

**Structure génomique et stratégie adaptative de
l'algue verte unicellulaire *Ostreococcus tauri***

ARAGO LABORATORY

Oceanological Observatory

- Biological oceanography
- Models in cell biology and evolution



Ostreococcus ecotype diversity and genome structure

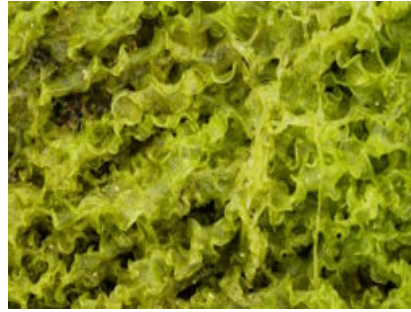
- Definition and diversity of algae
- Interest of picoeukaryotes/Prasinophytes
- Genomics of Prasinophytes

Definition of algae

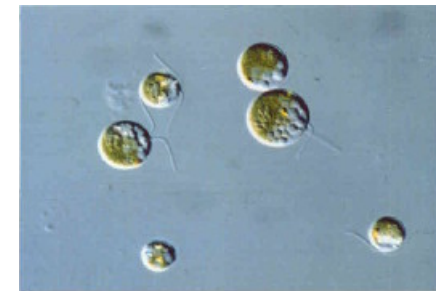
Red alga



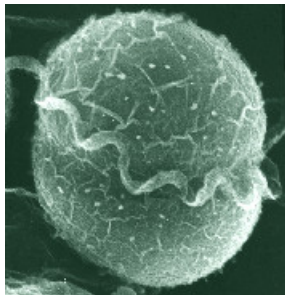
Green alga



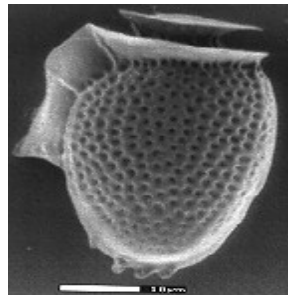
Brown alga



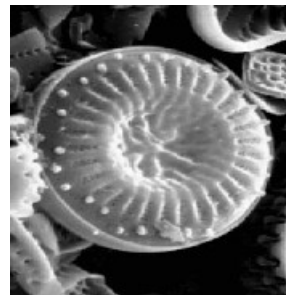
Dunaliella tertiolecta



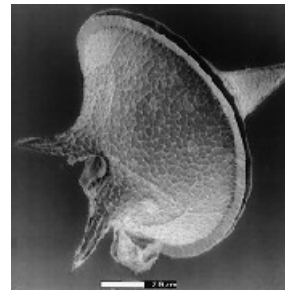
Crypthecodinium cohnii



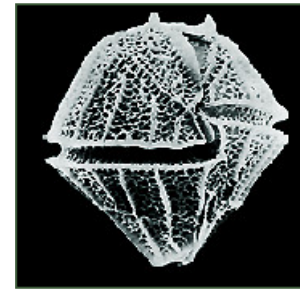
Dinophysis



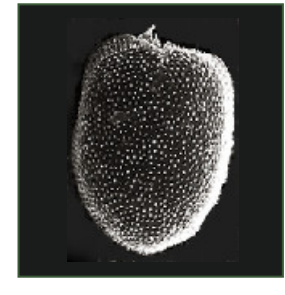
Cyclotella stelligera



Protopteridium



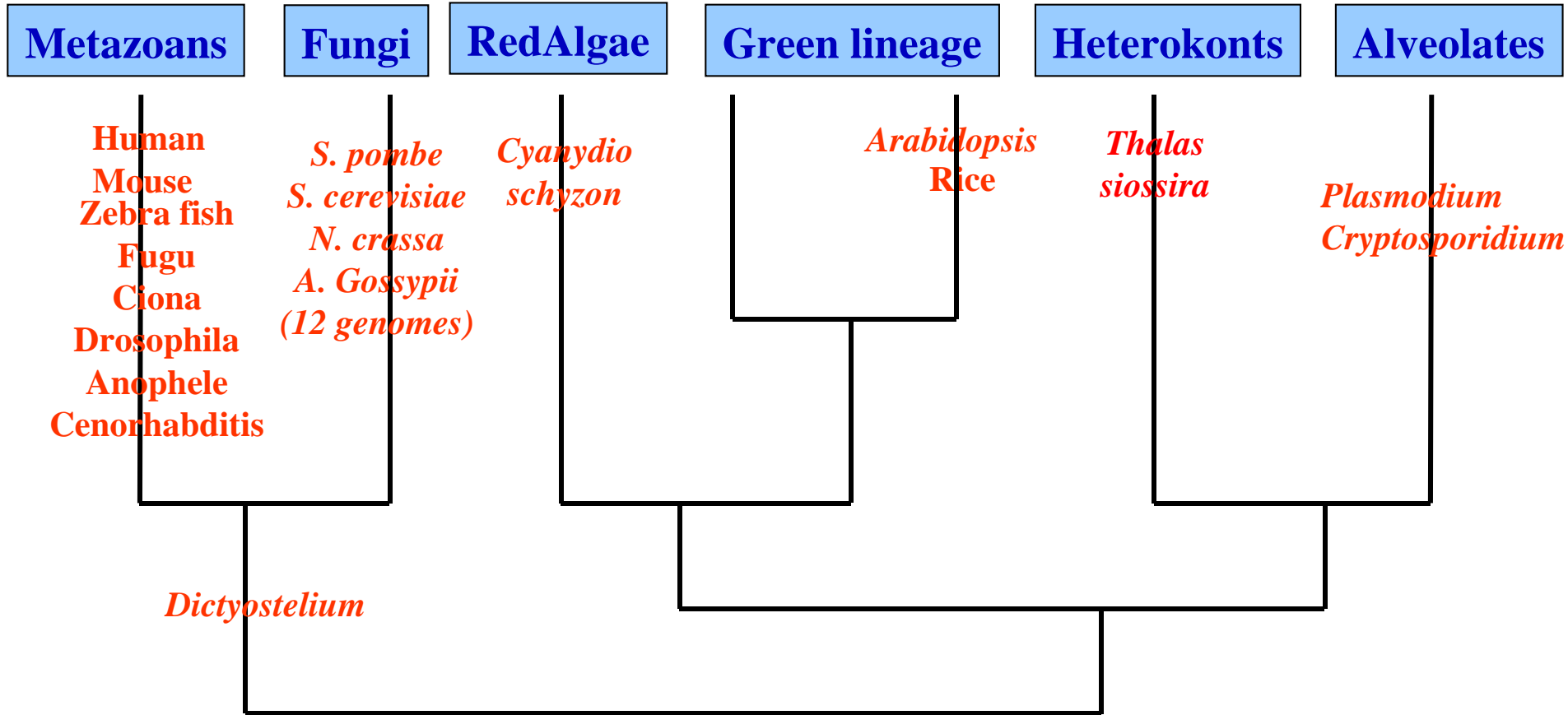
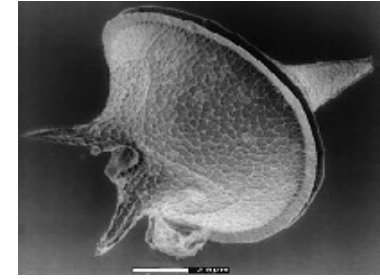
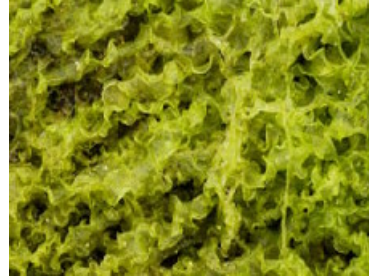
Gonyaulax polygramma



Prorocentrum minimum

Algae are a rather ill-defined assemblage that includes aquatic organisms, usually photosynthetic, having a simple vegetative apparatus (thalle)

This definition has no phylogenetic significance and algae represents many different evolutionary steps.



From Baldauf et al. Science 2000, 290:972-7; Van de Peer et al. J. Mol. Evol. 2000, 290:565-76, simplified



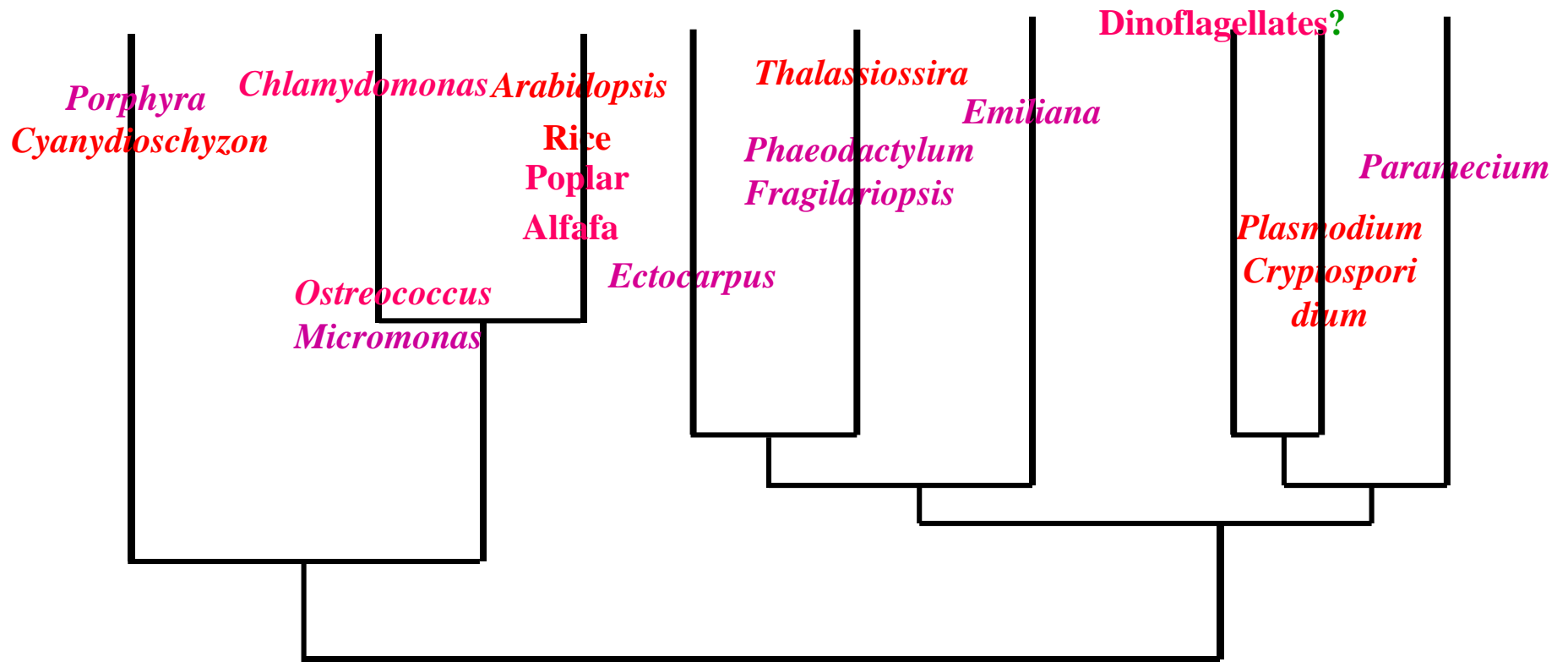
RedAlgae

Green lineage

Heterokonta

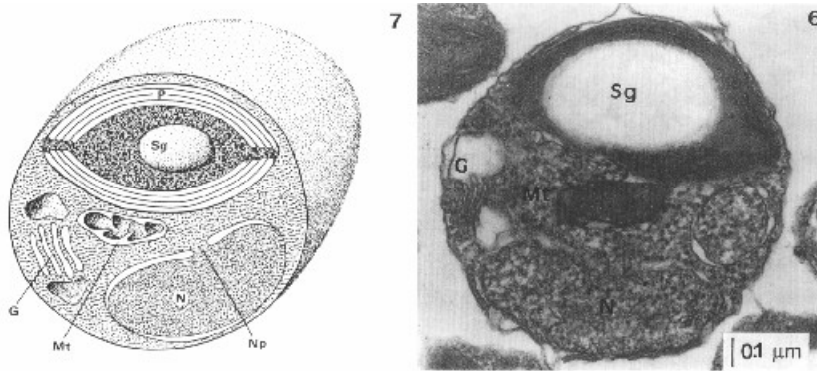
Haptophyta

Alveolata



Prasinophytes - Order Mamiellales

Ostreococcus



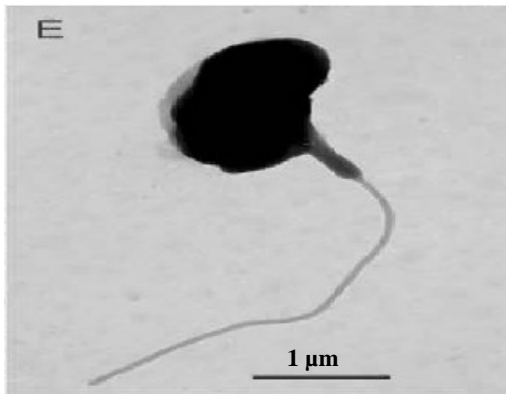
From Chrétiennot-Dinet et al. (1995)

