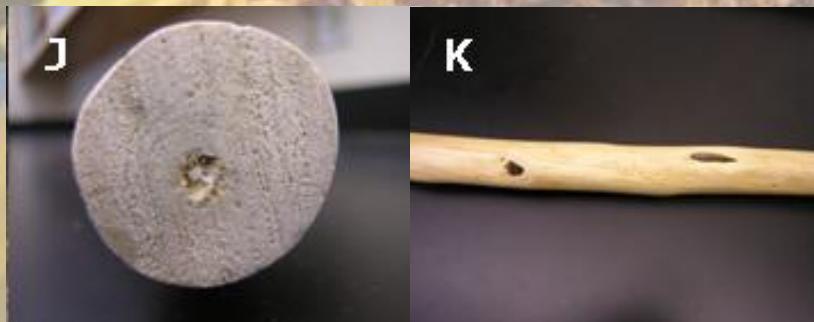




# Genomics of Cellulosic Biomass Traits in Sunflower

Loren Rieseberg,  
University of British  
Columbia

# Sunflower



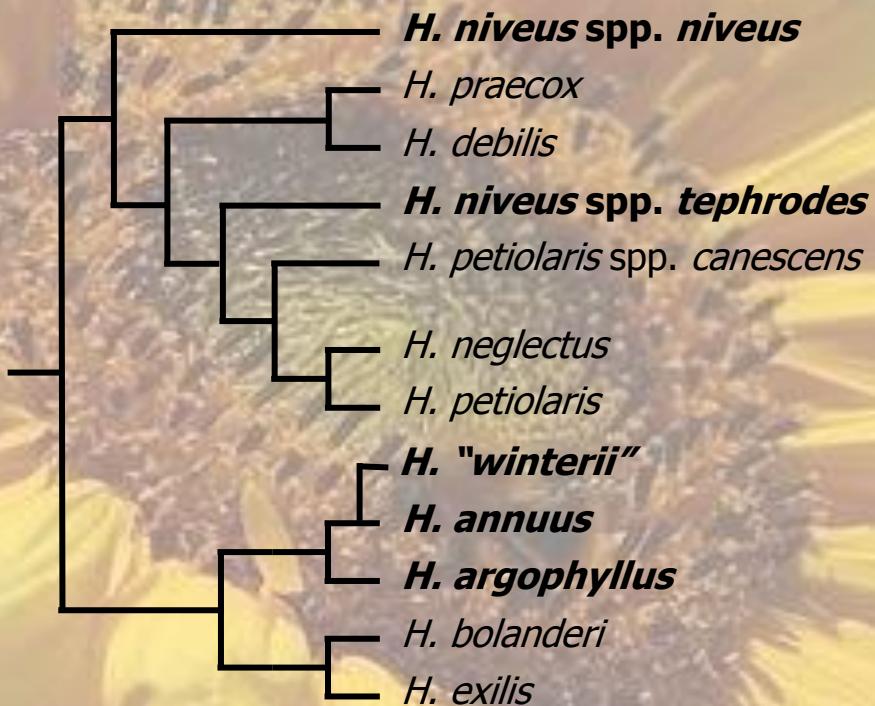
- Globally important oilseed crop ~ \$8 billion
- Ranks 11<sup>th</sup> among world food crops in terms of area harvested
- Only major crop to be domesticated in North America
- Potential for cellulosic biomass production
- Drought tolerant wild sunflowers have woody stems

