Duckweed (Lemnaceae): Biological design for alkane and biodiesel production

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Cold Spring Harbor Laboratory

Duckweed (Lemnaceae):

A clonal aquatic flowering plant

Existing energy crops

- Corn/sugarcane/swichgrass
  - Compete with food crops
  - High energy input
  - High lignin content
  - Production of bioethanol
- Algae
  - High biomass output
  - Difficult to harvest
  - Contamination problems
  - Can produce oil

Replacing fossil fuels

Biofuel feedstock	**Fossil energy balance**
Cellulosic ethanol (*Miscanthus* grass)	2 -36
Palm oil	9
Ethanol (sugarcane)	8
Rapeseed (canola) oil	2-3
Corn ethanol	1. 5

The best energy crops are clones…….


Oil palm is the most
productive oil crop…
But competes with rainfo
and food production
MPOB
Malaysia
Palm Oil Board

LETTER

nature

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The oil palm *SHELL* gene controls oil yield and encodes a homologue of SEEDSTICK

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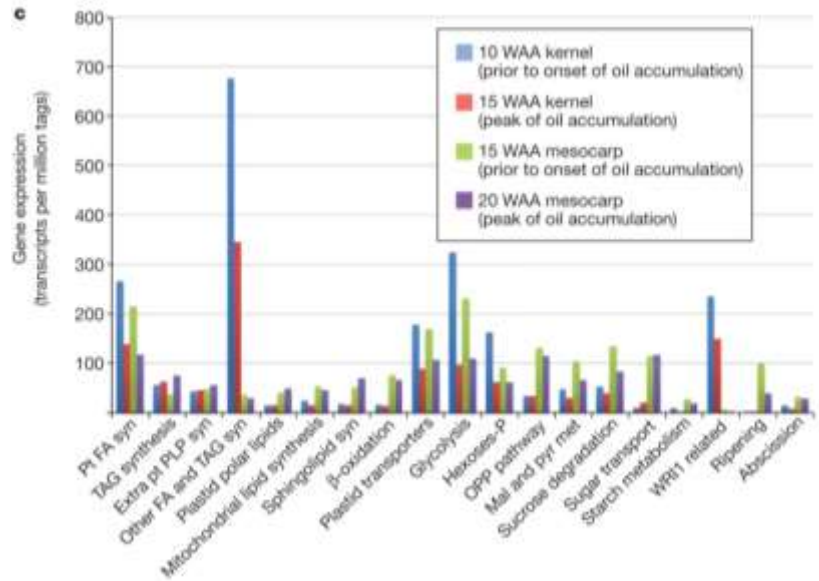

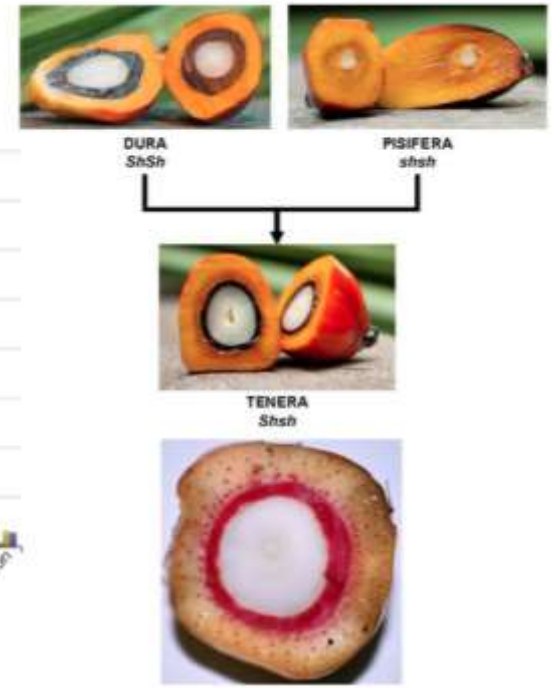

Lemnaceae as energy crop

Lemnaceae (duckweed) are the world's smallest, but fastest growing aquatic flowering plants

Used for basic research, environmental monitoring and waste water remediation

Very high rates of biomass accumulation make them an attractive target for engineering biofuel feedstocks

Lemnaceae as energy crop

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- Does not compete with food production
- Can be grown on wastewater
- Rapid growth
- Low lignin content
- Long production period
- Spread all over the world
- Cheap and easy to grow

