A new resource for studies in karyotype (genome) evolution of duckweeds

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Duckweed (Lemnoideae)

- 5 genera, 37 species; worldwide distribution, except arctic & antarctic zones
- Free floating on water surface; neotenous reduction of body plan
- Fast growing & rare sexual reproduction
- High economic potential for waste water treatment, biofuel production & livestock feeding
- Variable genome size (1C: 158 – 1880 Mbp) & chromosome numbers (2n: 20 – 126)

Drawings: Dr. K. Sowjanya Sree, Amity University Delhi.
Duckweeds are of interest for karyotype (genome) evolution studies

Nauheimer et. al. N. Phyto. 2012
Appenroth et al. CIBJ 2013
Wang et. al. J. Bot. 2011
Landolt VGI-ETH 1986
Geber Uni. Vienna 1989

DNA content pg / 1C

(*) Most common value in the genus

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Landolt VGI-ETH 1986
Geber Uni. Vienna 1989
FISH for (cyto)genome analysis

- **Multicolor** Fluorescence in situ Hybridization (mcFISH) is used:
  - to map genomic fragments/library clones to specific chromosomal regions
  - to validate the integrity of the WGS scaffolds & support the integration of WGS and other mapping data (e.g., physical maps) => across chromosome-size distance containing repetitive sequences
  - to detect chromosome rearrangements, chromosome homeology and karyotype evolution in related not-yet-sequenced species

- Cytogenetic maps from FISH could be used to integrate with NGS scaffolds and other genetic/physical maps to obtain chromosome-aligned genomes

- In addition to physical maps (optical maps, fingerprinting maps), cytogenetic maps is particularly important for duckweed sequencing projects because of the difficulty to obtain genetic maps
The *Spirodela polyrhiza* reference genome (clone 7498)

- 158 Mbp genome size
- 20 chromosome pairs
- 32 pseudomolecules contain 92% of the *Spirodela* genome assembly

Cao *et. al.* New Phytol. 2015

Strategy for chromosome alignment of pseudomolecules (Ψs) by FISH

Genomic and Genetic data

- WGS assembly scaffolds
- Fingerprinting maps
- 32 pseudomolecules
- 110 representative BACs

Cytogenetic integration

- 96 anchored BACs
- Test for integrity of Ψ by differently labelled BACs from each Ψ
- Chromosome alignment of Ψ by differently labelled BACs from pools of Ψs
Validation of integrity of pseudomolecules

Ψ6 (6.3 Mbp)

Example: Ψ6 is present on one chromosome pair

Ψ5

Example: Mis-aligned BACs in Ψ5 could be detected in a BAC pool
Validation results of pseudomolecules (Ψ) by FISH

Genomic and Genetic data

- WGS assembly scaffolds
- Fingerprinting maps
- 32 pseudomolecules
- 110 representative BACs

Cytogenetic integration

- 96 anchored BACs
- Integrity of 29 Ψs is confirmed
- 3 chimeric Ψs are detected
- 32 + 3 Ψs are integrated into 20 chromosome pairs
Integration of $32 + 3 \Psi$s into 20 chromosomes by probe pooling & rehybridisation.

$\Psi_{24,25,26}$, $\Psi_{30,31,32}$, $\Psi_{27,28,29}$

1st FISH

$\Psi_{25}$, $\Psi_{24,28}$, $\Psi_{27}$

2nd FISH

$\Psi_{25}$ & $\Psi_{28}$ belong one chromosome pair.

Fluorescent labelling:
- cy3
- a488
- TxR
The Spirodela cytogenetic map & chromosome-aligned version of genome sequence

Average distance between markers: 0.89 Mbp
Total number of genes: 17,714 (90% of the predicted genes)
Total length of chromosome sequences: 133.6 Mbp (92% genome assembly)

Cao et. al. New Phytol. 2015
BAC cocktail for Spirodela karyotyping

S1  S2  S3  S4  S5  S6  S7  S8  S9  S10  S11  S12  S13  S14  S15  S16  S17  S18  S19  S20

Alexa 488
cy3
Texas Red
41 labelled BACs for karyotyping

Cao et. al. New Phytol. 2015
Spirodela ancestral duplicated chromosomal segments


Cao et al. New Phytol. 2015
Outcome (availability)
A Spirodelas cytogenetic map which integrates the sequenced genome (BAC cocktail for karyotyping by FISH; ancestral origin of Spirodelas duplicated chromosome blocks)

Outlook
Studying chromosome homeology, genome and karyotype evolution of duckweed species by means of comparative chromosome painting

Example: Wolffia microscopica (L17)
Smallest flowering plant
Fastest doubling time: 30 -36 hours
„Sexual“ and vegetative reproduction
2n = 40; 743 Mbp genome size
7 natural clones from India / Bangladesh

Sree et. al. Flora 2015
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Cao, Hieu; Vu, Giang; Wang, Wenqin; Appenroth, Klaus; Messing, Joachim; Schubert, Ingo (2015). The map-based genome sequence of *Spirodela polyrhiza* aligned with its chromosomes, a reference for karyotype evolution. New Phytologist.

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**3rd ICDRA organizers**

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