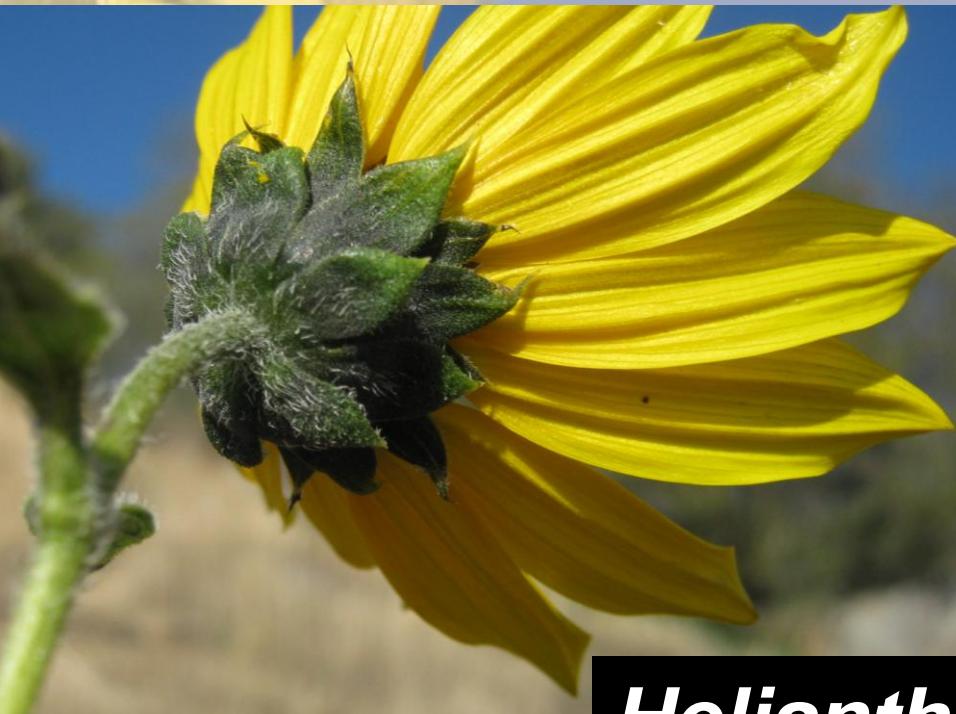
# Sunflower as a Biofuel

- Biodiesel – sunflower seed has high oil content, but oil more valuable as food than fuel
- Biogas – sunflower currently employed as a biogas (methane) crop in Europe
- Cellulosic biomass
  - Tremendous potential as dual use (food and fuel) crop, especially in Africa
    - Slow deforestation, eliminate need to search for wood, improve educational opportunities for girls and women
  - Cellulosic ethanol?