Lemnaceae *(family Araceae, monocots)*

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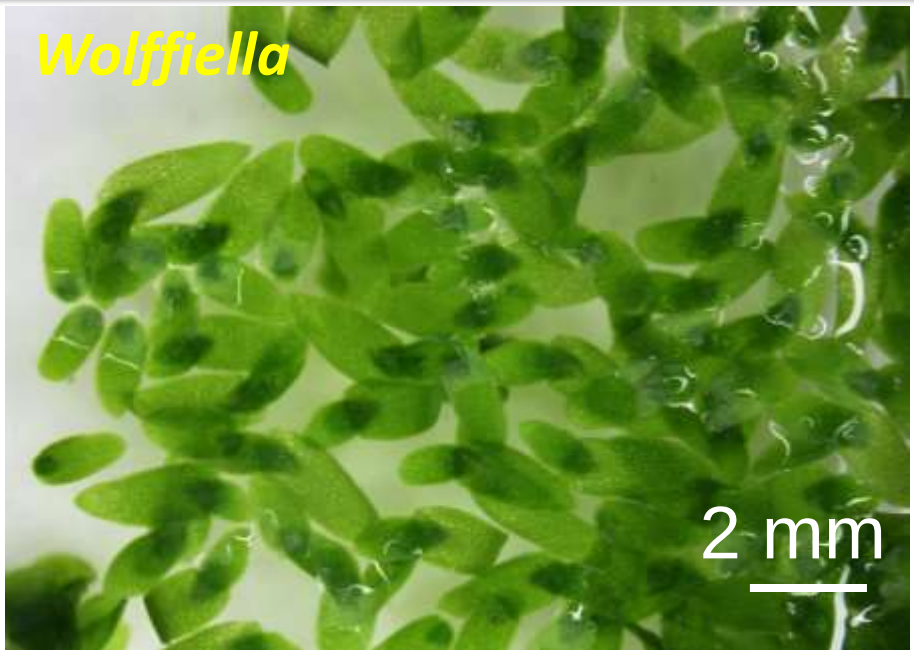
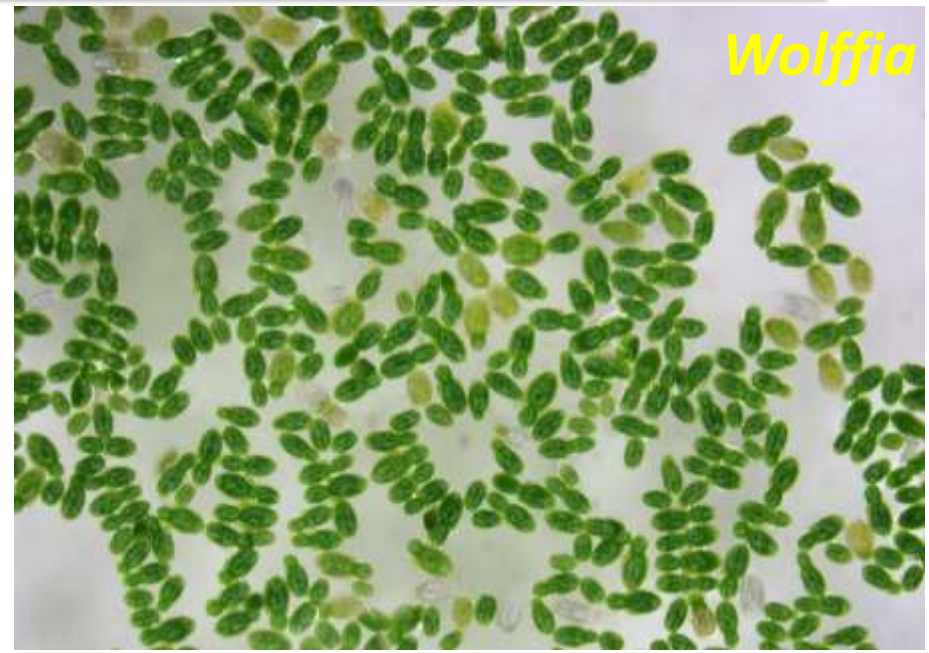
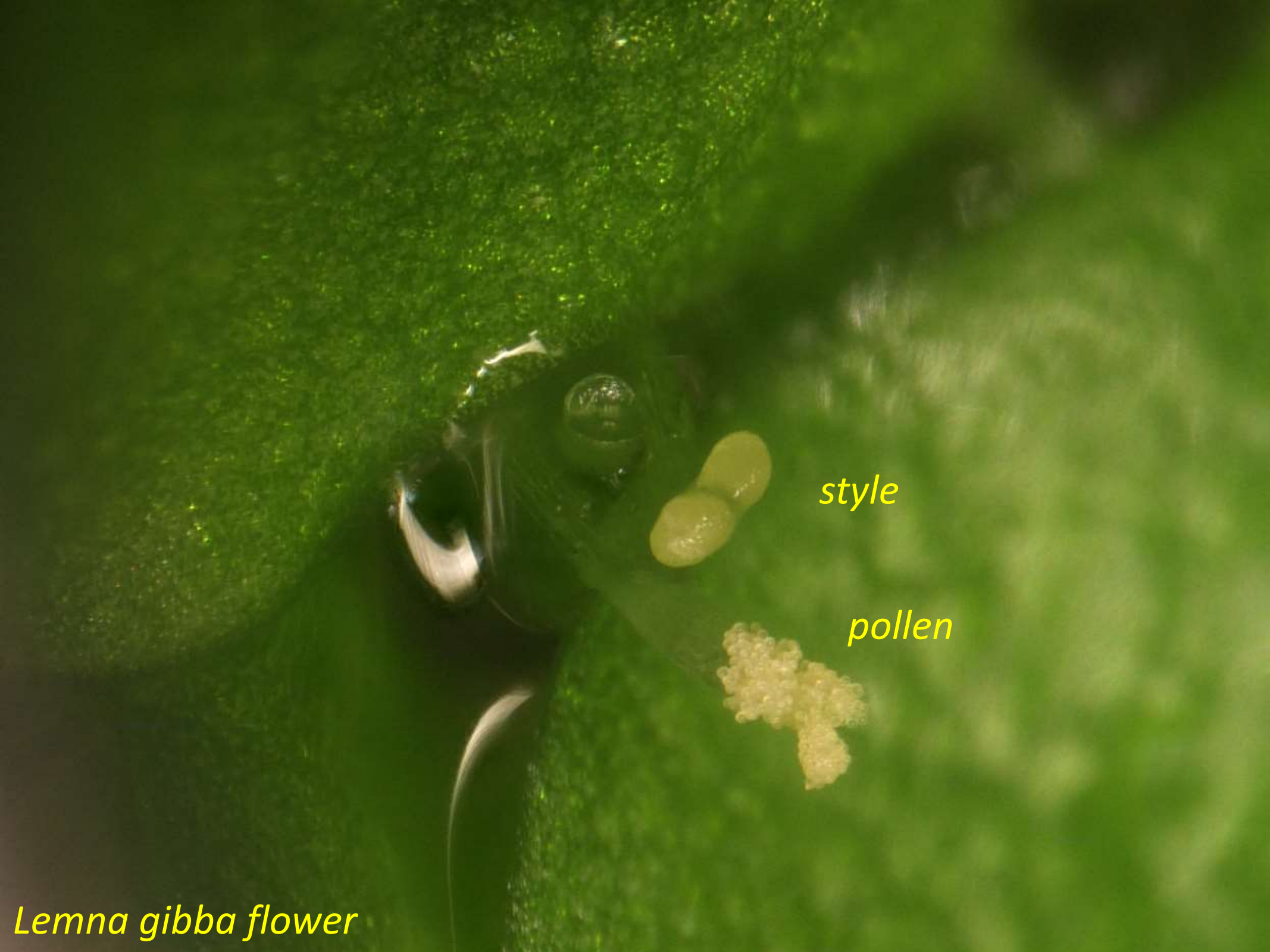