Bathycoccus



Courtesy MJ Dinet. N°170650704

Micromonas



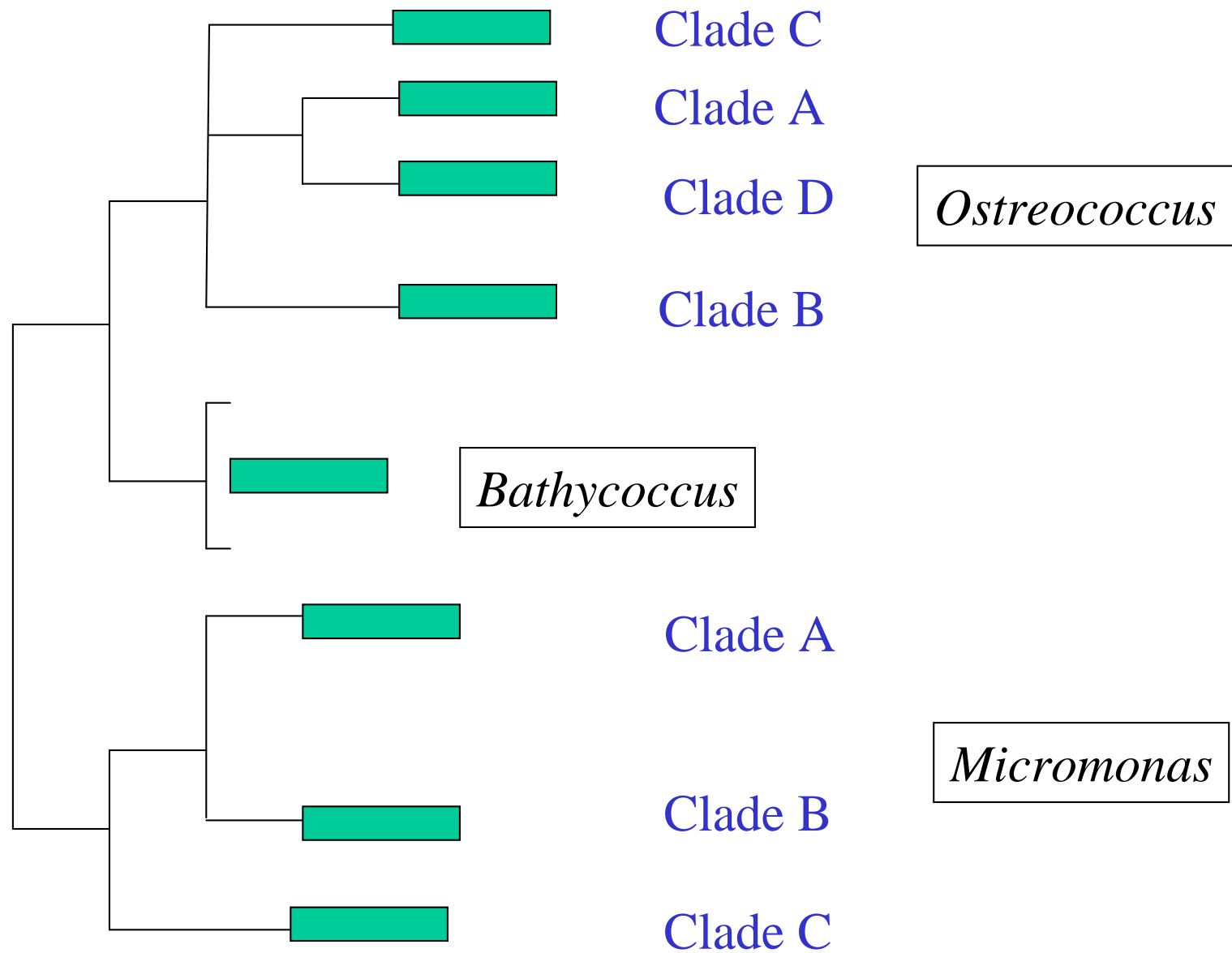
From Guillou et al. (2004)



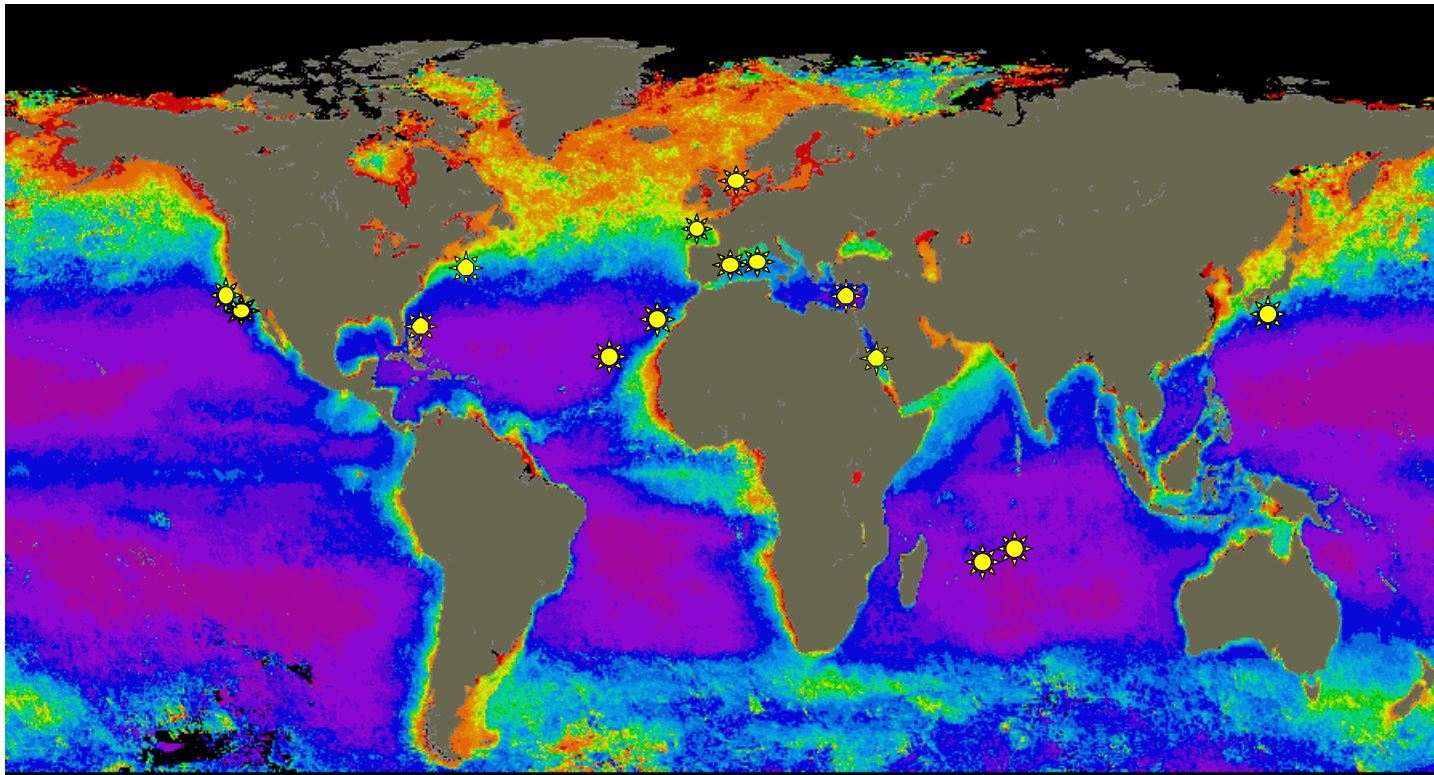
From S. Sym and D. Keats; <http://hypnea.botany.uwc.ac.za/>

Picoeukaryotes: size < 2-3 μm

Prasinophytes - Order Mamiellales

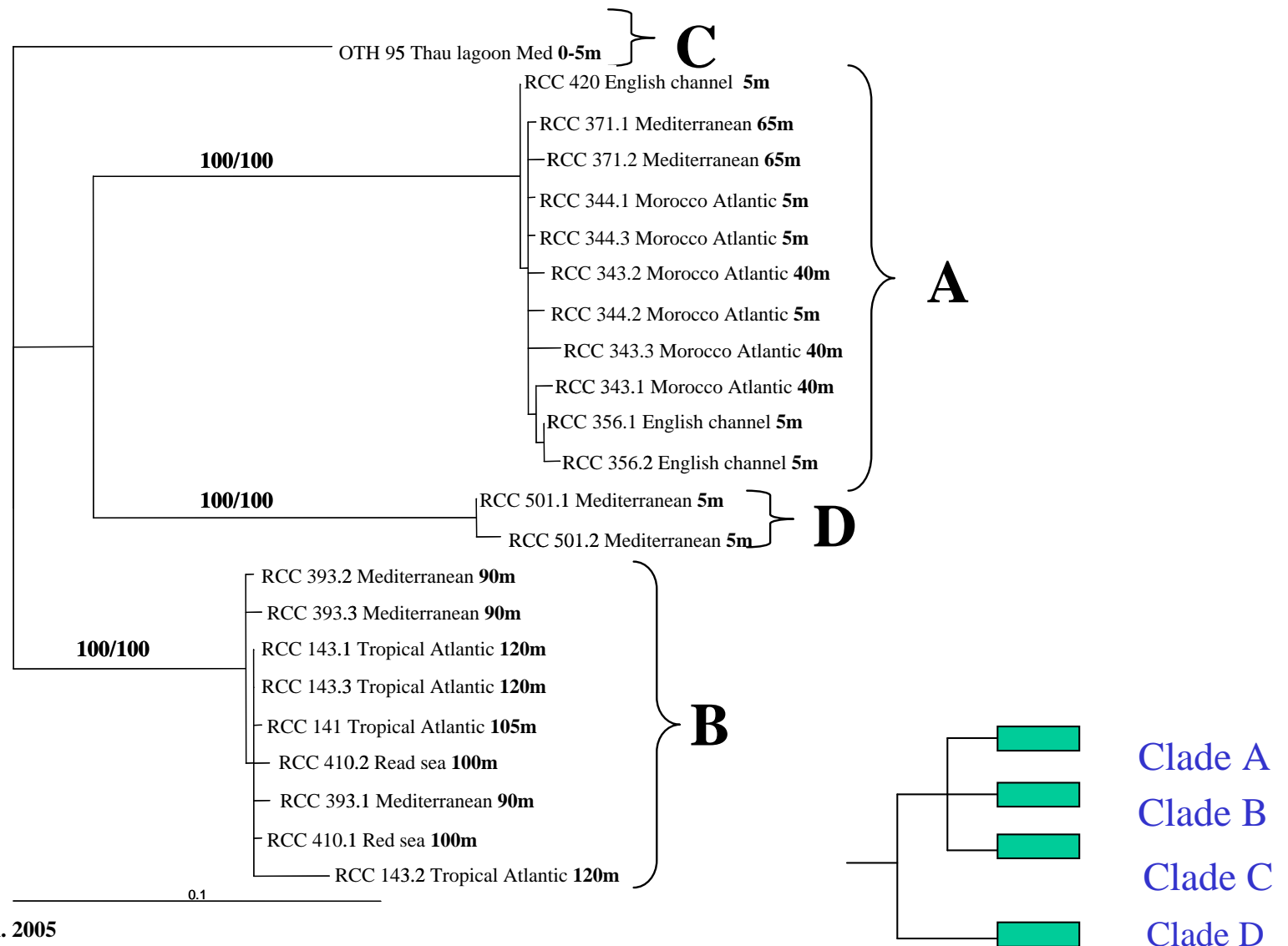


Geographical distribution of *Ostreococcus* isolates

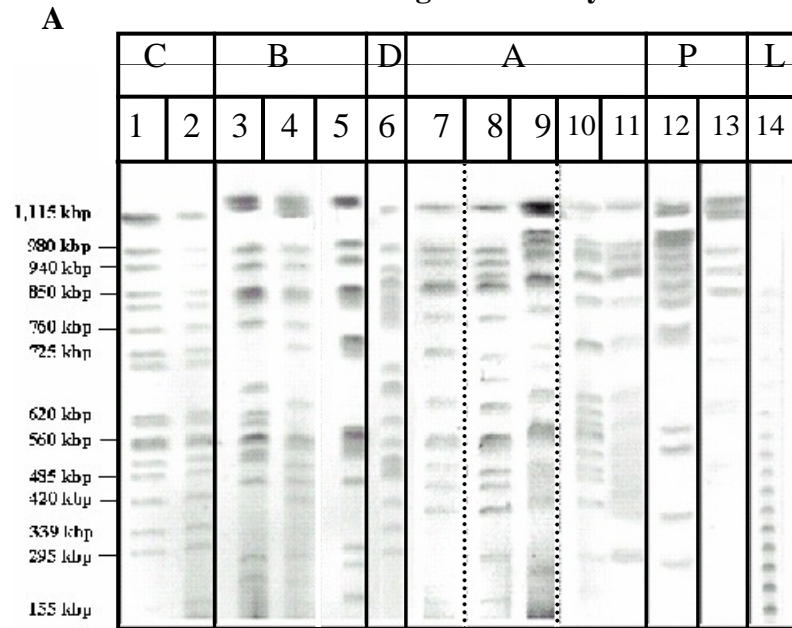


data from: Worden et al. 2004; Guillou et al. in press; Not et al. in prep.; Courties et al 1994.

