Genomic in situ hybridization to understand hybrid genomes

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GENOMIC IN SITU HYBRIDIZATION
GISH

- Background and history
  - What is it?
  - What can it do?

- Applications
  - Examples from our lab
    - Wheat
    - Brassica
    - Crocus
**GENOMIC IN SITU HYBRIDIZATION (GISH)**

- Uses total genomic DNA as a probe for *in situ hybridization* to chromosomes and nuclei.
- Identifies origin of chromatin.
- **Parental origin of hybrids**
  - Can be combined with other probes, most often repetitive DNA probes.
- **Alien chromatin in breeding lines**
  - Size and origin.
GENOMIC IN SITU HYBRIDIZATION
GISH

- Parental origin of hybrids
  - Auto or allo-poliploidy
- Alien chromatin in breeding lines
  - Size and origin
  - Recipient chromosome
- Meiosis and chromosome pairing
- Interphase cytogenetics
In Situ Localization of Parental Genomes in a Wide Hybrid

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Accepted: 7 March 1989
Addition of unlabelled, cold total genomic DNA to block common sequences between the parental genomes


Genomic in situ hybridization to identify alien chromosomes and chromosome segments in wheat

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Examples where GISH has been successful

- Triticeae
  - Triticum, Aegilops, Hordeum, Haynaldia, Thinopyrum, Secale, Hystrich, Leymus, Agropyron, Elymus, Elytrigia
- Brachypodium
- Oryza genomes
- Zea mays
- Pennesitum
- Tripsacum
- Saccharum
- Avena
- Lolium and Festuca hybrids Festulipa
- Eleusine
- Alstroemeria
- Aloe
- Lilium
- Allium
- Crocus
- Tulipa
- Musa genomes

- Asteraceae
  - Dahlia
  - Chrysanthemum, Dendranthema and Argyranthemum
- Brassicaceae
  - Brassica species, alien and hybrids with Eruca, Orchyphragmus, Sinapis, Raphanus
  - Brassica, Lesquerella fendleri, Arabidopsis species/hybrids
- Solanaceae
  - Solanum, potato, tomato
- Fabaceae
  - Arachis
  - Medicago
- Coffea arabica
- Gossypium
- Rubus
- Beta
- Zingeria
- Setaria
- Phalaenopsis
Calitriche platycarpa (2n=20) Water starworts

Savidge (1960): *allo-tetraploid* origin from *C. stagnalis* and *C. cophocarpa* (both 2n=10)

Schotsman (1967): *auto-tetraploid* from *C. cophocarpa*

<table>
<thead>
<tr>
<th>British Species</th>
<th>2n</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Calitriche hermaphroditica</em> L.</td>
<td>6</td>
</tr>
<tr>
<td><em>Calitriche truncata</em> Guss.</td>
<td>6</td>
</tr>
<tr>
<td><em>Calitriche stagnalis</em> Scop.</td>
<td>10</td>
</tr>
<tr>
<td><em>Calitriche obtusangula</em> Le Gall</td>
<td>10</td>
</tr>
<tr>
<td><em>Calitriche platycarpa</em> Kütz.</td>
<td>20</td>
</tr>
<tr>
<td><em>Calitriche palustris</em> L.</td>
<td>20</td>
</tr>
<tr>
<td><em>Calitriche brutia</em> Petagna</td>
<td>28</td>
</tr>
<tr>
<td><em>Calitriche hamulata</em> Kütz.</td>
<td>38</td>
</tr>
</tbody>
</table>

Gornall, Johnson and Schwarzacher 2004
‘GOLDEN YELLOW’ CROCUS
2n=3x=14
6 chr from *Crocus angustifolius*
2x4 chr from *Crocus flavus*
Saffron
*Crocus sativa*
$2n=3x=24$

*C. cartwrightianus* green
*C. thomasii* red
Crocus sativus Pachytene

DAPI

Silver nitrate
Crocus sativus Pachytene

C. hadriaticus
C. thomasii

DAPI
Rapeseed *B. napus* (AACC, 2n=4x=38) - hybridized with C-genome CACTA element red

*B. oleracea* (CC, 2n=2x=18)  *B. rapa* (AA, 2n=2x=20)