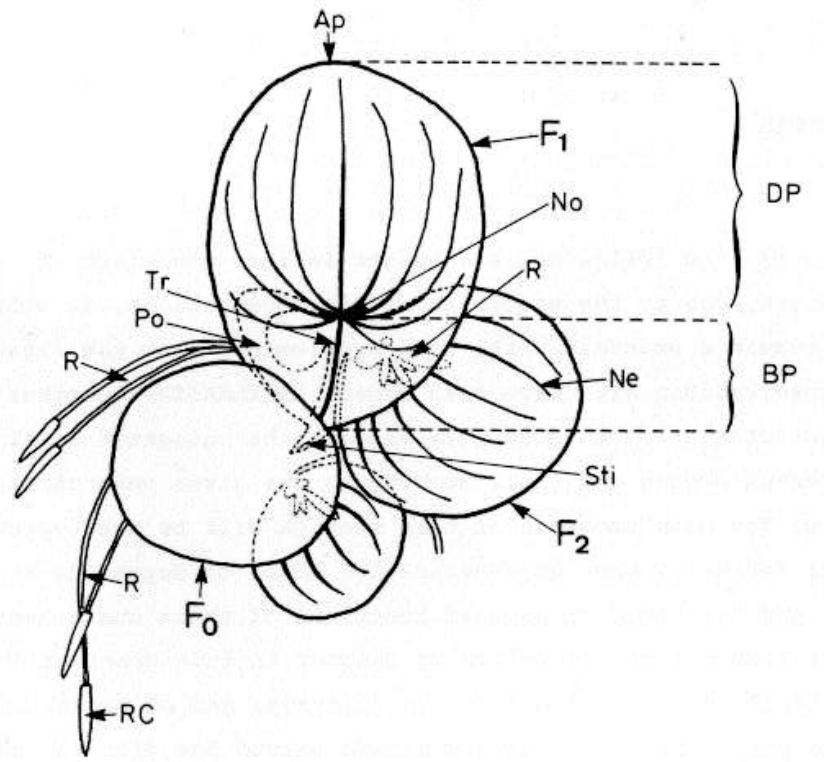


Lemna gibba flower

Lemnaceae

Liopsida	class
Aridae	subclass
Arales	order
Araceae	family

Wolfia microscopica



Spirodela polyrhiza
(Landolt, 1986)

- Reduced morphology
  - •Fronds (leaf-like structures)
  - •Meristem-like stem cell "pocket"
- Clonal reproduction
  - •48 hours duplication by budding
  - •Limited flowering

Lemnaceae

- Wide natural variation:
  - Growing rate
  - Starch 12-48%
  - Protein 11-40%
  - Lipid 2-9%
  - Low lignin content (5%)