# *Helianthus* Woody Taxa





## *Helianthus winterii*



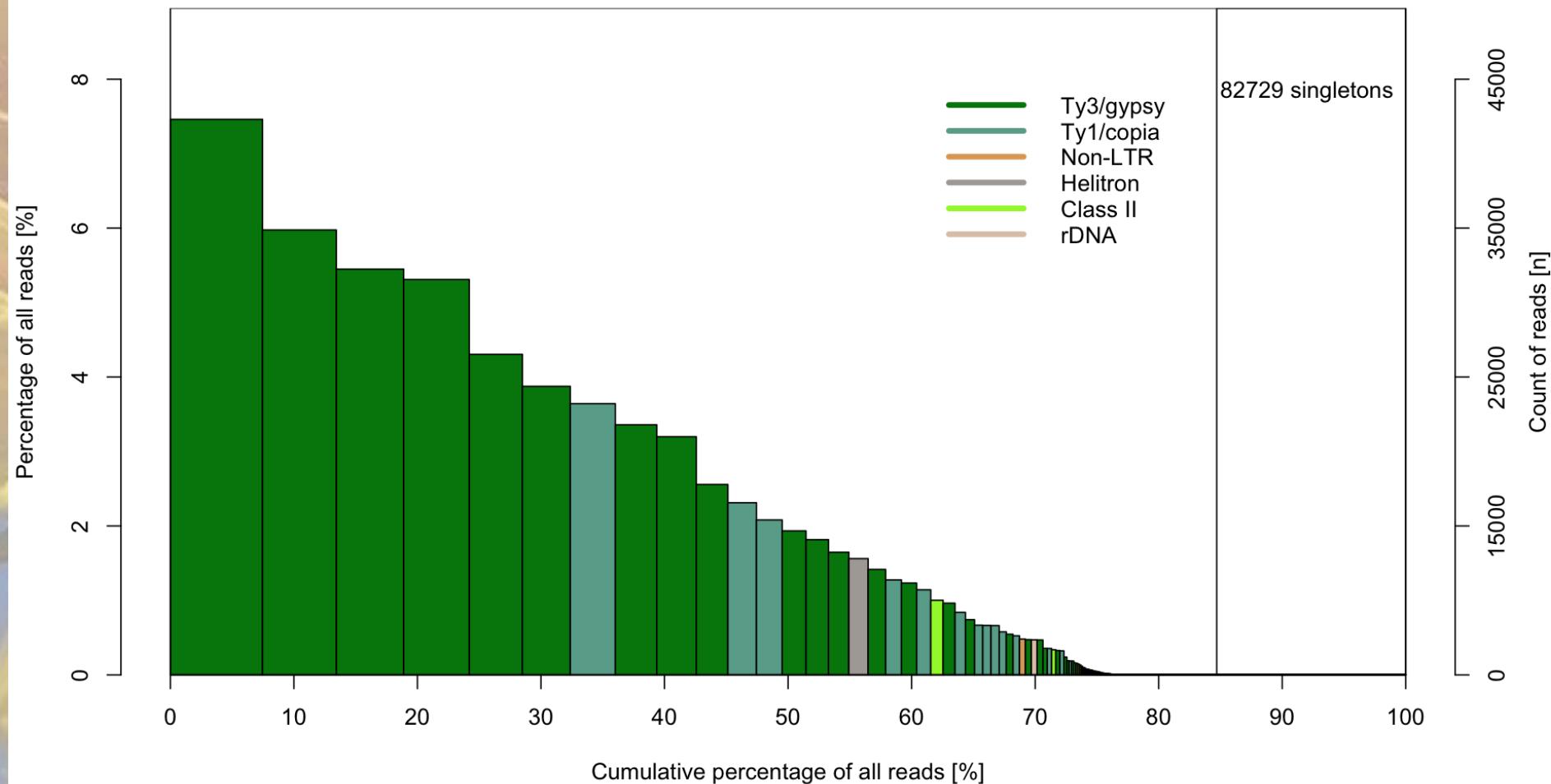
# Questions:

- When will we have a reference sequence for sunflower?
- Can we develop sunflower cultivars with favorable cellulosic biomass traits?