Genome Specificity of a CACTA (*En/Spm*) Transposon

Pat Heslop-Harrison
Karine Alix
Xianhong Ge
Brassica synthetic hexaploids

Brassica napus addition line Orychophragmus violaceus

Xianghong Ge, Farah Badakshi, Heslop-Harrison and Schwarzacher 2010
Brassica Monosomic Addition Line
Orychophragmus violaceus

DAPI, ASY-1
Brassica Monosomic Addition Line
*Orychophragmus violaceus*

DAPI, ASY-1 *O. violaceus* genomic DNA
partly unpaired and partly paired with Brassica chromosomes
Alien gene transfer, alien recombinant chromosomes
Total genomic DNA can be used as a probe to distinguish:

- Genomes in sexual hybrids
- Alien chromosome introgression
  - Additions
  - Translocations
- Used extensively in breeding programmes to introduce desirable traits from wild species
Rye DNA
pTa71-45S rDNA
4 major sites 1RS, 6BS
6 major sites 1RS, 1BS, 6BS

1BL.1RS

1DL.1RS
LINE 7-102 DERIVED FROM A TRITICALE X WHEAT CROSS

rye chromosome derivative 1R substitutes wheat chromosome 1D

Forsström and Schwarzacher 2000
Derivative chromosome 1R of Lines 7-102 and 7-169

1R

<table>
<thead>
<tr>
<th>5S rDNA</th>
<th>45S rDNA</th>
<th>pSc200</th>
<th>pSc119.2</th>
<th>AAC</th>
</tr>
</thead>
</table>

der1R

AAC
pSc200

Forsstrom and Schwarzacher 2000

pSc119.2
pTa71
Wheat 5AS.5RL at meiosis

Schwarzacher 1997
Characterization of new sources of Wheat streak mosaic virus resistance

24 resistant and non-resistant sister lines from six different populations of wheat lines that potentially include an alien chromosome arm from *Thinopyrum intermedium* carrying WSMV resistance (Wsm-1 gene)

Bob Graybosch, USDA-ARS, University of Nebraska, USA
WHEAT WITH T4DL.4JS TRANSLOCATION:

Th. intermedium DNA-green
Afa Thin-red

Niaz Ali
Niaz Ali, PhD thesis 2012
Thin DNA
The alien Thin fragment is not heavily methylated

Thin DNA  5-Methyl Cytosine

Niaz Ali
The alien Thin fragment is not heavily methylated
Orychophragmus violaceus genomic DNA
Homozygous partly unpaired and partly paired with Brassica chromosomes

Xianghong Ge, Farah Badakshi, Heslop-Harrison and Schwarzacher 2010
Parental origin of hybrids
- Auto or allo-poliploidy

Alien chromatin in breeding lines
- Size and origin
- Recipient chromosome

Meiosis and chromosome pairing

Interphase cytogenetics

Understanding hybrid genomes
- Chromosome behaviour
- Chromatin function
Thank you to members of the Molecular cytogenetics lab

- Collaborators and past members of the lab
  - Richard Gornall, Leicester
  - Bob Graybosch, Nebraska, USA
  - Karine Alix, INRA/Univ Paris-Sud, France

www.molcyt.com
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Xianhong Ge, Huazhong Agricultural University, Wuhan, China
The Origin of Species begins with an example of how humans have domesticated certain species and used artificial selection, in contrast to natural selection, to produce extraordinary variation in a short time. Darwin focused on “fancy” pigeons, but it is apparent in many domestic species, including dogs, cattle and crop plants like wheat (see below).

At that time, it was not known how such variation arose or was maintained. Now we know that this variation is due to genes and chromosomes, and a team here in Leicester investigate how the number and organisation of chromosomes varies across wheat varieties.

Dr Trude Schwarzacher and Prof Pat Heslop-Harrison investigate the evolution of cereals by examining their chromosomes. On the right are the chromosomes from a root cell of rye. The red and green spots are DNA sequences which can be used to trace the ancestry of different cereal species and varieties.

“Species are only strongly marked and permanent varieties”

Below are examples of wheat ears from different hybrids and wild varieties of wheat.