Maximum likelihood phylogeny of *Ostreococcus* strains inferred from ITS-1 and ITS-2



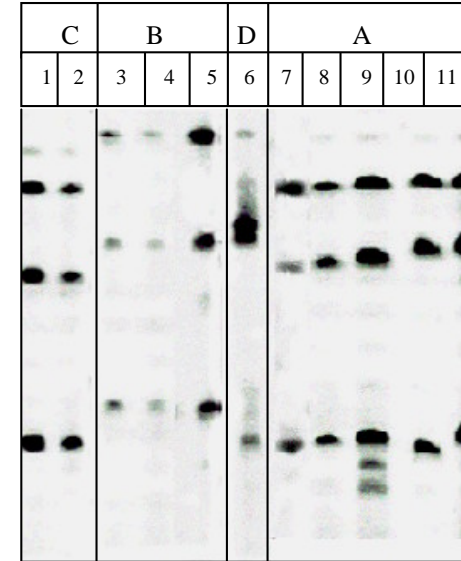
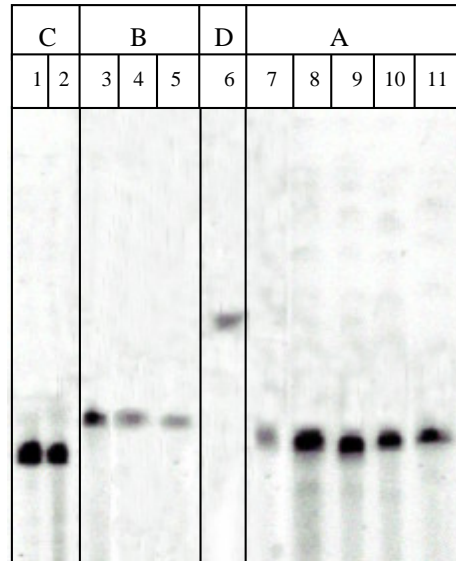
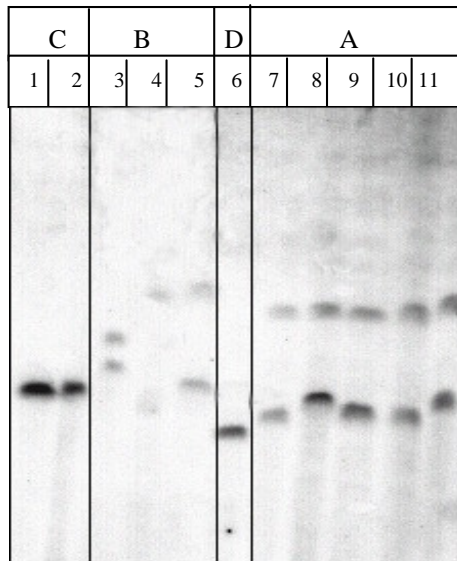
PFGE genome analysis



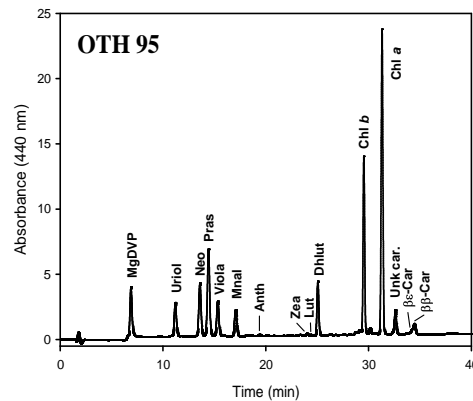
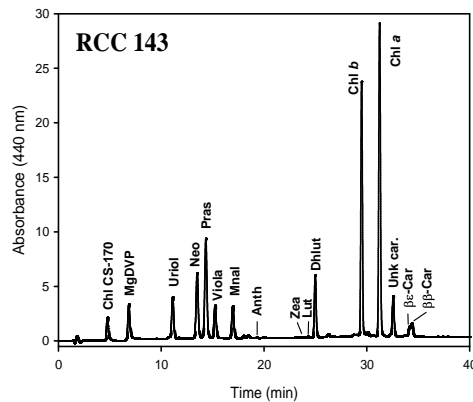
Hybridisations : B : ribosomic (28S)

C : Actin

D : Chlorophyl binding protein

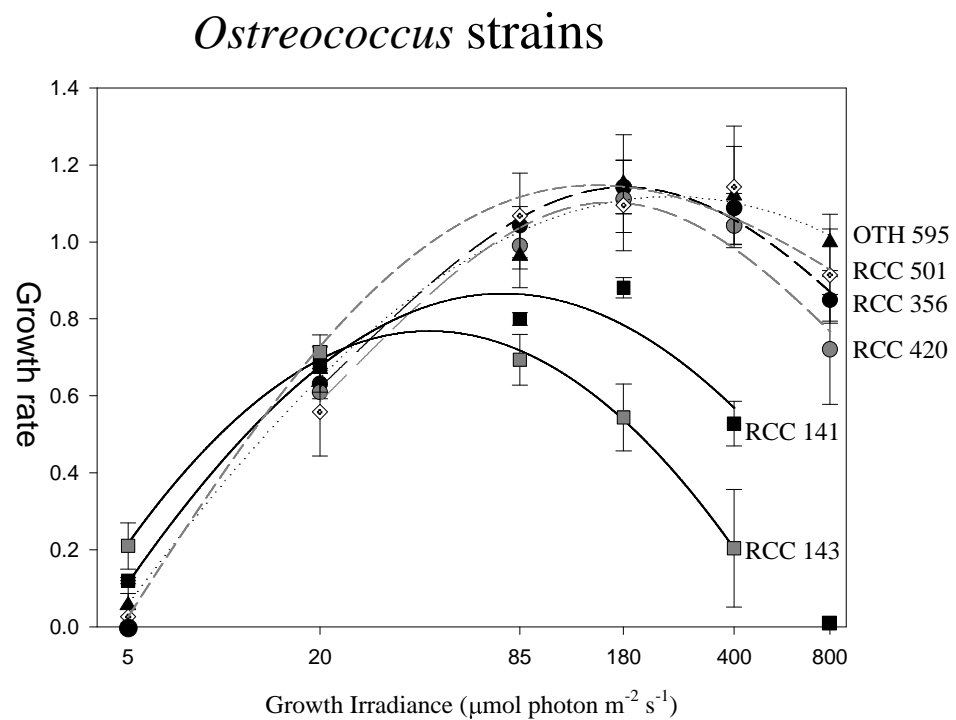


Pigment composition and ITS characteristics of the *Ostreococcus* strains



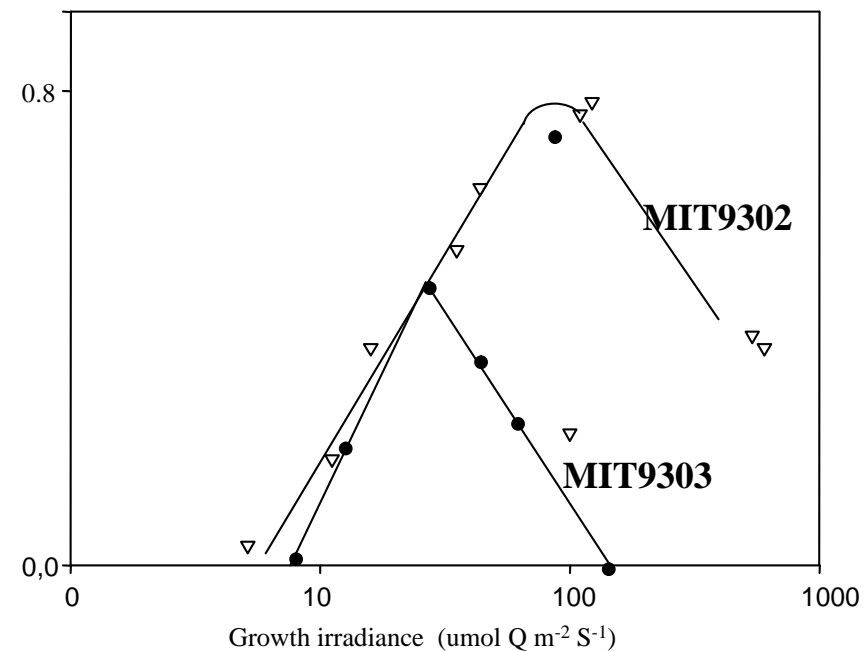
<i>Ostreococcus</i> strains	Clade	Pigments : Chl <i>a</i>		ITS sequences			
		Chl CS-170	Chl <i>b</i>	ITS-1 (G+C % content)	ITS-2 length	ITS-2 (G+C % content)	
RCC 343	A		0.648	155-156	53.7-56	255	57.6-58
RCC 344	A		0.680	153-155	55-55.8	255	57.6-58
RCC 356	A		0.622	153-155	53.7-54.4	255	57.6-57.2
RCC 371	A		0.664	155-156	54.6-55	255	57.6
RCC 420	A		0.613	155	54.4	255	57.6
RCC 141	B	0.021	0.967	143	48.9	264	54.9
RCC 143	B	0.019	0.846	143-148	46.7-48.9	264-265	54.5-55.5
RCC 393	B	0.010	1.011	139-142	48.5-49.3	264	54.1-55.3
RCC 410	B		0.916	142	48.5	264	54.5-54.9
OTH 95	C		0.627	162	50.6	267	56.6
RCC 501	D		0.595	151-156	54-54.5	264-265	58.5-59.1

Growth rate of *Ostreococcus* strains with light intensity



Rodriguez et al. *Env. Microbiol.* 2005

Sargasso Sea *Prochlorococcus* isolates

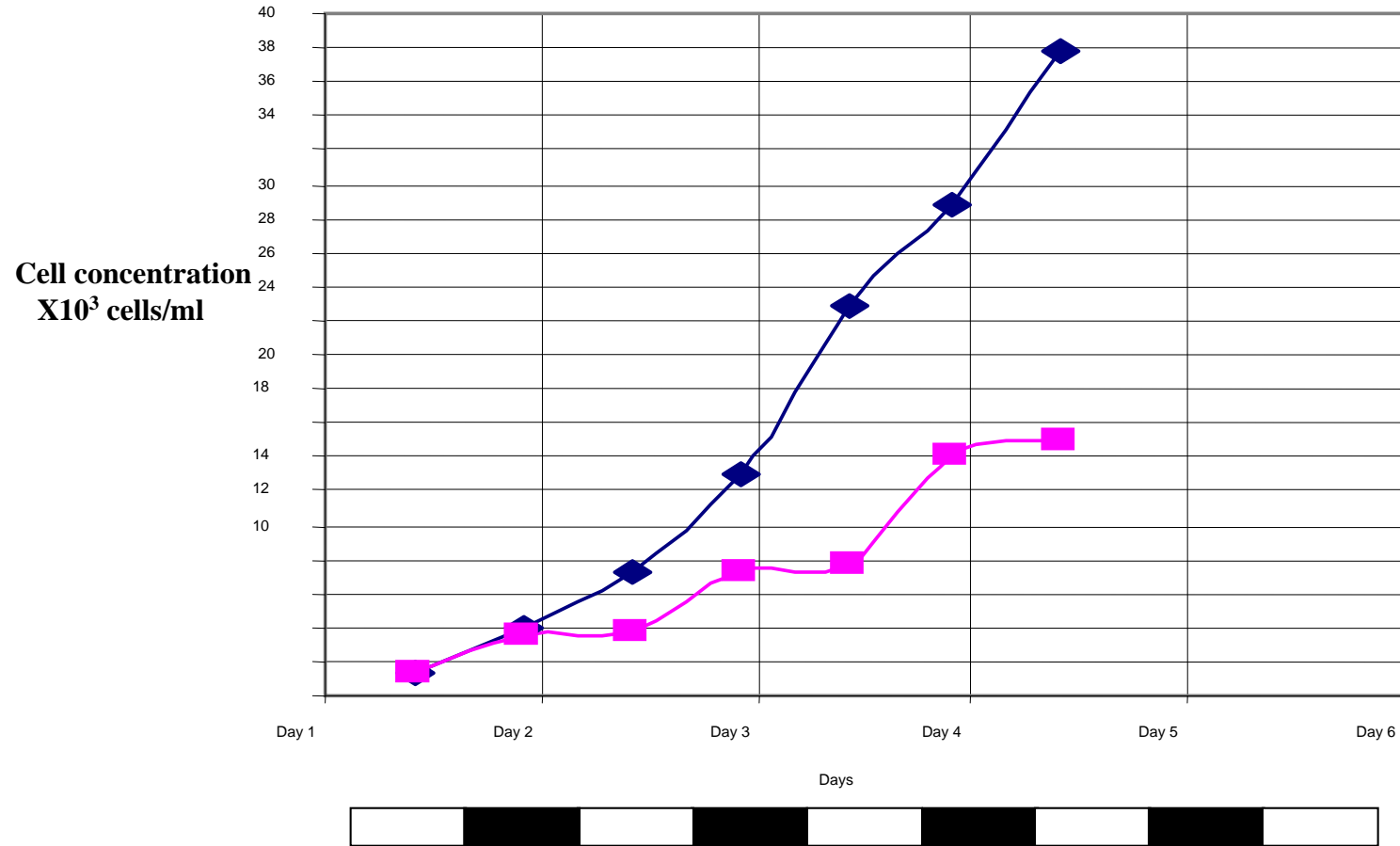


From Moore et al., *Nature* 1998

***Ostreococcus* as a model for cell biology and evolution**

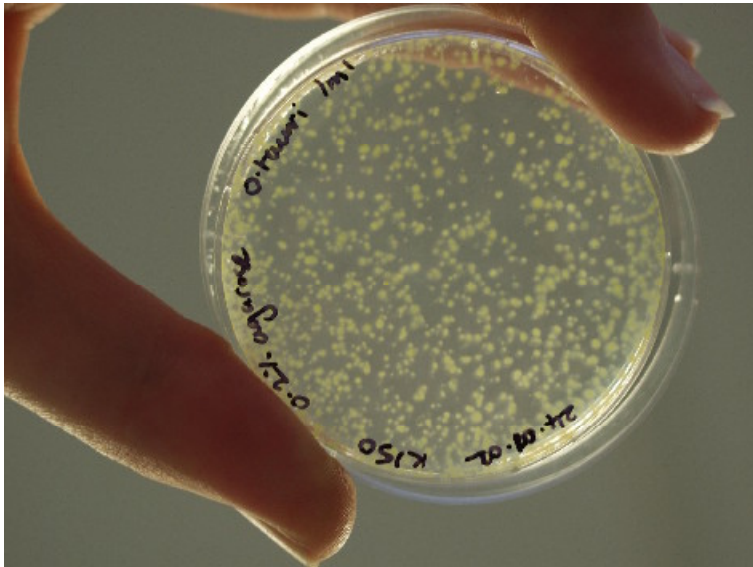
- Relevant phylogenetic position
- Cell biology
- Genomic data available

Cell growth and dark/light cycles



Transformation of *Ostreococcus* cells

Culture on solid phase



Sexuality?

