Clones, Carbon and Climate Change: The Epigenetics of Oil Production

Rob Martienssen
Cold Spring Harbor Laboratory
Agricultural Biotechnology: Emerging Technologies and Insights
January 28th, 2020
Oil Production: the long term view

World History (Years)
Replacing fossil fuels

Fossil Reserves (ancient plants) → Energy → Biofuels → Carbon Fixation → Atmospheric Carbon

200MYrs

200Yrs
Replacing fossil fuels

<table>
<thead>
<tr>
<th>Biofuel feedstock</th>
<th>Fossil energy balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulosic ethanol <em>Miscanthus</em> grass</td>
<td>2 -36??</td>
</tr>
<tr>
<td>Palm oil</td>
<td>9</td>
</tr>
<tr>
<td>Ethanol (sugarcane)</td>
<td>8</td>
</tr>
<tr>
<td>Rapeseed (canola) oil</td>
<td>2-3</td>
</tr>
<tr>
<td>Corn ethanol</td>
<td>1.5</td>
</tr>
</tbody>
</table>

The best energy crops are clones.......

*Worldwatch Institute*
On Feb. 24, 2008, Virgin Atlantic flew a 747-400 from London to Amsterdam with one engine partially powered by palm oil.
Oil palm is the world’s biggest edible oil crop but occupies precious land.
Oil Palm (*Elaeis Guineensis*)

- Human use dates back 5,000 years in W. Africa
- Found in Egyptian tomb at Abydos (3000 BCE)
- 4 palms (Bogor botanical garden) introduced into Malaysia and Indonesia in mid 1800s
- Oil from fruit (mesocarp) and seed (kernel) provides 45% of global edible oil
- Relatively high in saturated (but not trans-) fat: palm oil is the most efficient source of biodiesel
The oil palm genome

- 1,800,000,000bp
- 34,802 RefSeq genes
- 16 chromosomes
- *E. guineensis* and *E. oleifera*
- Ancient segmental duplications (also found in date palm)

Singh et al., 2013 Nature
Oil Palm hybrid vigor depends on the *Shell (Sh)* gene

- **Dura Sh/Sh**
- **Pisifera sh/sh** (usually female sterile)
- **Tenera Sh/sh** (25-30% higher yield)

Singh et al, Nature 2013
George Shull and Hybrid Corn (CSHL, 1909)

http://www.weedtowonder.org/
Cloning elite oil palm hybrids

Cell culture
Cloning can lead to “Mantled” abnormality

Normal fruit bunch

“Mantled” fruit bunch

Guthrie Biotech (Malaysia)
Transposable elements (jumping genes) were discovered in corn.
DNA methylation (methyl C)

• Transposons are chemically modified by cytosine modification in plants, many fungi and mammals

Chomet, Dellaporta, Chandler, Bennetzen, Walbot, Freeling, and many others
DNA chip reveals “Karma” a transposable element responsible for mantled

DNA methylation chip

High-Resolution DNA methylation maps of genetically identical trios

Jared Ordway, Steve Smith (Orion Genomics), Mei Ong-Abdullah (MPOB, Malaysia)
Karma LINE transposon in *deficiens* gene loses RNA-dependent DNA methylation in *mantled* palms

![Image of a diagram showing genomic sites and DNA methylation patterns]
Simple DNA tests predict yield

Ong-Abdullah et al., *Nature* 2015
Oil palm vs rainforest: the debate

24 July 2013 Last updated at 13:44 ET
Genome of oil palm sequenced
By Rebecca Morelle Science reporter, BBC World Service

Sequenced palm oil genome paves the way for sustainable plantations
Researchers pinpoint a gene that could be used to boost yields and reduce competition between forests and oil palms
By Emma Bryce “world on a plate” July 26th 2013
Theguardian.com

Can Indonesia increase palm oil output without destroying its forest?
Environmentalists doubt the world's biggest palm oil producer can implement ambitious plans without damaging woodland
Paige McClanahan
theguardian.com, Wednesday 11 September 2013 12.18
Duckweed (Lemnaceae): A clonal aquatic flowering plant
Duckweed: a tiny aquatic plant with enormous potential for agriculture and environment (FAO, 1999)
Lemnaceae

<table>
<thead>
<tr>
<th>Liopsida</th>
<th>class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aridae</td>
<td>subclass</td>
</tr>
<tr>
<td>Arales</td>
<td>order</td>
</tr>
<tr>
<td>Araceae</td>
<td>family</td>
</tr>
</tbody>
</table>

**Wolfia microscopica**

- Reduced morphology
  - Fronds (leaf-like structures)
  - Meristem-like stem cell “pocket”

- Clonal reproduction
  - 48 hours duplication by budding
  - Limited flowering
  - 10cm² to 1 hectare in 50 days

*Spirodela polyrhiza* (Landolt, 1986)
Growing duckweed (*Lemna gibba*) in constant light conditions
Deciphering duckweed genomes

- *Lemnaceae* have 21 chromosomes (we have 23)
- 19,000 genes
**Lemna minor (clone 8627)** is a natural triploid hybrid

- Inter-specific hybrid
- 21 chromosomes from each parental species (42+21)
- "hybrid vigor"