# Complication #1: sunflower genome is very large

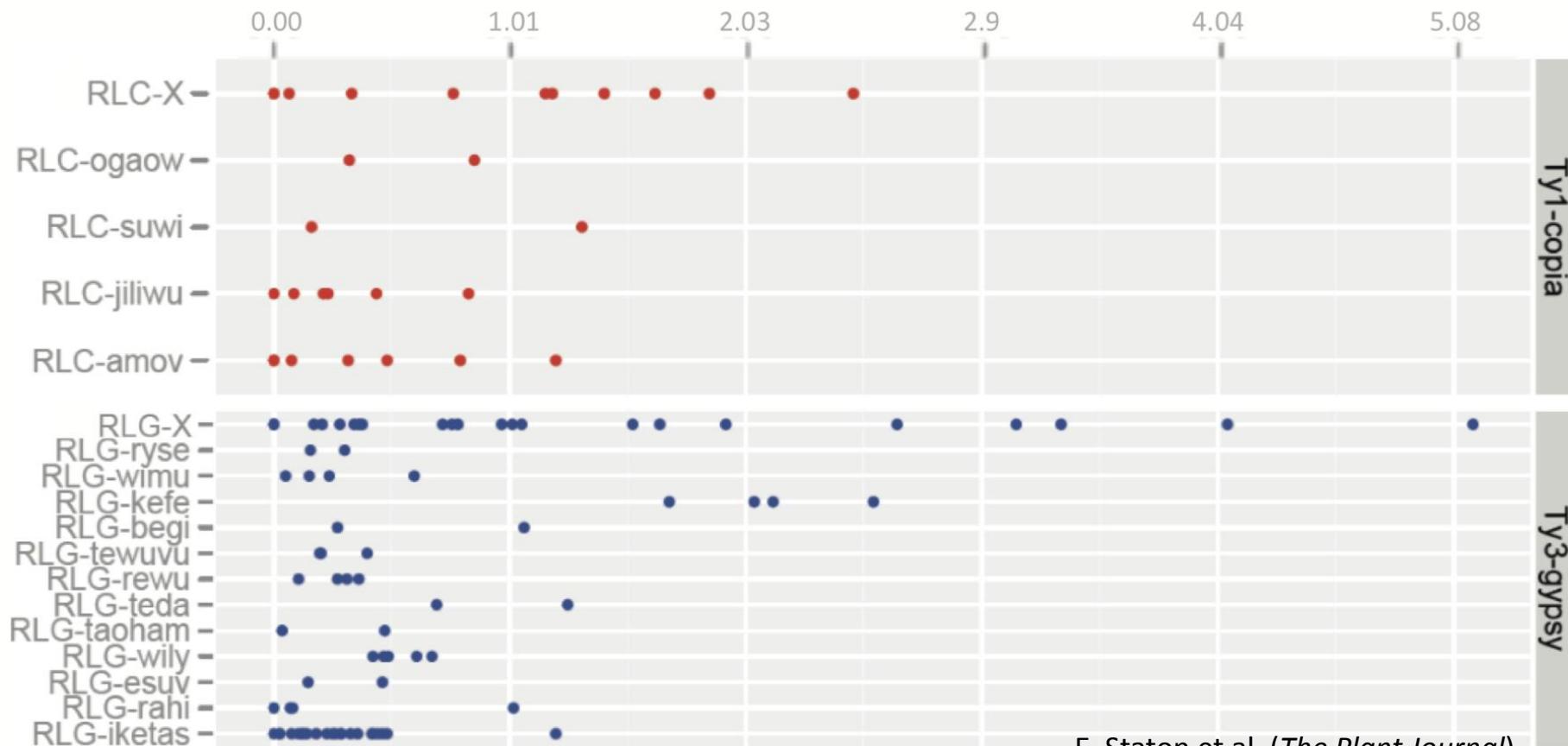
| Organism                      | Genome Size     | Completed    |
|-------------------------------|-----------------|--------------|
| Arabidopsis                   | 119 Mb          | 2000         |
| Oryza (rice)                  | 420 Mb          | 2002         |
| Vitis (grapevine)             | 490 Mb          | 2007         |
| Populus                       | 550 Mb          | 2006         |
| Solanum (potato)              | 844 Mb          | 2011         |
| Glycine (soybean)             | 1,100 Mb        | 2010         |
| Zea mays (maize)              | 2,800 Mb        | 2009         |
| Homo sapiens (human)          | 3,200 Mb        | 2006         |
| Monodelphis (opossum)         | 3,500 Mb        | 2007         |
| <b>Helianthus (sunflower)</b> | <b>3,600 Mb</b> | <b>2013?</b> |