Introduction of DNA inside the cells

- Electroporation
- Lipofection

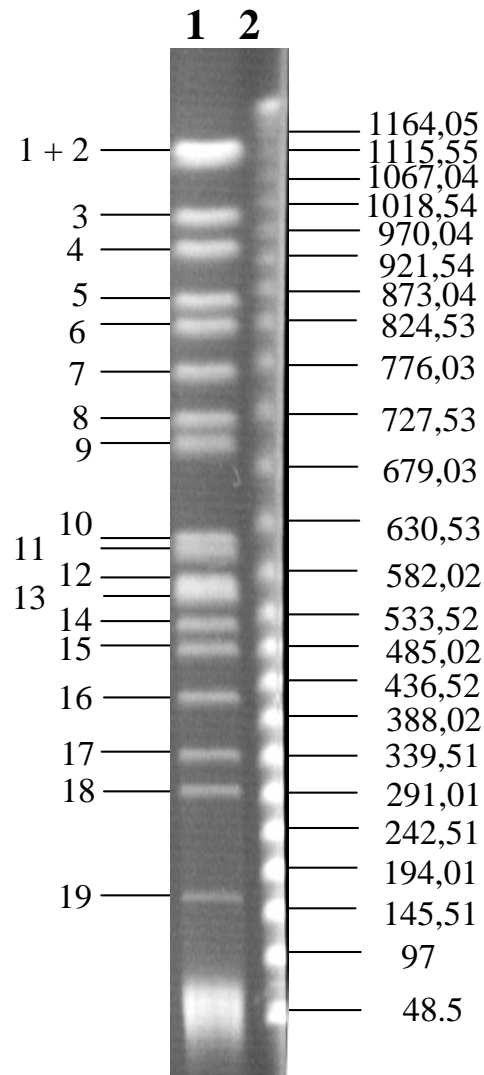
Design vectors with active promoters

- Heterologous promoters
- Homologous promoters
- Reporter/selection genes

Transient/stable expression

- Transient expression
- Genomic integration

Caryotype of *Ostreococcus tauri* by pulsed field gel electrophoresis



✓ 19 chromosomes : 12.6 Mbp

Chlamydomonas reinhardtii : ~100 Mbp

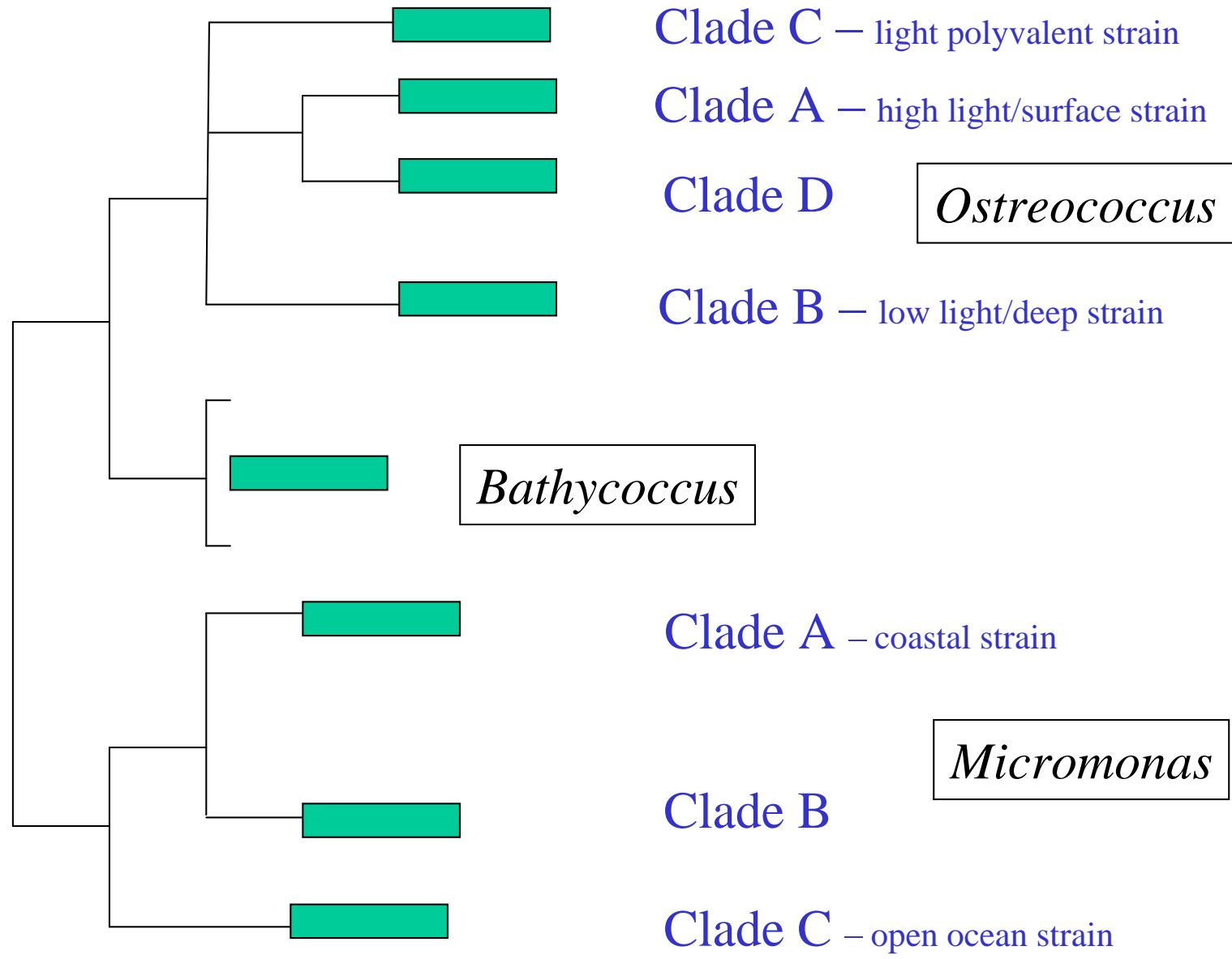
Cyanidioschyzon merolae : 16.5 Mbp

Arabidopsis thaliana : 115.5 Mbp

Sacharomyces cerivisiae : 12.5 Mbp

Human : 3,000 Mbp

Prasinophytes - Order Mamiellales



European consortium

IGH Montpellier

J. Demaille
S. Echeynié
C. Ferraz

BEG Ghent

S. Degroeve
S. Robbens
S. Rombaut
P. Rouzé
Y. Van de Pier

GDP Perpignan

R. Cooke
M. Delseny

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A. Picard
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University of Maryland

Charles Delwiche
Virginia Sanchez Puerta

Academy of Natural Sciences

Rick McCourt

University of Iowa

Debashish Bhattacharya

Shot-gun, BAC and cDNA libraries

Genome sequencing strategy

***Ostreococcus tauri* genome sequenced : 8-fold coverage**

Orientated sequencing

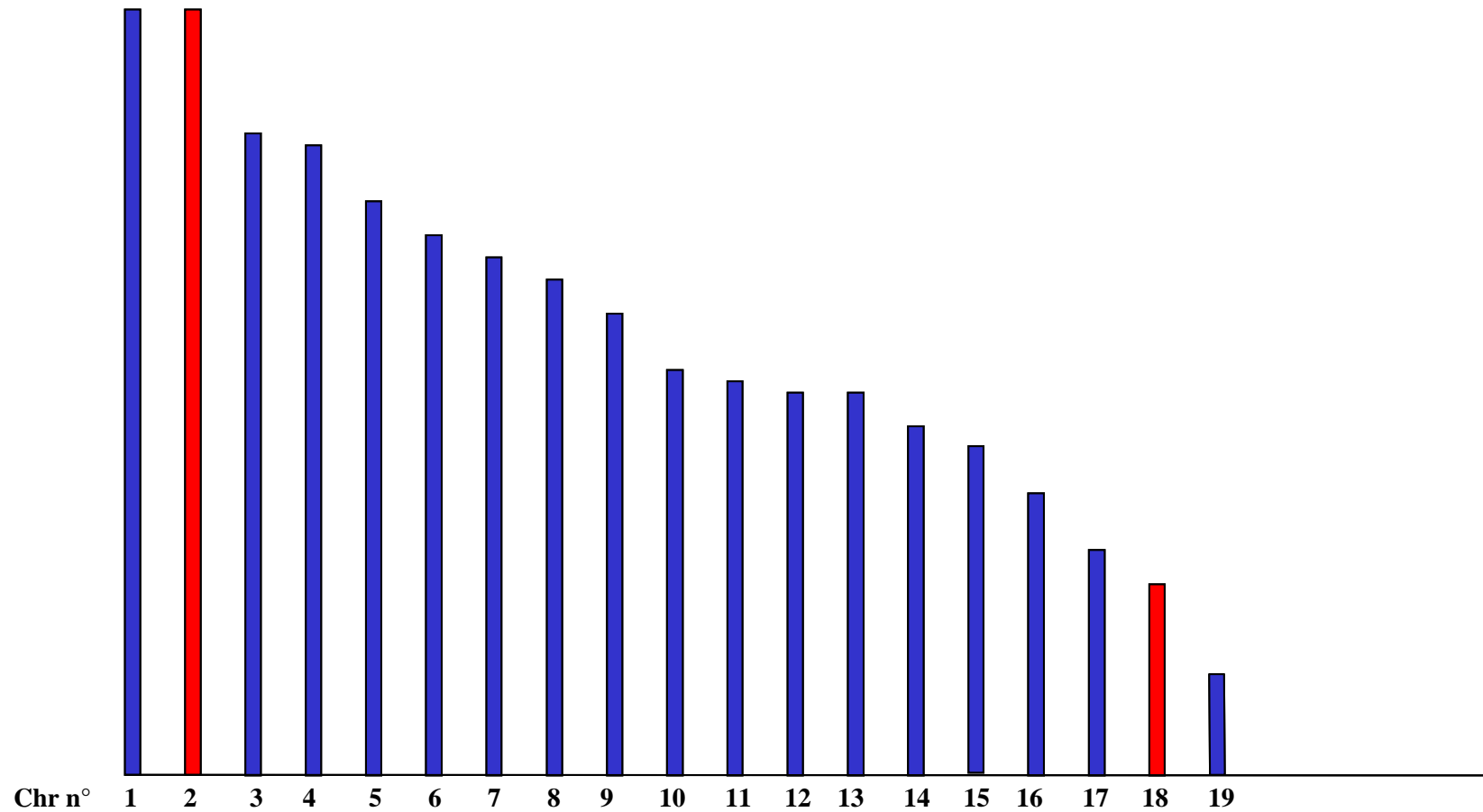
- Few repeated sequences
- Gene sequences closely related to plants or green algae
- Telomeric sequences identical to *Arabidopsis thaliana*
- No obvious centromeric sequences

Some general characteristics of the *O. tauri* genome

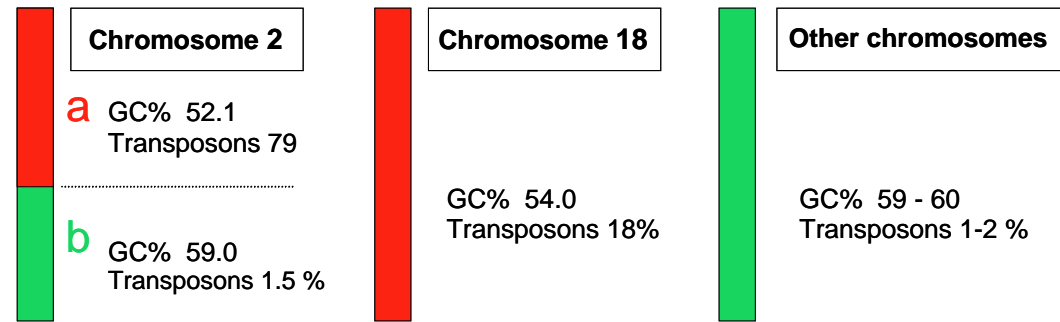
	<i>C. merolae</i>	<i>O. tauri</i>	<i>C. reinhardtii</i>	<i>A. thaliana</i>	<i>S. pombe</i>	<i>S. cerevisiae</i>	<i>A. gossypii</i>	<i>N. crassa</i>	<i>P. falciparum</i>	<i>C. parvum</i>
Size (Mbp)	16.5	12.6	100	115.5	12.5	12.5	9.2	38.6	22.8	9.1
Chromosome number	20	19		5	3	16	7	7	14	8
(G + C) content (%)	55	59	65	34,9	36	38	52	50	19,4	30
Gene number (coding proteins)	5331	8236		25 498	4929	5770	4718	10 082	5628	3807
Gene density (bp per gene)	3099	1535		4526	2528	2165	1950	3700	4338	2382
Mean gene length (excluding introns)	1552	1134		1310	1426	1424	1900	1673	2283	1795
Mean inter-ORF distance (bp)	1543	396		3220	952	515		1953	1694	566
Genes with introns (%)	0,5	25		79	43	5	4.7	100	53,9	5
Mean length of introns (bp)	248	187*		170	81	NA		134	179	
% coding	44.9	74		28.8	57.5	70.5		44	52.6	75.3
Number of 18S, 5,8S and 28S rRNA	3	4		700-800	200-400	100-150	40	175-200	7	5

