

3. Does the neutral polymorphism is reduced within the *E. siliculosus* male sex locus as in the other studied organisms sex chromosomes?
   ➔ **Study of molecular diversity**
1. Development of sex linked markers

- Comparative genome hybridization → 6 regions potentially flanked supercontig 68
- Development of markers with Primer 3 and e-PCR
- Sex linkage test and cartography

Identification of two other sex linked regions within the same linkage group:
- Supercontig 285
- Supercontig 439
2. Recombination study

- Expected: few or no recombination within the sex locus (Bull, 1978)
- On ≈ 2000 individuals (meiosis products)

Recombination suppressed:
- within supercontig 68
- between supercontigs 68 and 285
- between supercontigs 68 and 439
- between supercontigs 285 and 439

Linkage group 30

Non recombining region of about 1 Mpb= male sex locus

Without recombination: same profile for every marker

With recombination: some markers have a different profile
3. Molecular diversity study

- Expected: the sex locus exhibits a lower molecular diversity than a classical chromosomic region (Charlesworth et al., 1993, Barton, 2000)
- Available material: 37 strains from natural populations
- Need to have male haploids

7 male gametophytes + the sequenced strain 32m + one « outgroup » sampled in Italy
3. Molecular diversity study

- **Study of neutral** molecular diversity: no selection influence
- Sequences of about 2000 bp length, containing the same proportions of introns and exons, from three genes
  1. one gene within the sex locus
  2. one gene in the same linkage group as the sex locus but outside the sex locus
  3. one gene located elsewhere in the genome
- Primers design, sequences amplification, sequencing and analysis of molecular diversity with the software DNAsp

<table>
<thead>
<tr>
<th>Gene</th>
<th>Region length</th>
<th>Number of individuals</th>
<th>Polymorphic sites within non coding regions</th>
<th>Polymorphic sites within coding regions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>synonymous</td>
</tr>
<tr>
<td>68.3</td>
<td>1925</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>313.11</td>
<td>1216</td>
<td>7</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>159.81</td>
<td>1922</td>
<td>8</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

No polymorphism within the sex locus!
Conclusion and perspectives

- Molecular diversity study on more individuals from populations of the Western Channel
- Genome of the female strain now available
  - Female sex markers available to study natural populations

Linkage group 30

Non recombining region of about 1 Mbp = male sex locus

Ladder  F  F  F  M  M  M  F  M
Tools for studying Western Channel population structure

1. Gametophyte or sporophyte?
   - Gametophyte (congo red positif)
   - Sporophyte (congo red negatif)

2. Male or female?
   - ♀ marker
   - ℋ marker
Tools for studying Western Channel population structure

CONGO RED

Gametophyte

SEX MARKERS

Female gametophyte
Male gametophyte
Male parthenosporophyte
Female parthenosporophyte
Heterozygous sporophyte

Sporophyte
• Molecular diversity study on more individuals from populations of the Western Channel
• Genome of the female strain now available
• Sex markers for other brown algae
Thank you for listening
<table>
<thead>
<tr>
<th>Markers that amplify</th>
<th>size</th>
<th>Sex linked</th>
<th>sequenced</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. crouaniorum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>68-27</td>
<td>200</td>
<td>yes</td>
<td>Yes</td>
</tr>
<tr>
<td>285-12</td>
<td>200</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Deg. 285-10</td>
<td>1500</td>
<td>Yes?</td>
<td>no</td>
</tr>
<tr>
<td><strong>E. fasciculatus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>68-79</td>
<td>200</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Deg. 285-10</td>
<td>1500</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td><strong>E. sil Naples</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>200</td>
<td>Yes all except 68-79</td>
<td>no</td>
</tr>
<tr>
<td>Deg. 285-10</td>
<td>1500</td>
<td>yes</td>
<td>no</td>
</tr>
</tbody>
</table>