- Needs genetic modification for lipid production

Chemical properties of biodiesel

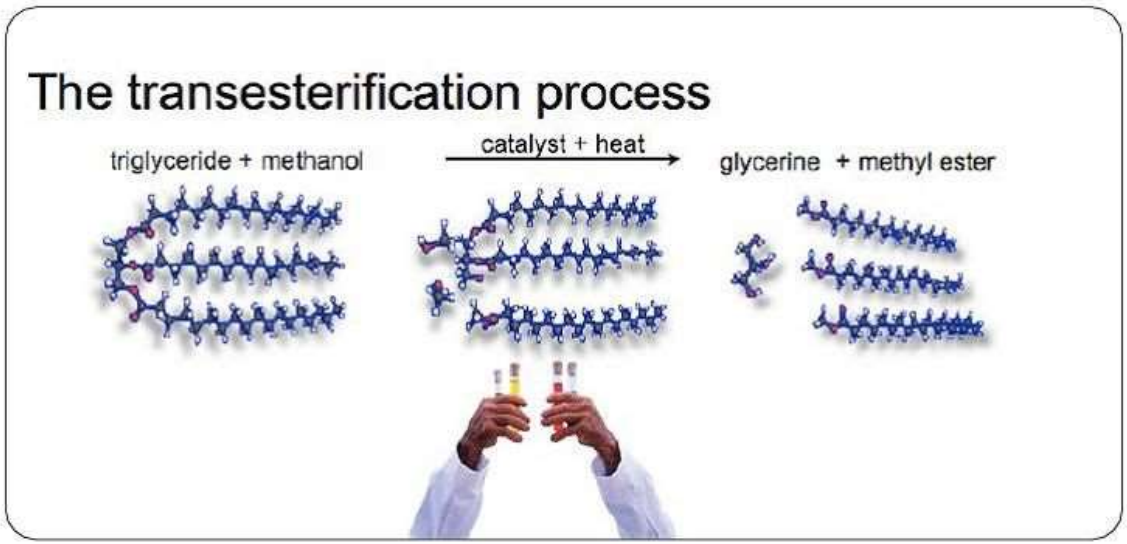
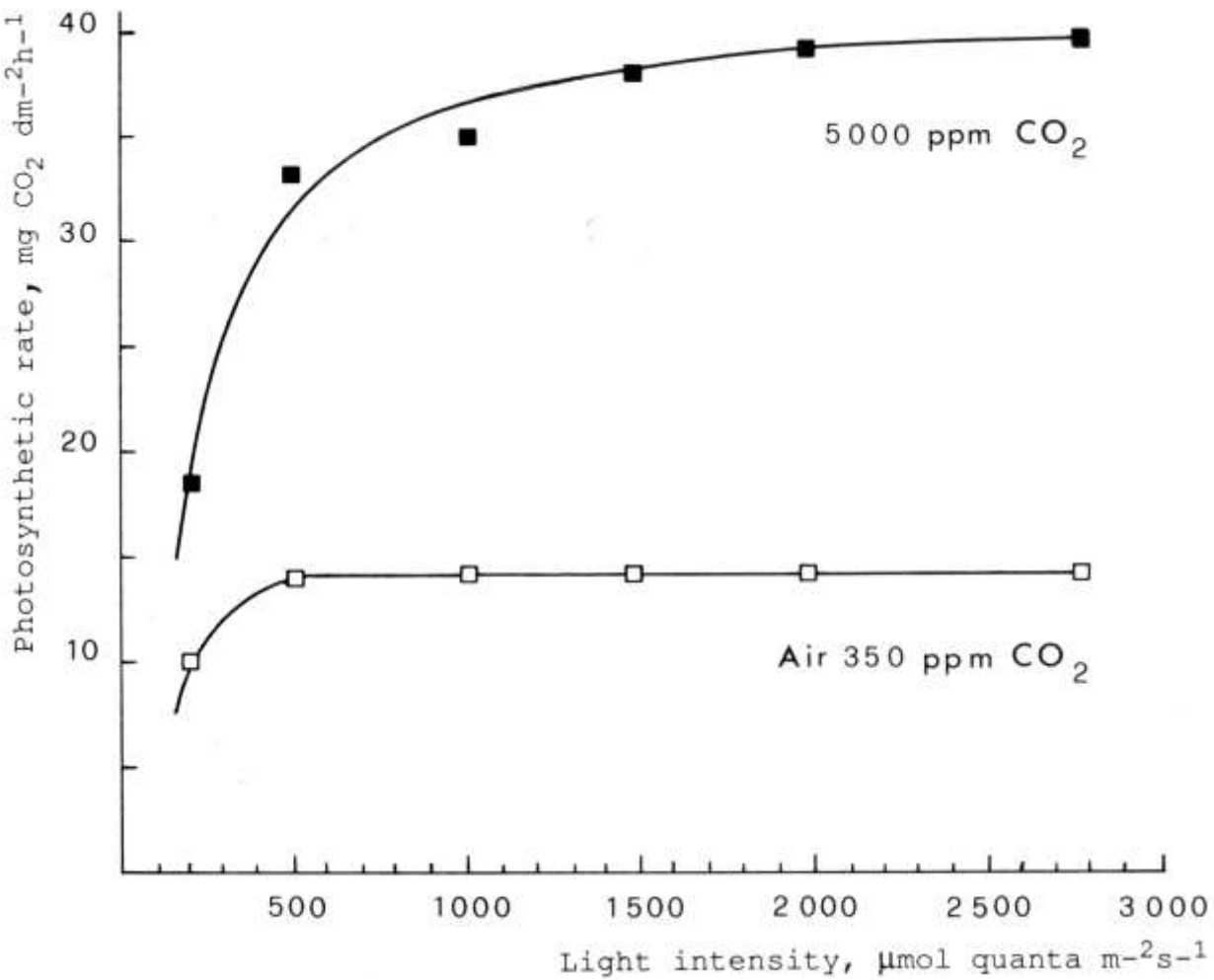


Table 1. *Fuel Properties as a Function of Fuel Composition in Diesel Engines*

Chemical property	Fatty acids		
	Saturated 12:0, 14:0, 16:0, 18:0, 20:0, 22:0	Monounsaturated 16:1, 18:1, 20:1, 22:1	Polyunsaturated 18:2, 18:3
Cetane number	High	Medium	Low
Cloud point	High	Medium	Low
Stability	High	Medium	Low
NO_x emissions	Reduction	Slight increase	Large increase