# Complication #2: sunflower genome is highly repetitive



# Complication #3: many of the LTR repeats are very young

Age of LTR retrotransposon insertions  
Millions of years



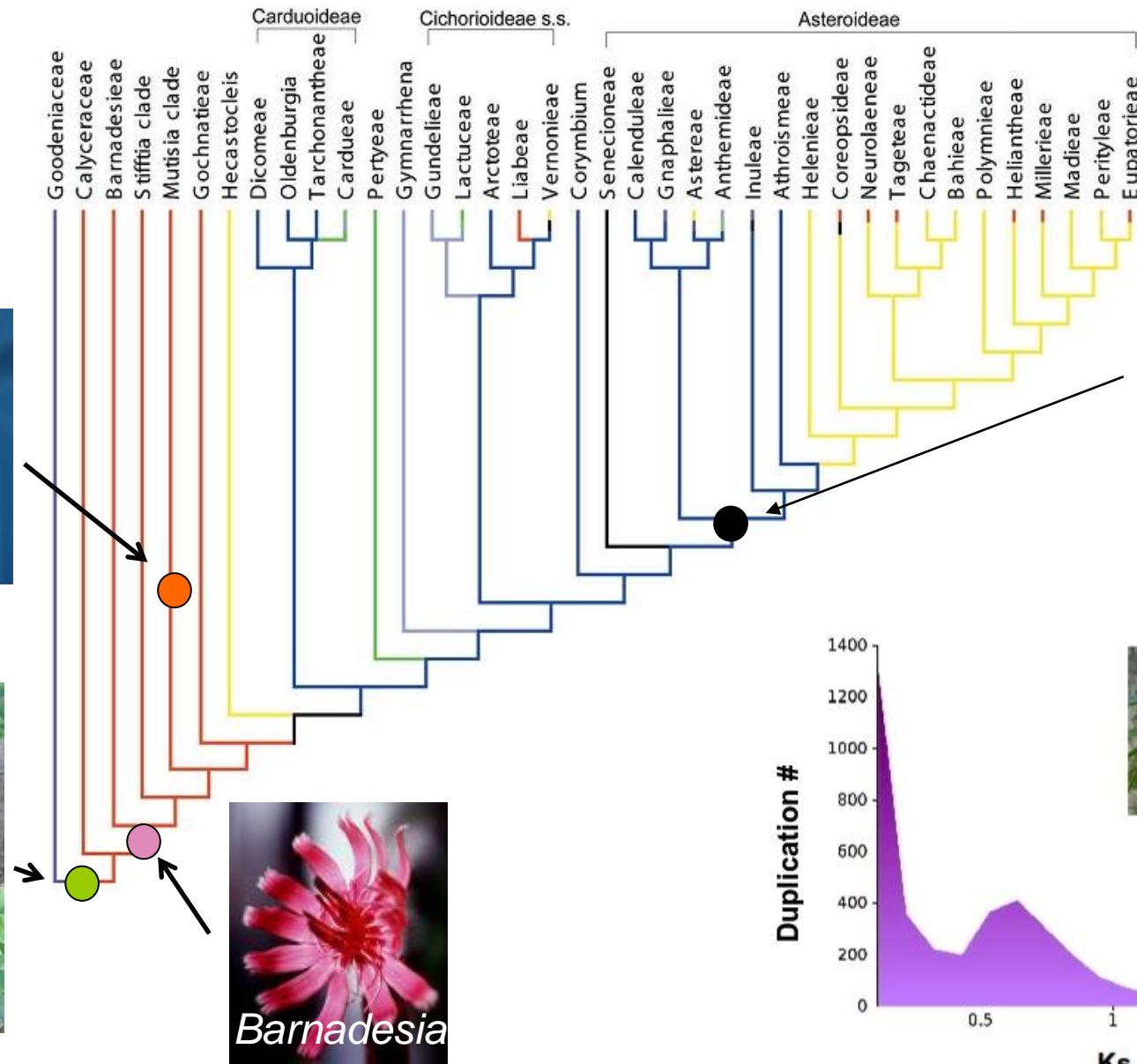
# Complication #4: sunflower is an ancient octaploid