* Excluding chromosomes 2 and 18

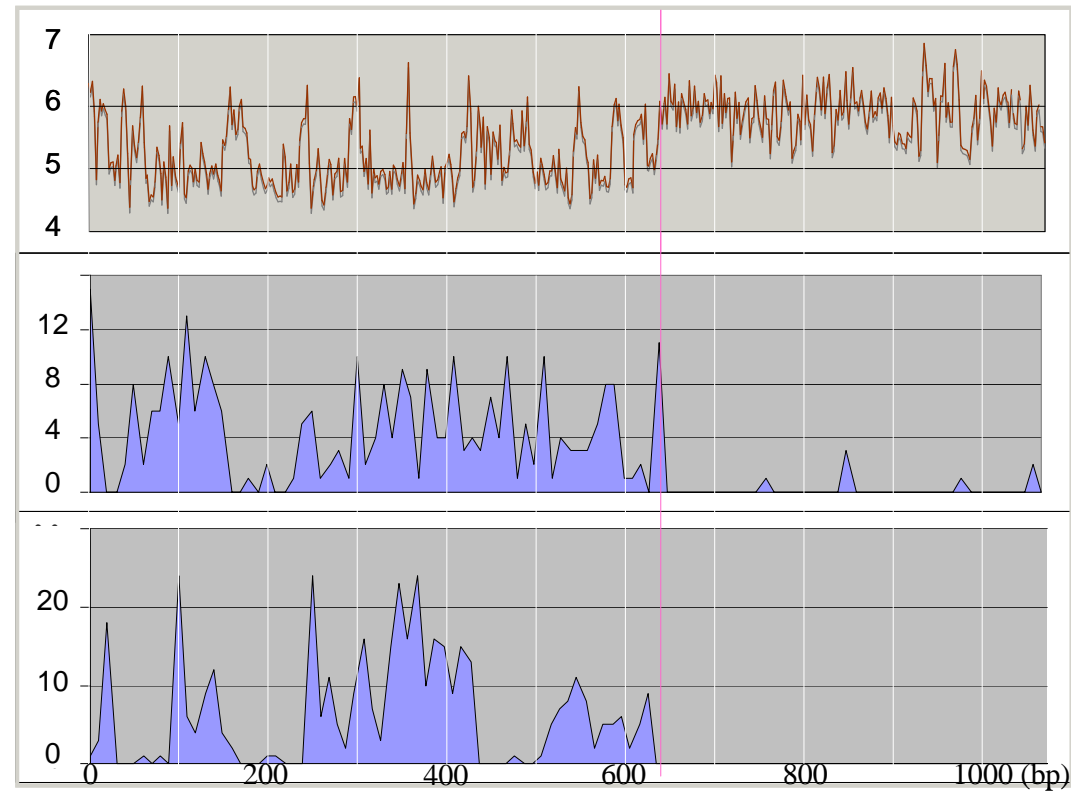
Chromosome heterogeneity in the *Ostreococcus tauri* genome

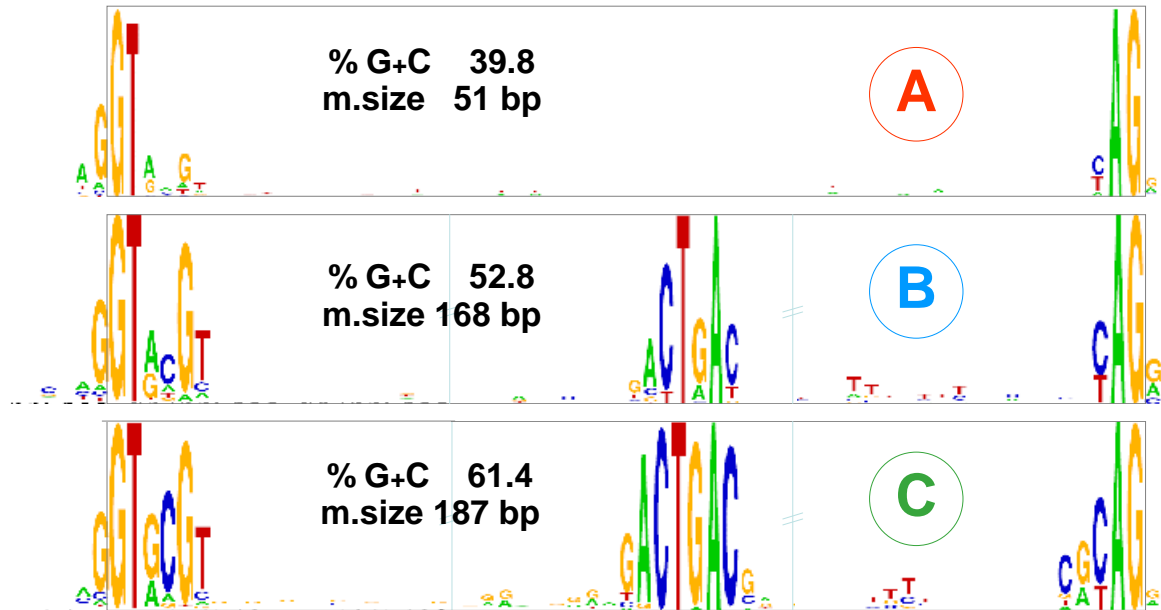
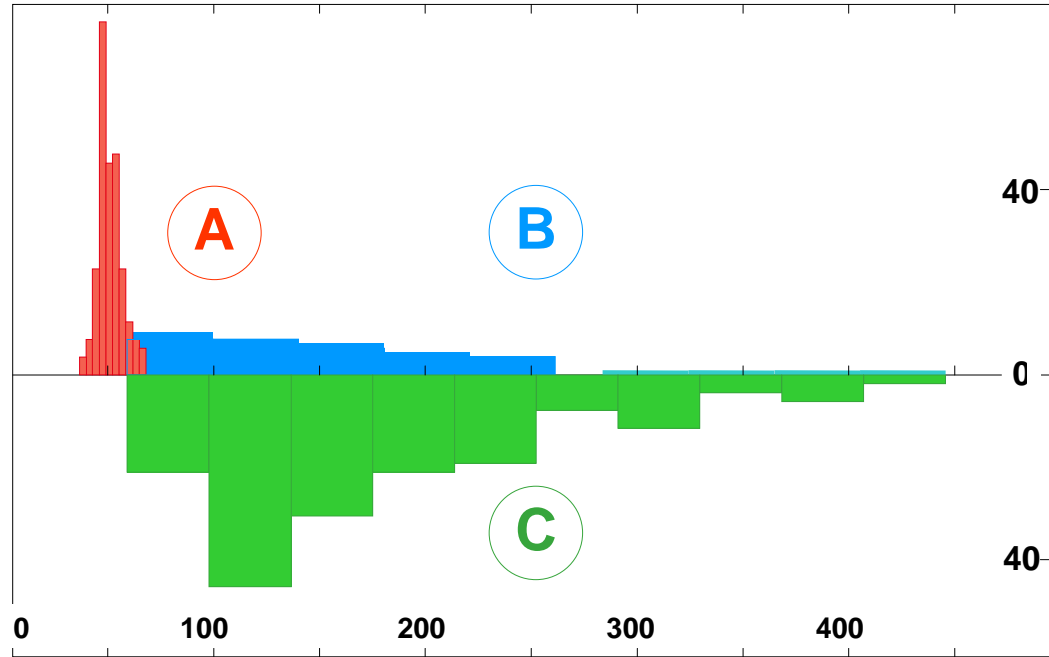