Photosynthetic rate responds well to increased Carbon



Andersen, 1985

Our goal

- Develop duckweeds as biofuel feedstock
  - Create/improve molecular tools to study duckweed
  - Study duckweed development and metabolism
  - Generation of transgenic lines for biofuel production
    - Increase expression of genes related to the production of TAG
    - Silence the genes that have a role in the oxidation of lipid bodies
    - Redirect the starch metabolism silencing the key genes that lead to its accumulation

Lemna gibba genome sequence
http//www.lemna.org

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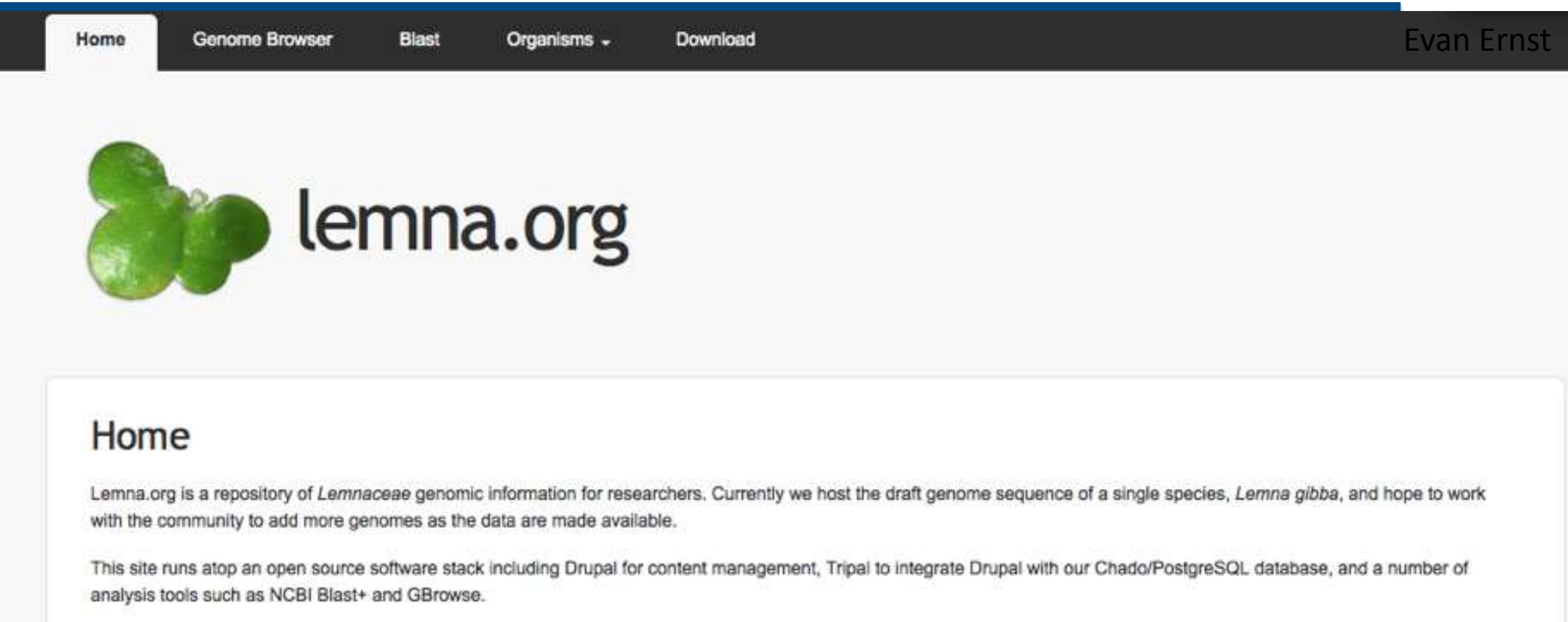
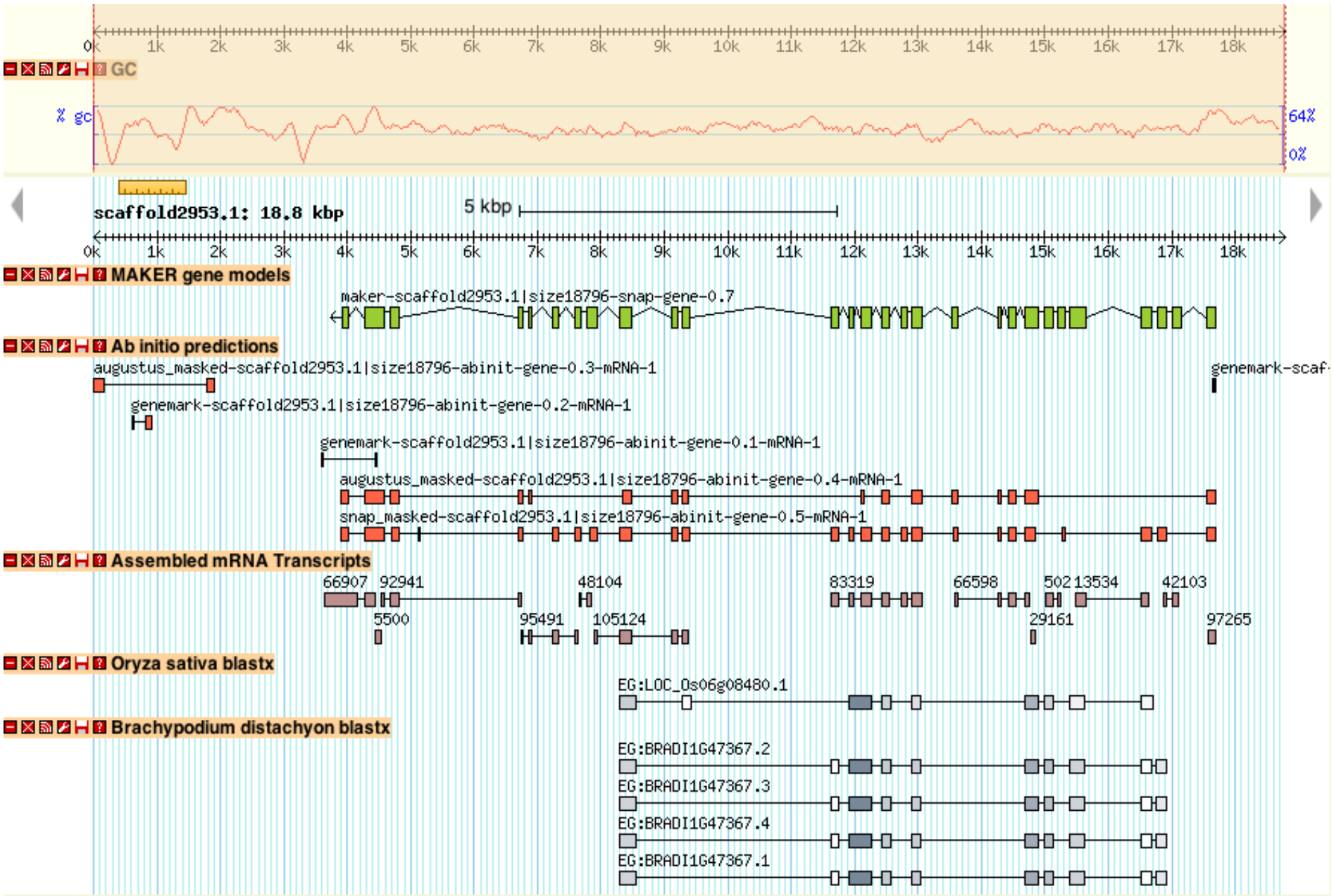
Evan Ernst



