Unreduced Gametes in *Brassica*: Effects of Genotype, Temperature and Interspecific Hybridization

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Summary

• What is an unreduced gamete?
• Why are unreduced gametes interesting?
• Experimental material
• Hypotheses: hybridisation, genotype and temperature effects
• Results from pollen measurements
• Results from sporad observations
• Results from hybridisation experiment
What is a reduced gamete?

Maternal chromosome
Paternal chromosome

Pre-meiosis DNA content doubles; two identical chromatids formed

Normal meiosis

“2n” DNA content

Reduced gametes

“n” DNA content
What is an unreduced gamete?

Pre-meiosis DNA content doubles; two identical chromatids formed

"2n" DNA content

Abnormal meiosis

"2n" DNA content

Unreduced gametes
Evolution of polyploid plants through union of unreduced gametes

Species A

2n = AA

Species B

2n = BB

Pollen

New polyploid species

2n = AABB

Ovules
U’s Triangle of *Brassica*

- **B. juncea**
- **B. nigra**
- **B. carinata**
- **B. oleracea**

Diagram:
- **B. rapa**
- **B. napus**

Genotypes:
- AA
- AABB
- AACC
- BB
- BBCC
- CC

**U’s Triangle**

**Brassica**

- **AA**
- **B. rapa**
- **B. napus**

**Genotypes**

- **AABB**
- **AACC**
- **BB**
- **BBCC**
- **CC**
U’s Triangle of Brassica

B. juncea

B. rapa

B. carinata

B. nigra

B. oleracea

AABB x AACC

BB → BBCC ← CC
Interspecific hybrids

B. juncea

B. napus

B. carinata

~2400 bud pollinations

13 genotypes total
Unreduced gametes: hypotheses

• Do interspecific hybrid *Brassica* produce more unreduced gametes than their natural parents?

• Are there differences between *Brassica* genotypes in unreduced gamete production?

• Will unreduced gametes contribute to form higher ploidy progeny in interspecific crosses?

• Does temperature influence unreduced gamete production?
Estimating unreduced gamete production

Method 1: Pollen measurements

“Normal” viable pollen (reduced)

“Giant” viable pollen (unreduced)
Hybrids produce high frequencies of viable unreduced pollen grains.
Normal Meiosis

Parallel Spindles (a common means of 2n gamete formation)

Dicotyledonous plants
Estimating unreduced gamete production

Method 2: Sporad observation

- Tetrad (4 nuclei) reduced
- Dyads (2 nuclei) unreduced
Sporad results: interspecific hybrids produce more unreduced gametes than their parent species

0.02%
4 dyads out of ~10,000 sporads

1.32%
680 dyads out of ~27,500 sporads

Genotypic differences

2n gametes in viable pollen
2n gametes in sporads

Does temperature affect unreduced gamete production?

Temperature treatments – four controlled environment rooms, night/day temperature

- “cold” – 5°C / 10°C
- “cool” – 13°C / 18°C
- “warm” – 15°C / 25°C
- “hot” – 25°C / 30°C

- Five genotypes (B. napus, B. juncea, B. carinata) and five of their interspecific hybrids
- Two plants x 10 genotypes in each room
- 2 x 300 sporad counts per plant

The effect of temperature on unreduced gamete production

### Allohexaploid *Brassica*

| **B. rapa** | **B. nigra** | **B. oleracea** | Diploid  

<table>
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<tr>
<th>AA</th>
<th>BB</th>
<th>CC</th>
<th>2n = 2x</th>
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| **B. juncea** | **B. napus** | **B. carinata** | Tetraploid  

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<th>AABB</th>
<th>AACC</th>
<th>BBCC</th>
<th>2n = 4x</th>
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| AABBBCC? | Hexaploid  

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<th>2n = 6x</th>
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Can we use unreduced gametes to produce this hexaploid from the tetraploid species?
Indicate the three basic genomes with A=10, B=8 & C=9 chromosomes.
DNA content (estimated by flow cytometry) for second cross hybrid population

Relative DNA content: ~AABBCC = 6.0x
Approximate ploidy level of progeny

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<th>Cross</th>
<th>Plants</th>
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| AABC x BBCC | 46     | All ~4x  
| BBAC x AACC | 64     | All ~4x  
| CCAB x AABB | 2      |  

Phenotype of “~6.5x” plant

Pollen viability: 0-22%
Self seed set: 0
OP seed set: 13
“~6.5x” plant chromosome spreads

2n ~ 58

Expected AABBC = 54 chromosomes
Phenotype of “5.5x” plant

Pollen viability: 25%
Self seed set: 166
OP seed set: 458
“5x” plant chromosome spreads

2n ~ 50

Expected AABBC = 54 chromosomes
Molecular marker results

6.5x plant

$A^jB^j$ from *B. juncea* + $2(0.5A + 0.5B + C^n \text{ or } c)$ from $C^nC^cA^nB^c$ hybrid

Explanation: Meiosis II failed to separate sister chromatids

5.5x plant

$A^jB^j$ from *B. juncea* + $C^{n/c}C^{n/c}A^nB^c$ from $C^nC^cA^nB^c$ hybrid (minus a few chromosomes)

= $A^jA^nB^jB^cC^nC^c$

Explanation: Meiosis I failed to separate homologous chromosomes
Unreduced gametes: conclusions

- Interspecific hybrid *Brassica* produce more unreduced gametes than their natural parents.
- Genotype influences unreduced gamete production.
- Unreduced gametes can be used to produce higher ploidy progeny in interspecific crosses, depending on genotype.
- Cold ambient temperature increases unreduced gamete production in some genotypes.
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