A

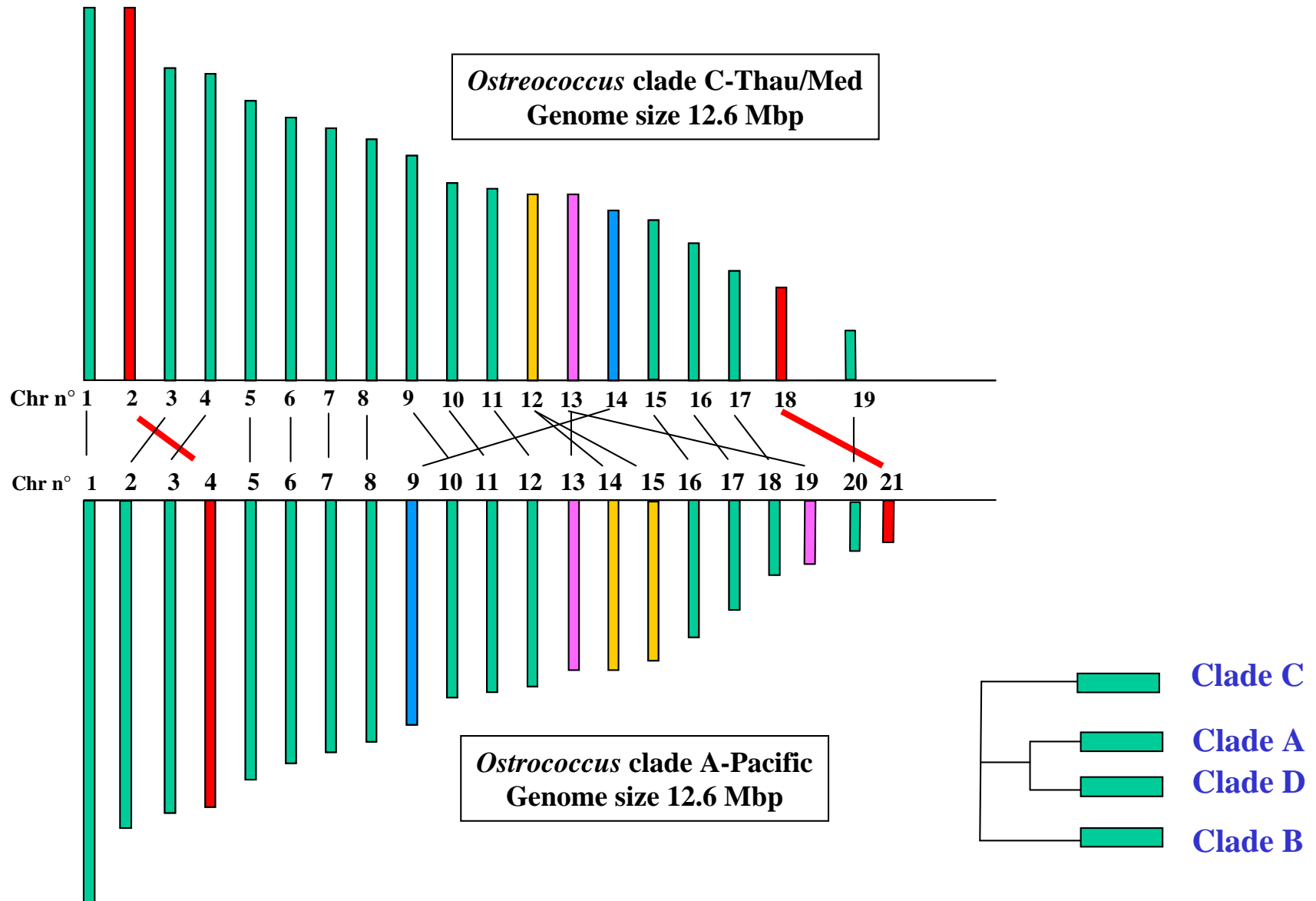


B





Preliminary comparison between two *Ostreococcus* strains



Comparative genomics of cell functions

- **Functions well conserved in all lineages**

Basic metabolism

- **Functions conserved with specific adaptations**

Cell division

Transcription factors

Signaling pathways

- **Functions specific to some lineages**

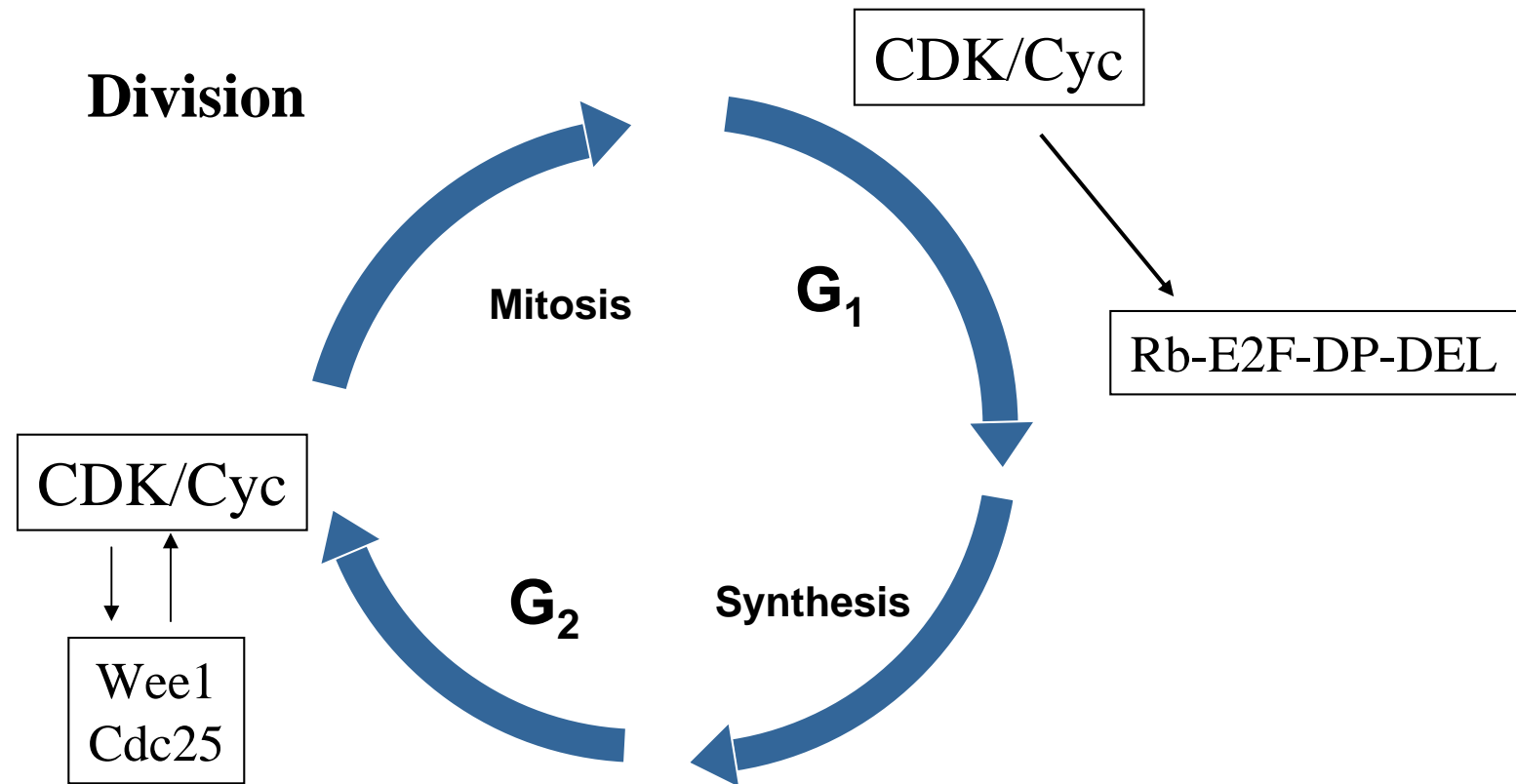
Photosynthesis

Cell wall synthesis

Starch metabolism

Comparative genomics of the cell division cycle

Comparative genomics of the cell division cycle



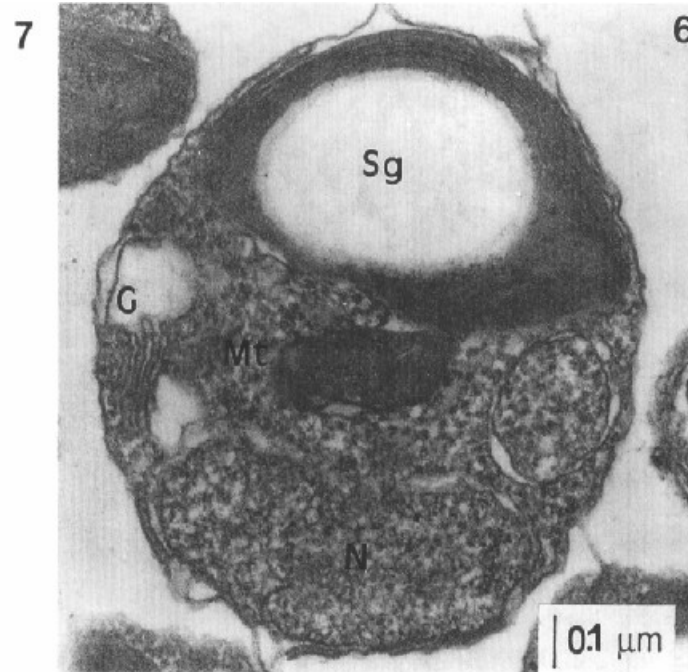
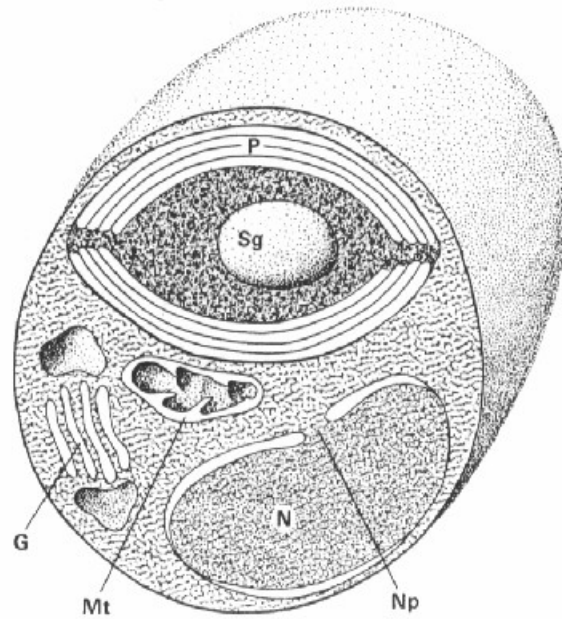
Comparative genomics : the core cell cycle

Gene names	Copy number		
	Hs	At	Ot
CDKA (CDK1,2,3)	3	1	1
CDKB	0	4	1
CDK 4	1	0	0
CDK 6	1	0	0
CDKD or F	2	4	1
CycA	2	10	1
CycB	4	9	1
CycD	5	10	1
CycH	2	1	1
Rb	3	1	1
E2F	6	3	1
DEL	1	3	1
DP	2	2	1
CKS	1	2	1
CDC25	3	0	1
WEE1	1	1	1

Comparative genomics of starch metabolism

Collaboration S. Ball and JP Raï. CNRS-University of Lille

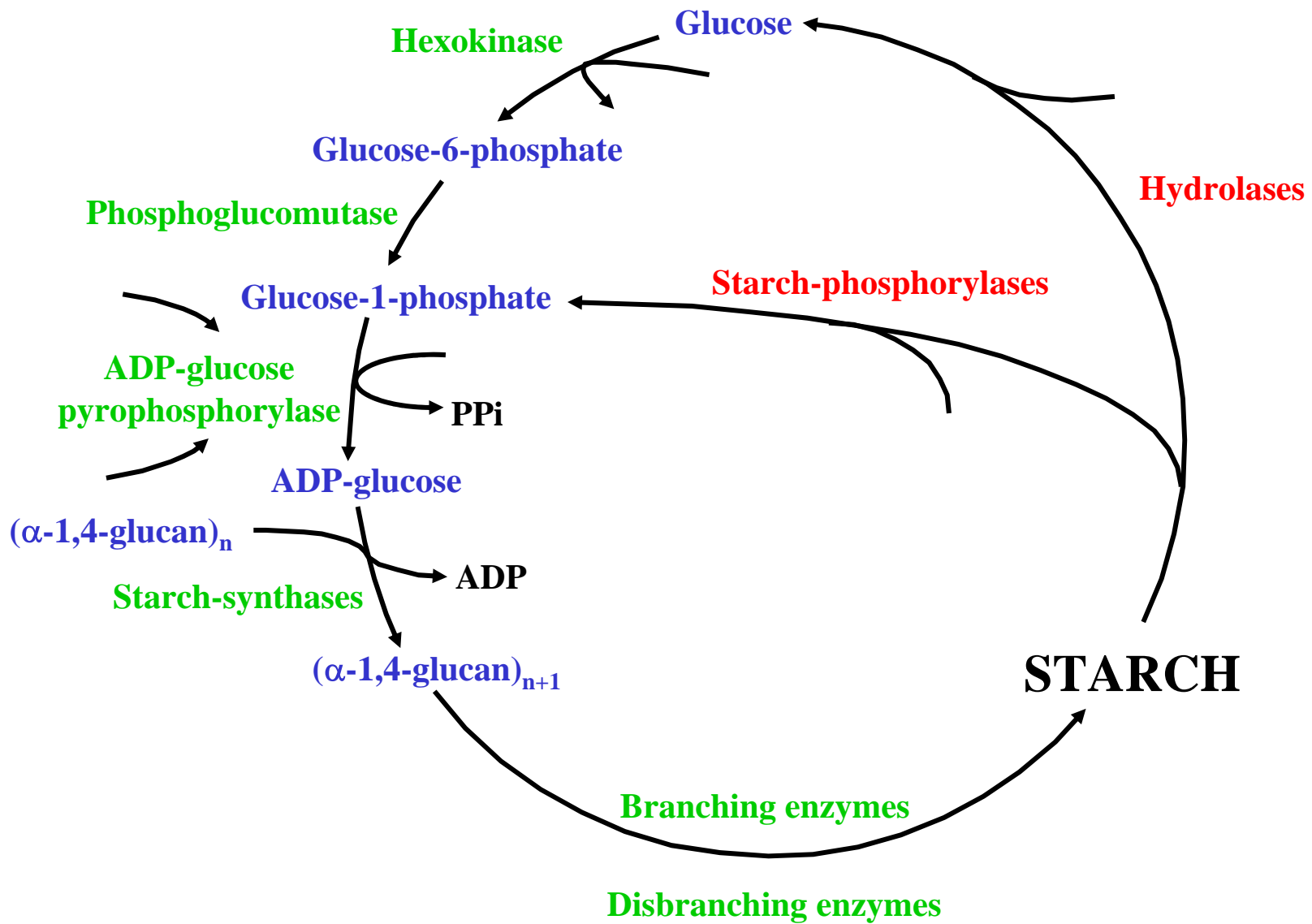
Starch metabolism



From Courties et al. (1994) and Chrétiennot-Dinet et al. (1995)

Collaboration S. Ball and JP Ral. CNRS-University of Lille (Ral et al. Plant Physiol, 2004)

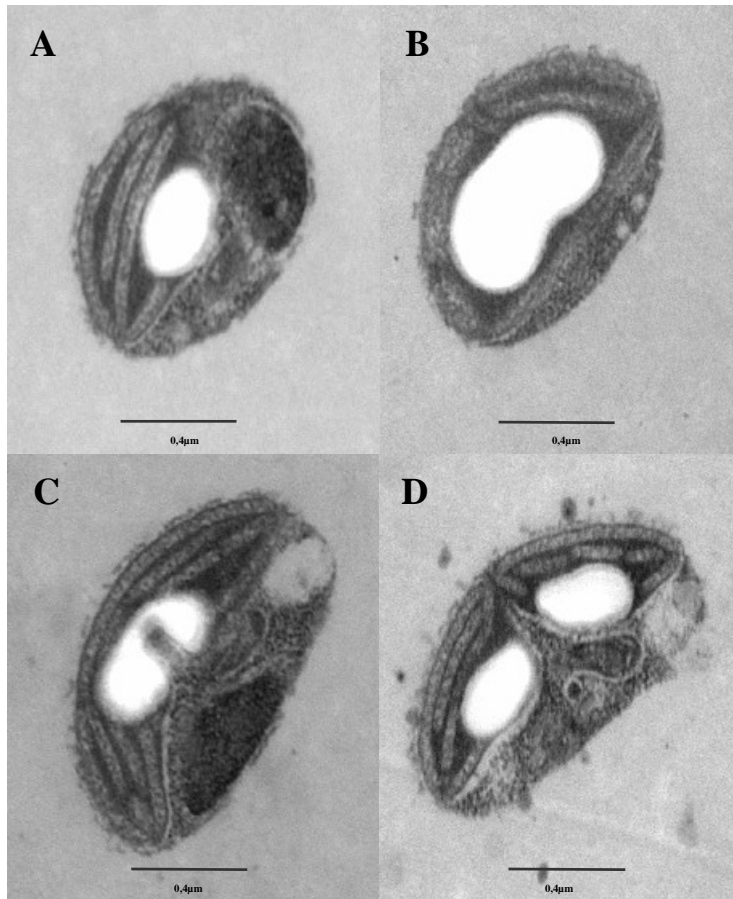
Starch metabolism



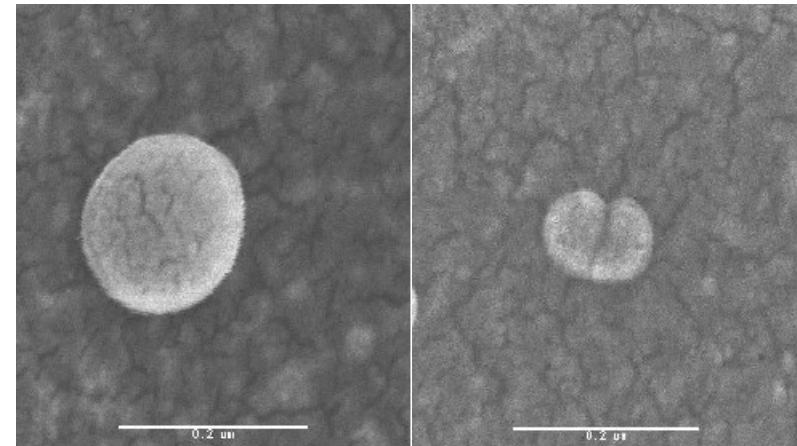
Starch metabolism genes

Nucleotidic sequences <i>C. reinhardtii</i>	Nucleotidic sequences <i>A. thaliana</i>	Nucleotidic sequences <i>O. tauri</i>
ADP- glc Pyro. (Large subunit)	ADP- glc Pyro. (Large subunit)	ADP- glc Pyro. (Large subunit)
ADP- glc Pyro. (Small subunit)	ADP- glc Pyro. (Small subunit)	ADP- glc Pyro. (Small subunit)
CD 142 (GBSSI)	Starch Syn thase 1	GBSSI
CD191 (Starch Synthase)	Starch Syn thase 2	SSI
SS III (Starch Synthase)	Starch Syn thase 3	SSII
CD 192 (Starch Synthase)	Starch Syn thase 4	SSIII -a
EST 43 (Starch Synthase)	Starch Syn thase 5	SSIII -b
SS V (Starch Synthase)	Starch Syn thase 6	SSIII-c
Branching Enzyme I	Branching Enzyme 1	Branching Enzyme I
Branching Enzyme II -a	Branching Enzyme 2	Branching Enzyme II -a
Branching Enzyme II -b	Branching Enzyme 3	Branching Enzyme II -b
DBE9-I4 (Isoamylase)	Isoamylase 1	Isoamylase 1
Isoamylase	Isoamylase 2	Isoamylase 2
	Isoamylase 3	
Pullulanase	Pullulanase	Pullulanase
D- Enzyme	D- Enzyme	D- Enzyme
PHO A (Phophorylase)	Phophorylase 1	Phophorylase 1
PHO B (Phophorylase)	Phophorylase 2	Phophorylase 2
Protein R1	Protein R1	Protein R1
α - Amylases	alpha amylases (3)	α - Amylases (3)
β - Amylases	beta amylases (8)	β - Amylase (1)
	glycogenins (7)	