Lemna gibba G3 DWC131 Assembly (450 Mbp)

	# >200nt	# >100Knt	N50 (bp)	NG50 (bp)	Longest (bp)	Size (Mbp)
Contigs	471,436	-	1,876	1,476	33,244	401
Scaffolds	140,499	54	16,085	18,907	270,981	507

PICKLE: a regulatory gene for Oil biosynthesis

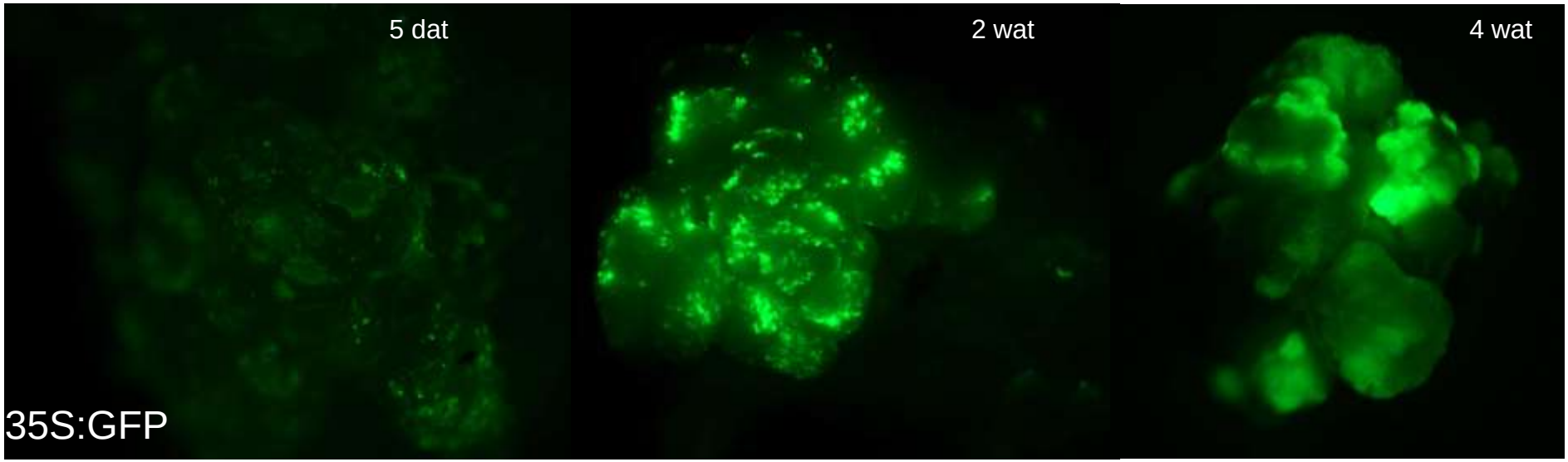


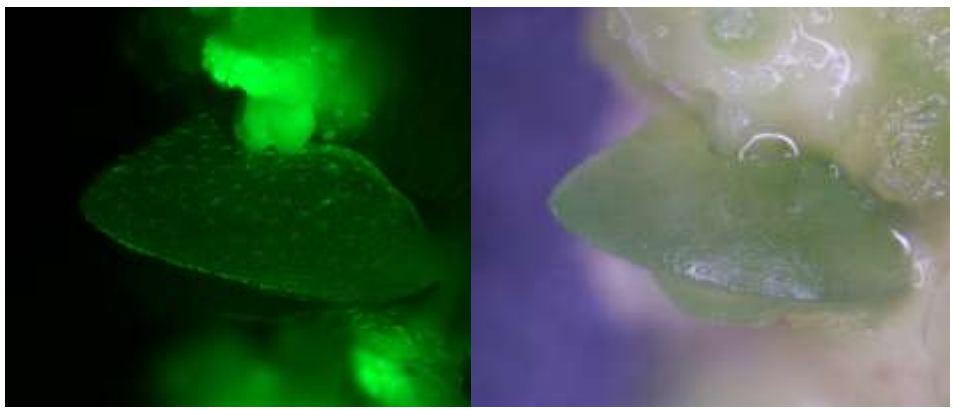
Molecular tools

Aim:

- Develop a stable transformation protocol suitable for high throughput application
  - Study of promoters for overexpression of genes of interest
  - Design artificial microRNA using endogenous miRNA precursors to silence undesired pathways

Transformation: regeneration





- Green Fluorescent Protein (GFP) gene from Jellyfish
- Efficiency of stable transformants 90%
- 6 week regeneration
- Comparable with Arabidopsis

Alex Cantó Pastor, Almudena Molla Morales

Diverting carbon from starch to oil

Cold Spring Harbor Laboratory

Plant Biotechnology Journal

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Increasing the energy density of vegetative tissues by diverting carbon from starch to oil biosynthesis in transgenic Arabidopsis