Evan Ernst
TABLE II

TEMPERATURES OF CHROMOSOMAL DUPLICATION, GAMETIC AND SOMATIC FORMULAE FOR PLANTS HETEROGONOUS FOR FACTOR PAIR A AND a AND RATIO

OBTAINED WHEN SUCH PLANTS ARE SELFED TOGETHER

WITH DIAGRAMS ILLUSTRATING THE CHROMOSOMAL CONDITION IN SOMATIC CELLS

<table>
<thead>
<tr>
<th>No. of Extra Chromosomes in Set</th>
<th>No. of Sets Affected</th>
<th>Gametic Formula</th>
<th>Selfed Ratios</th>
<th>Somatic Formula</th>
<th>Somatic Diagram</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>12</td>
<td>AA + Aa</td>
<td>1A : 0a</td>
<td>AAAAA</td>
<td>R POSSIBLE ION</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA + 4Aa + aa</td>
<td>35A : 1a</td>
<td>AAAa</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>An + aa</td>
<td>3A : 1a</td>
<td>Aaa</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(12 + 12)</td>
<td></td>
<td>(12 + 12) + 1</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>2A + a + AA</td>
<td>NOR. 8A : 1a</td>
<td>AAAa</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A + 2a + 2Aa</td>
<td>MUT. 9A : 0a</td>
<td>Aaa</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ aa</td>
<td></td>
<td>(12 + 12) + 1</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>12</td>
<td>12, (12 + 1)</td>
<td></td>
<td>(12 + 12) + 12</td>
<td></td>
</tr>
</tbody>
</table>

No. of chromosomes Frequencies
12 + 0 1 2 3 4 5 6 7 8 9 10 11 12

12, 66 220, 495, 792 924, 792, 495, 220, 66, 12, 1

The corresponding figures show the trend of the expected thermal condition in the evening primrose and the theory of mutation connected with it are by many considered to furnish an example of a valuable theory founded upon incorrect interpretations. The belief is growing that most of the new forms which have appeared in cultures of the Enotheras are not mutations at all and that the evening primroses, as an abnormal group of plants, are not to be seriously considered as representative of the processes of evolution in normal forms.

In the short time at my disposal, I wish to outline some recent findings in the jimson weed (Datura Stramonium) which it is hoped may throw incidentally some light on the more highly involved phenomena in the Enotheras, and which may serve as a basis of a brief discussion of their possible evolutionary significance.

The jimson weed is not supplied with a wide range of obvious Mendelian characters. The early studies of

1 A paper presented before the American Society of Naturalists at the Chicago meeting, December 26, 1929.
**Lemna as an oil (and protein) crop**

- Excellent metabolic profile:
  - Growth rate
  - Starch 12-50%
  - Protein 11-40%
  - Lipid 2-9%
  - Low lignin content (5%)

- Needs genetic modification for oil production
Algae make oil: can duckweed?

• Algae can accumulate lots of oil, but only slowly under nutrient-limiting conditions
• Slow growth requires sterile water tanks and artificial illumination to avoid contamination
• Duckweed grows robustly in much harsher environments
• Can duckweed make oil instead of starch?

Duckweed *turions* contain 40% starch

John Shanklin, Brookhaven National Laboratory
Oil (triacyl glycerol or TAG) is made from free fatty acids (FFA)

Step 1: make FFA

Vanhercke et al. 2014
Genetic transformation of *Lemna minor* increases oil content.

<table>
<thead>
<tr>
<th>TAG (%DW)</th>
<th>0.05</th>
<th>0.1</th>
<th>0.15</th>
<th>0.2</th>
<th>0.25</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.6</td>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
But free fatty acids (FFA) are toxic.

- Decreased growth rate
- Impaired frond development
  - size and shape
  - frequency and distribution

NORMAL

Wri1

Alex Canto-Pastor, Almudena Molla-Morales
Oil (triacyl glycerol or TAG) is made from free fatty acids (FFA)

Step 1: make FFA
Step 2: make TAG
Step 3: prevent breakdown

Vanhercke et al. 2014
Engineered duckweed has normal growth: (Oilweed? Lemnola?)

Lm8627 (WT)

O+D(inWT)-2

In collaboration with John Shanklin, Jorg Schwender BNL
Open stomata ensure optimal access to atmospheric CO2, reducing photorespiration at the cost of water loss.

Because duckweeds grow atop ample H₂O, they do not face this constraint in optimizing photosynthesis – duckweed stomata remain open at all times.
Photosynthetic rate responds well to increased Carbon

Andersen, 1985
CO$_2$ effect on biomass

- Biomass fold change after 12 days on SH medium:
Climate change: we’ve been here before...

- 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

- Arctic sediment core samples revealed alternating layers of freshwater *Azolla* fossils measuring 8-20 meters thick.

- *Azolla* blooms along with other aquatic plants 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.

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 photorecords 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 decommissioning. 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.

![Chart](image-url)
Acknowledgements

Ravi Santhanamurthi
Rajinder Singh
Leslie Low
Meilina Ong-Abdullah
Nathan Lakey
Jared Ordway
Steve Smith
Evan Ernst
Joe Simorowski
Uma Ramu
Joanne Saldanha
Seung Cho Lee
Will Dahl
Almudena Mollá-Morales
Alex Canto-Pastor

John Shanklin
Jorg Schwender