Micrographs of dividing starch granule



Transmission electron
microscopy

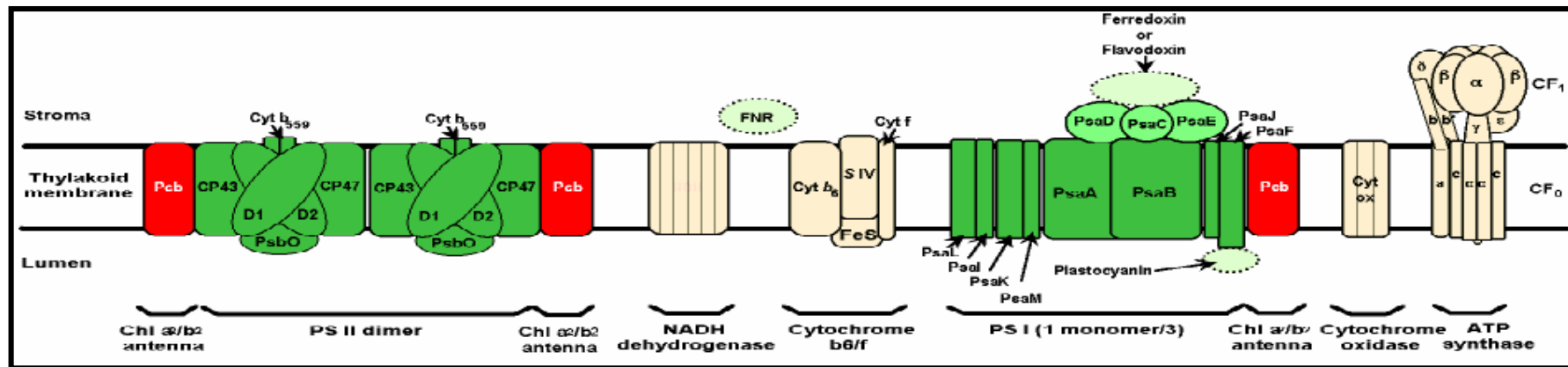


Scanning electron
microscopy

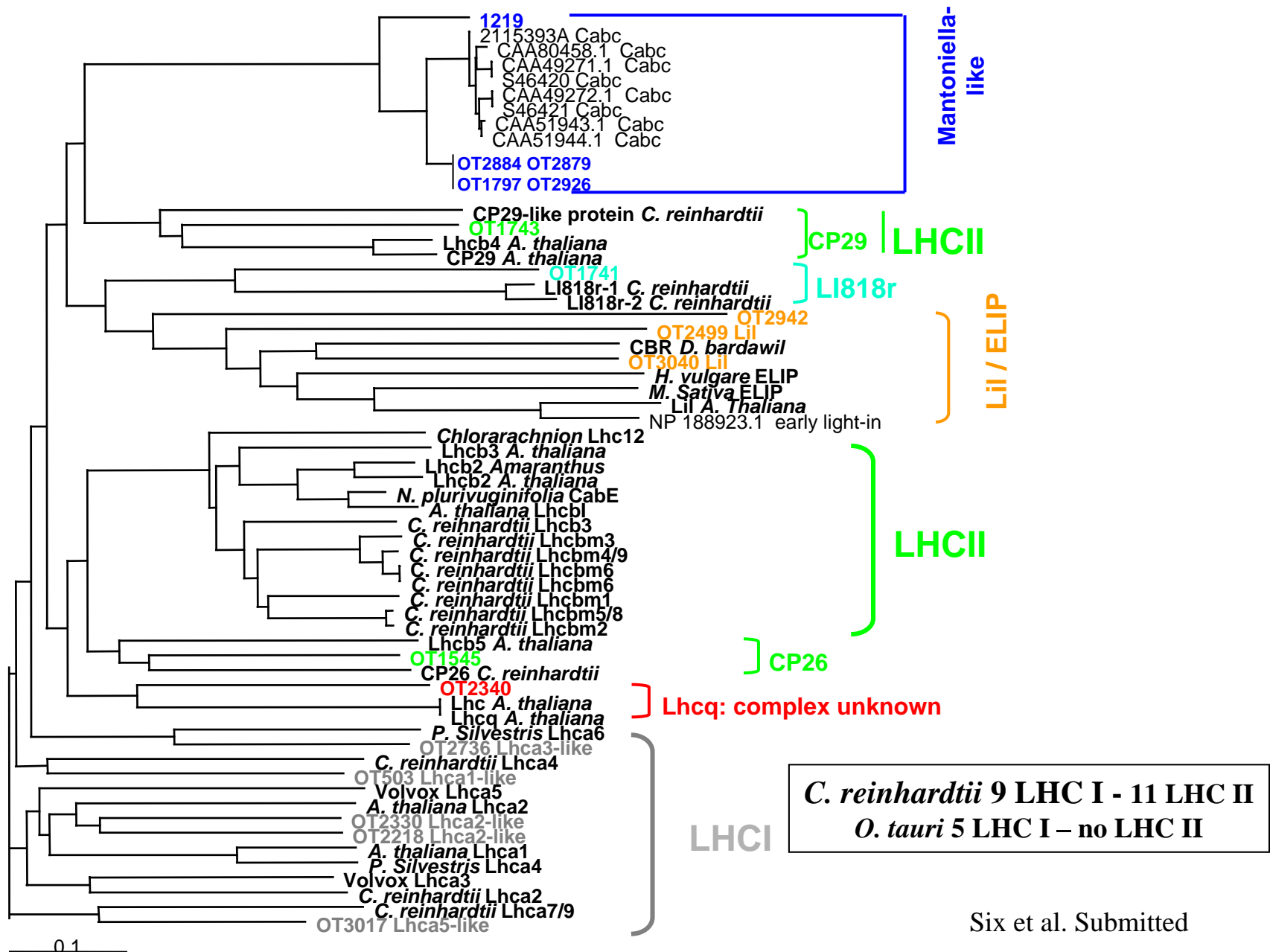
Comparative genomics of photosynthesis

Collaboration with F. Partensky, C. Six and A. Worden

Photosynthesis complexes



Ostreococcus LHC phylogeny



Mammiellales as model organisms in the green lineage

- Relevant phylogenetic position
- Cell biology
- Small typical eukaryote genomes
- Wide geographical distribution
- Surface/deep strains and coastal/open ocean strains
- Comparative genomics and functional studies
- Gene content and ecological niche

What's next ?

- Genome comparison between the Mediterranean and the Pacific *Ostreococcus* strains
- Characterisation of the «deep/surface» ecotypes
- Genome sequencing of a « deep » *Ostreococcus* strain
- Other mammiellales species (*Micromonas* and *Bathycoccus*)
- Comparative genomics and functional studies
- Gene content and ecological niche

European consortium

IGH Montpellier

J. Demaille
S. Echeynié
C. Ferraz

BEG Ghent

S. Degroeve
S. Robbens
S. Rombaut
P. Rouzé
Y. Van de Pier

GDP Perpignan

R. Cooke
M. Delseny

ENS Paris

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K. Jabbari

Arago Banyuls

A. Camasses
E. Derelle
Y. Desdevises
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H. Moreau
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D. Vaultot

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Asaf Salamov
Andrea Aertz
Yigong Lou
Astrid Terry
Nik Putnam

University of Maryland

Charles Delwiche
Virginia Sanchez Puerta

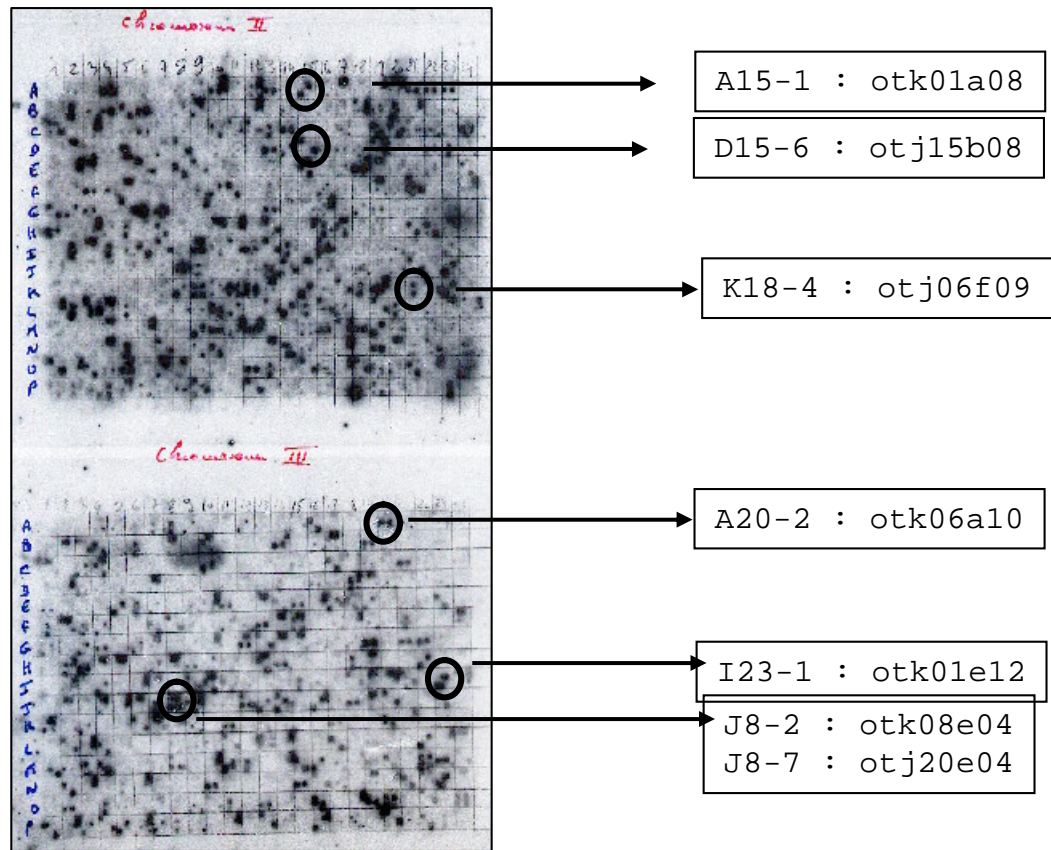
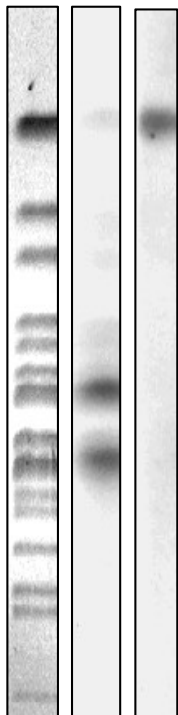
Academy of Natural Sciences