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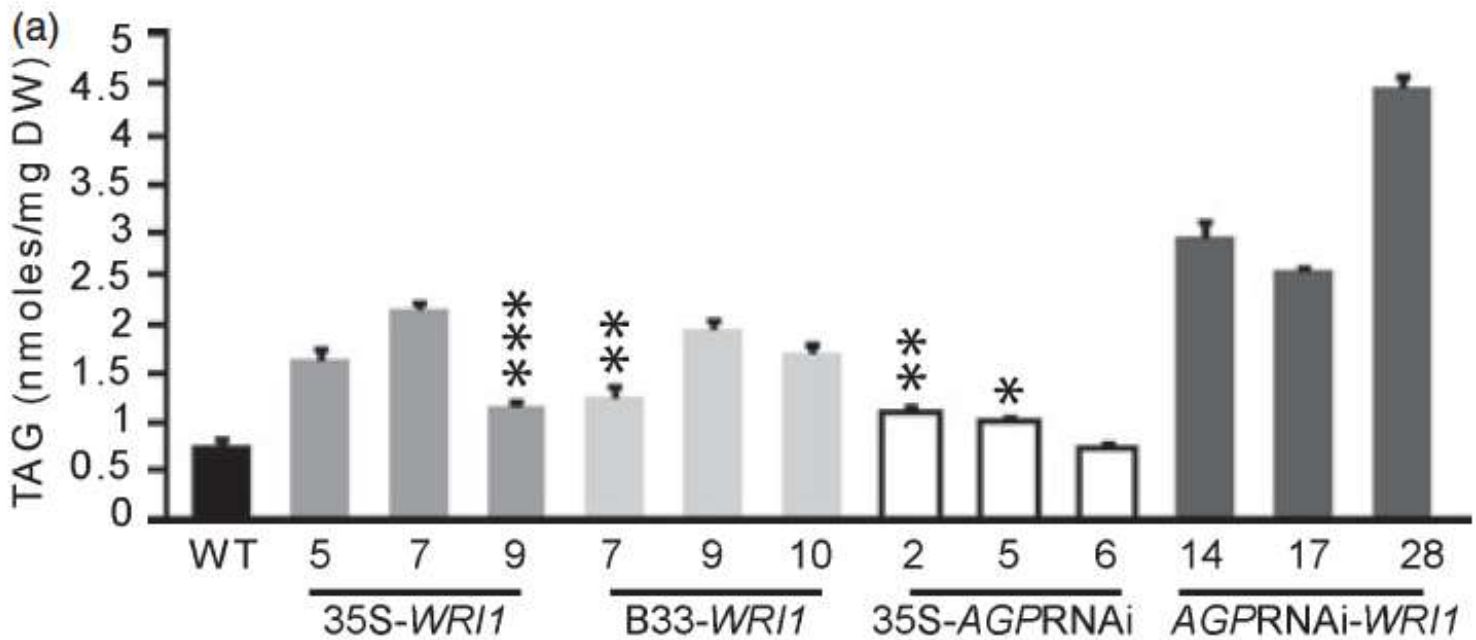


Climate change: we've been here before…

Moran *et al (2006) Nature* 441:601-605.

- In the early Eocene (~49 million years ago), atmospheric CO_2 concentrations were 5 times current levels.
- Arctic sea surface temperatures averaged 13° C in contrast to today's -9° C.
- Isolation of the Arctic Ocean from deep water currents led to a surface layer of fresh water.

“Azolla event” & global climate change

Azolla on a river in New Zealand.

- Arctic sediment core samples revealed alternating layers of freshwater *Azolla* fossils measuring 8-20 meters thick.
- *Azolla* blooms **alone** may have drawn 80% of the CO_2 out of the atmosphere contributing to the climate change that converted the ancient Greenhouse to the current Icehouse.

Moran *et al (2006) Nature* 441:601-605, Sluijs, A. et al. Nature 441, 610–613 (2006).
Speelman et al (2009) Geobiology 7(2):155-70..