Rick McCourt

University of Iowa

Debashish Bhattacharya

Genome assembling strategy

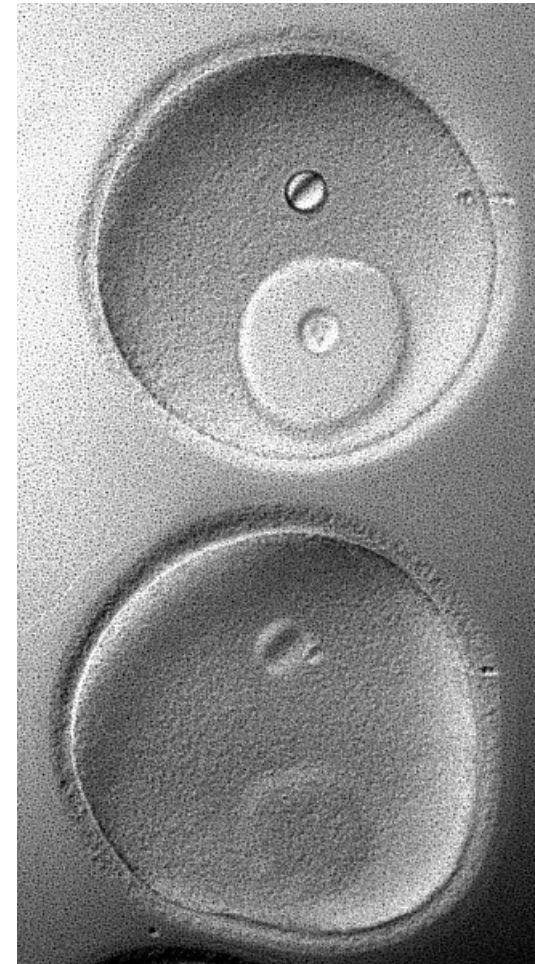


Functionality of *O. tauri* Cdc25

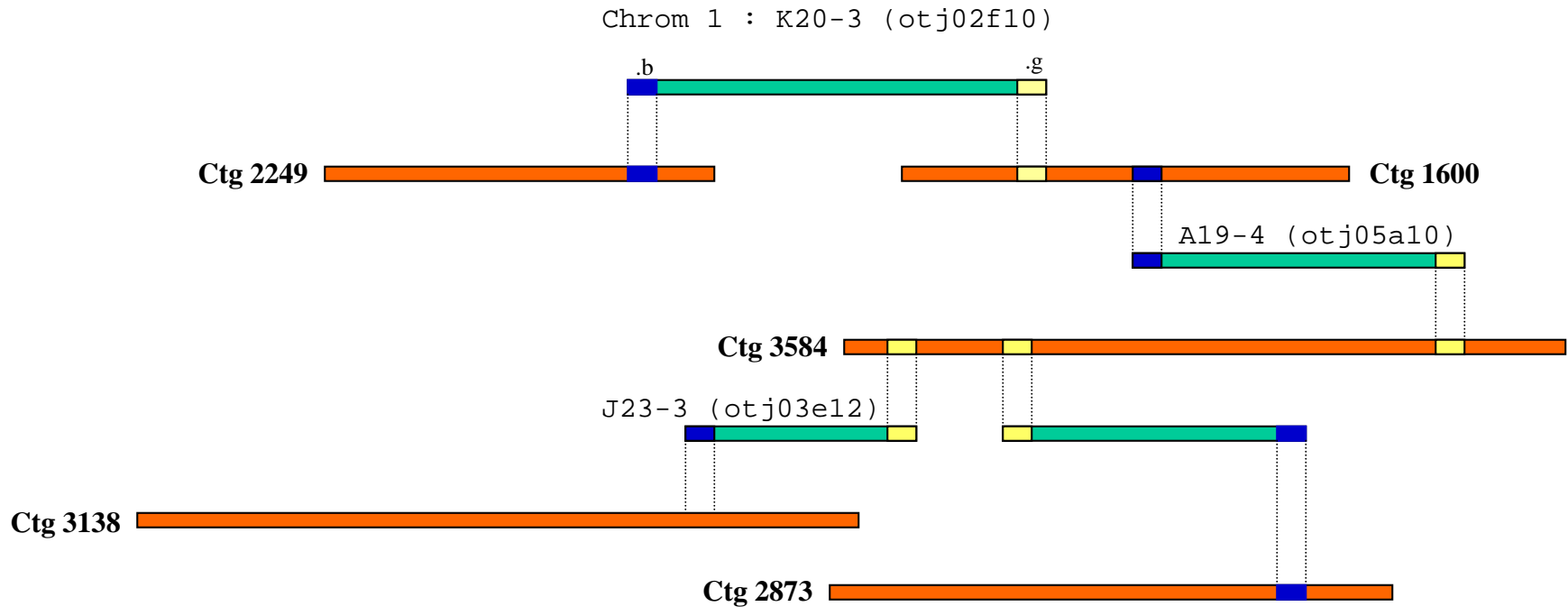
S. pombe



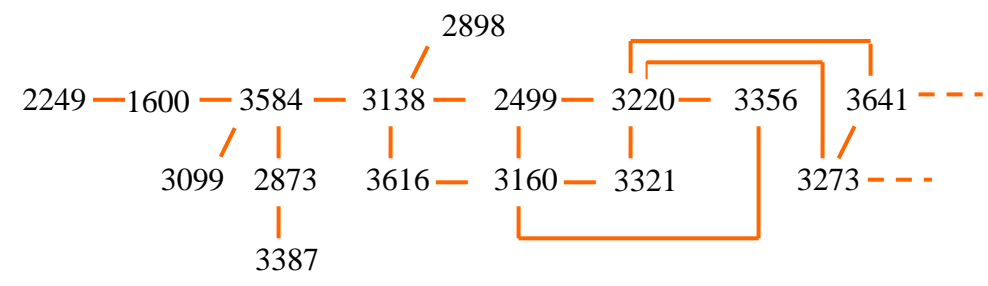
Star fish oocyte



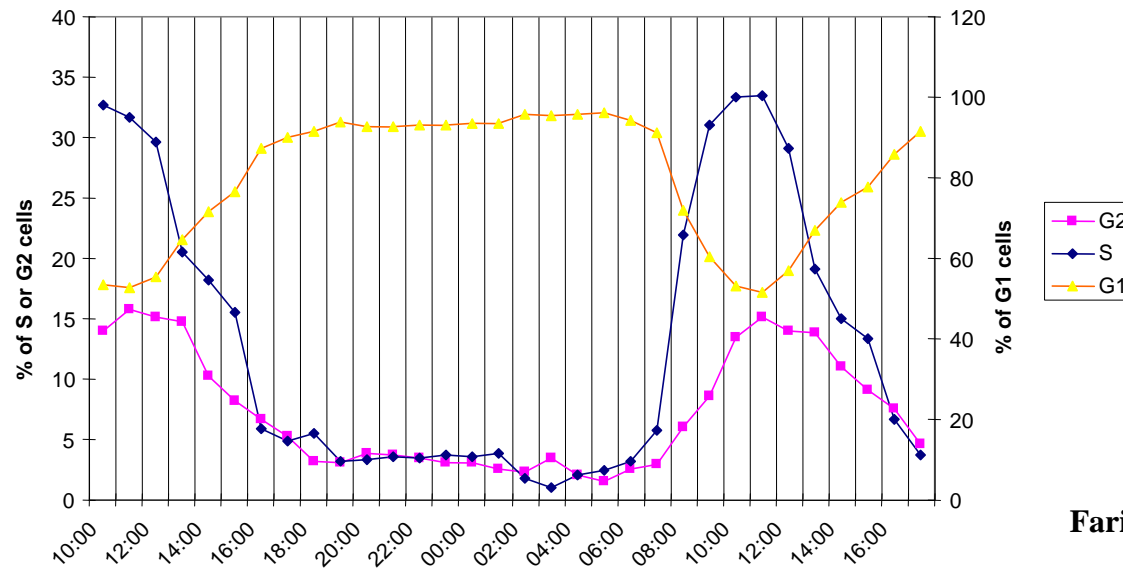
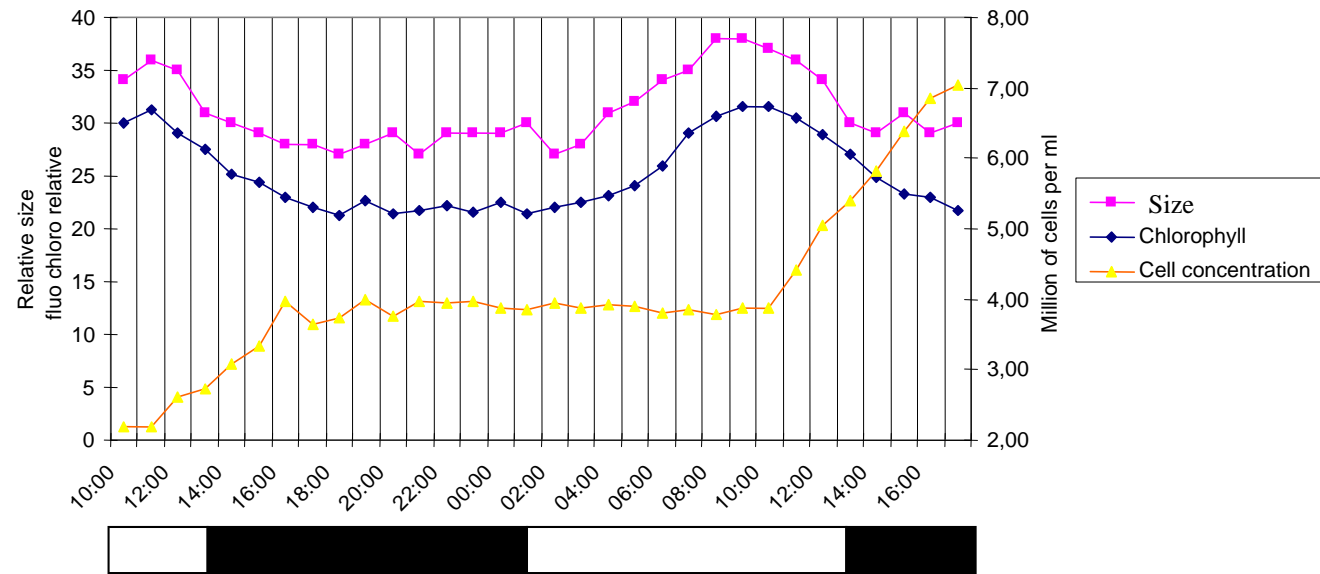
Kadharoo et al., Cell Cycle (2004)



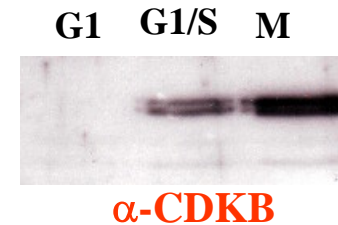
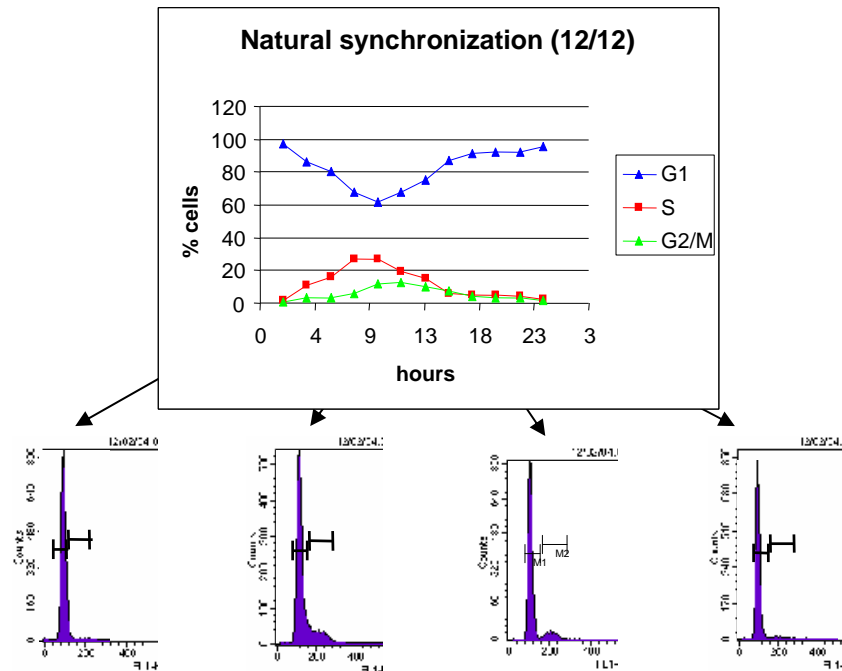
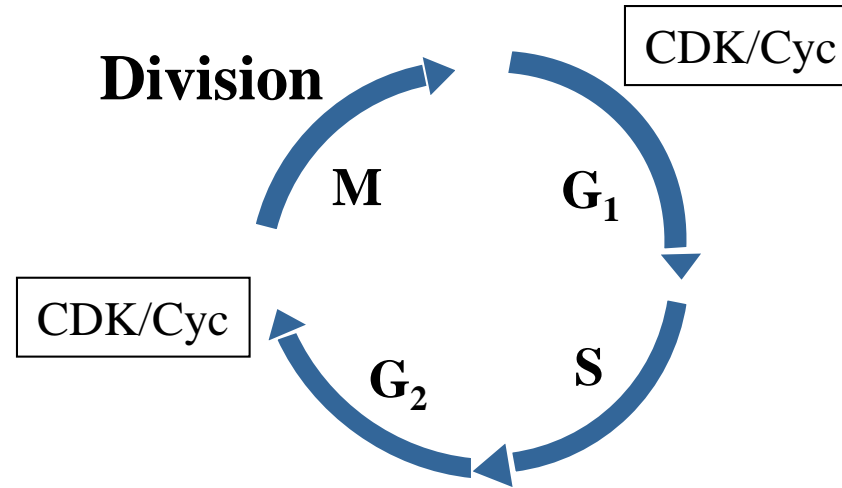
→ **Contig «clustering» on chromosome I**



Characterization of 12/12 light/dark cycle



Functional characterisation of the cell division cycle



Expression of CDKB gene specifically in S, G2 or M phases