50 years of duckweed in space

Effects of Prolonged Near Weightlessness on Growth and Gas Exchange of Photosynthetic Plants

C. H. Ward, S. S. Wilks, and H. L. Craft

Rice University, Houston, Texas and USAF School of Aerospace Medicine, Brooks Air Force Base, Texas

An experiment was designed to determine the effects of long-duration (30 days) exposure to near weightlessness on growth and gas exchange of the unicellular green alga *Chlorella sorokiniana* and the giant duckweed *Spirodela polyrhiza*. Instrumentation was provided for in-flight monitoring of carbon dioxide, oxygen, temperature, and pressure. Transmittance of light through the cultures was measured with photocells to indicate relative growth. Twelve hour light-dark cycles and data acquisition were controlled by programmer. The experiment was launched into near circular east-west orbit at Vandenberg Air Force Base on 30 March 1966 as part of the Air Force Office of Aerospace Research nonrecoverable OV-1 satellite program. Data were taken every 3 hours, stored on a satellite tape recorder, periodically transmitted to tracking stations, and accumulated at Cape Kennedy for decommutation. Computer reduction of data was performed at Brooks Air Force Base. Following data reduction, programmed control experiments were performed to simulate conditions, especially temperature, experienced in orbit. The alga experiment developed a gas leak during launch and lost pressure rapidly upon exposure to the vacuum of space. Data from the duckweed experiment were obtained for 230 hours prior to failure of the satellite power system. A nonstatistical comparison of flight and ground control data indicates that photosynthetic and respiratory gas exchange of *Spirodela polyrhiza* was not affected by exposure to near weightlessness for a period of 230 hours. Accuracy of comparison of flight and ground control data was compromised because of inability to quantitatively duplicate the amount of experimental plant material under conditions required for maintenance of axenic culture.

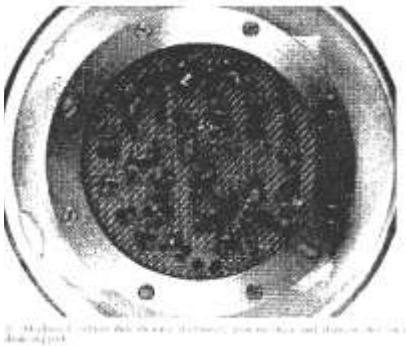
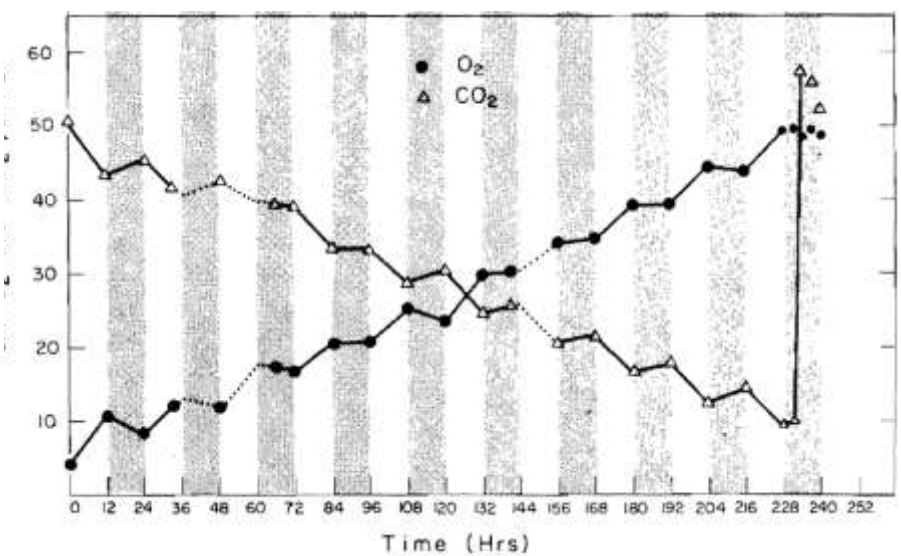
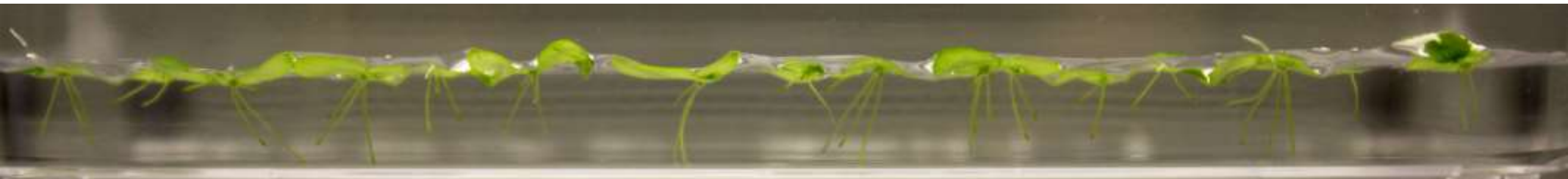


Fig. 14. Artist's concept of separation of OV1 satellites from Atlas Retaining Structure



Conclusions

- *Lemnaceae* are perfect candidates for biofuel production
- The use of *Lemnaceae* as an oil source requires genetic modification
- Genetic tools for *Lemnaceae* transformation are ready
- We are testing strategies for overexpression and silencing of genes involved in TAG and starch metabolism, respectively



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