Gerbera



Acicarpha



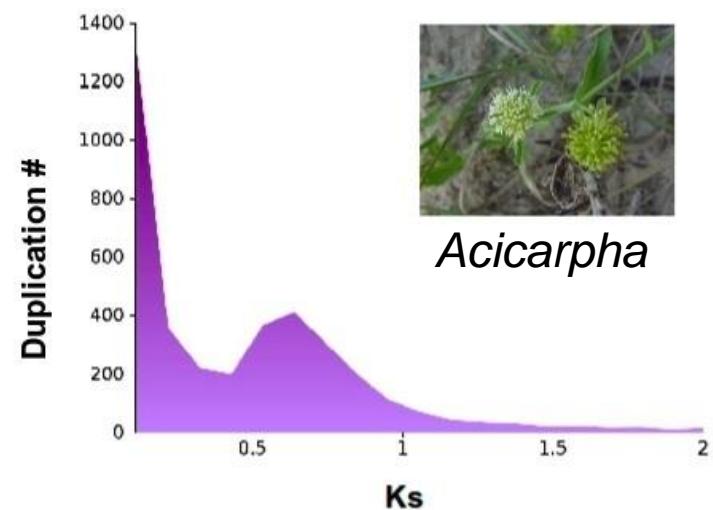
Barnadesia



Mike Barker

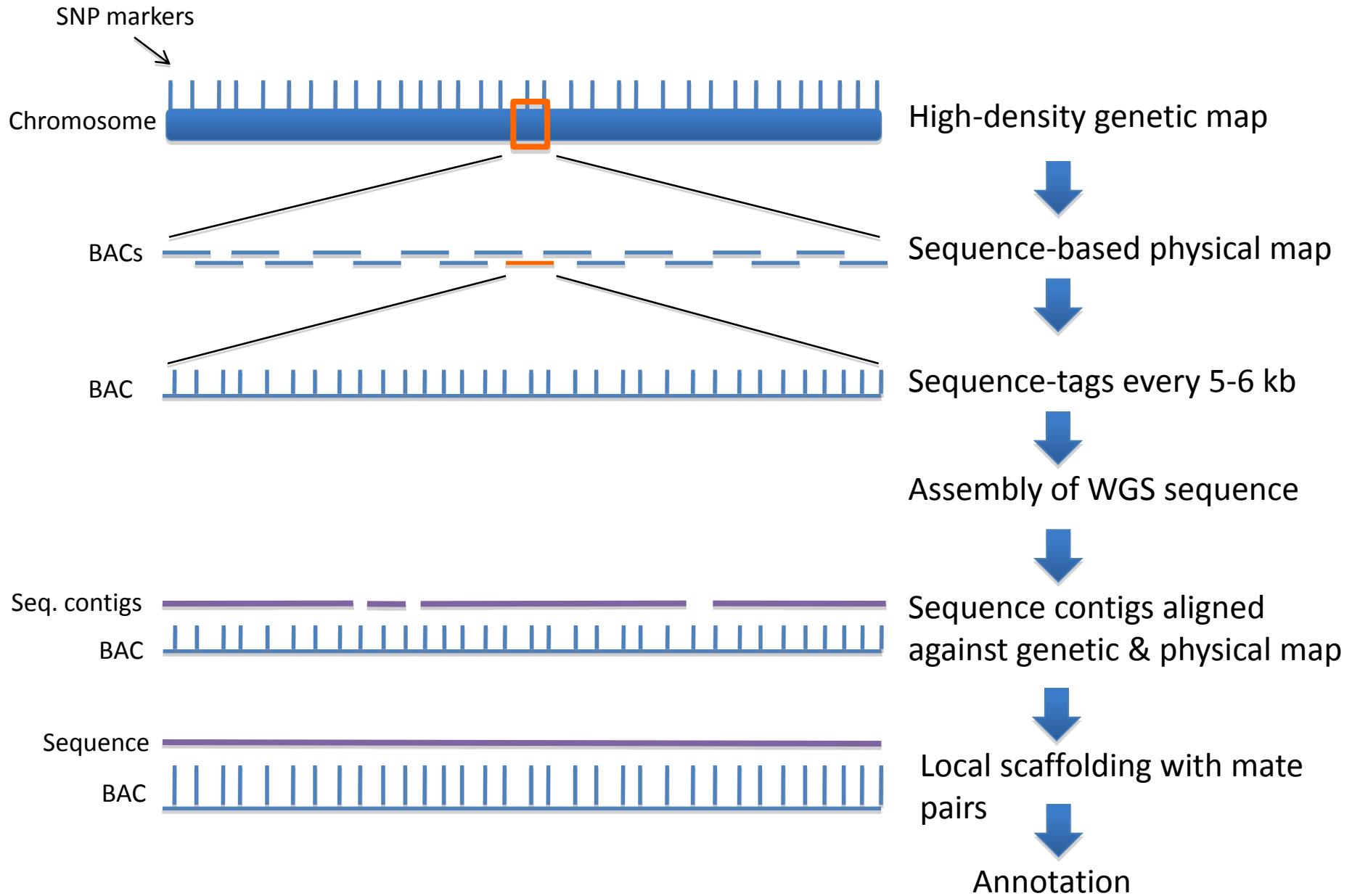


Sunflower



Acicarpha

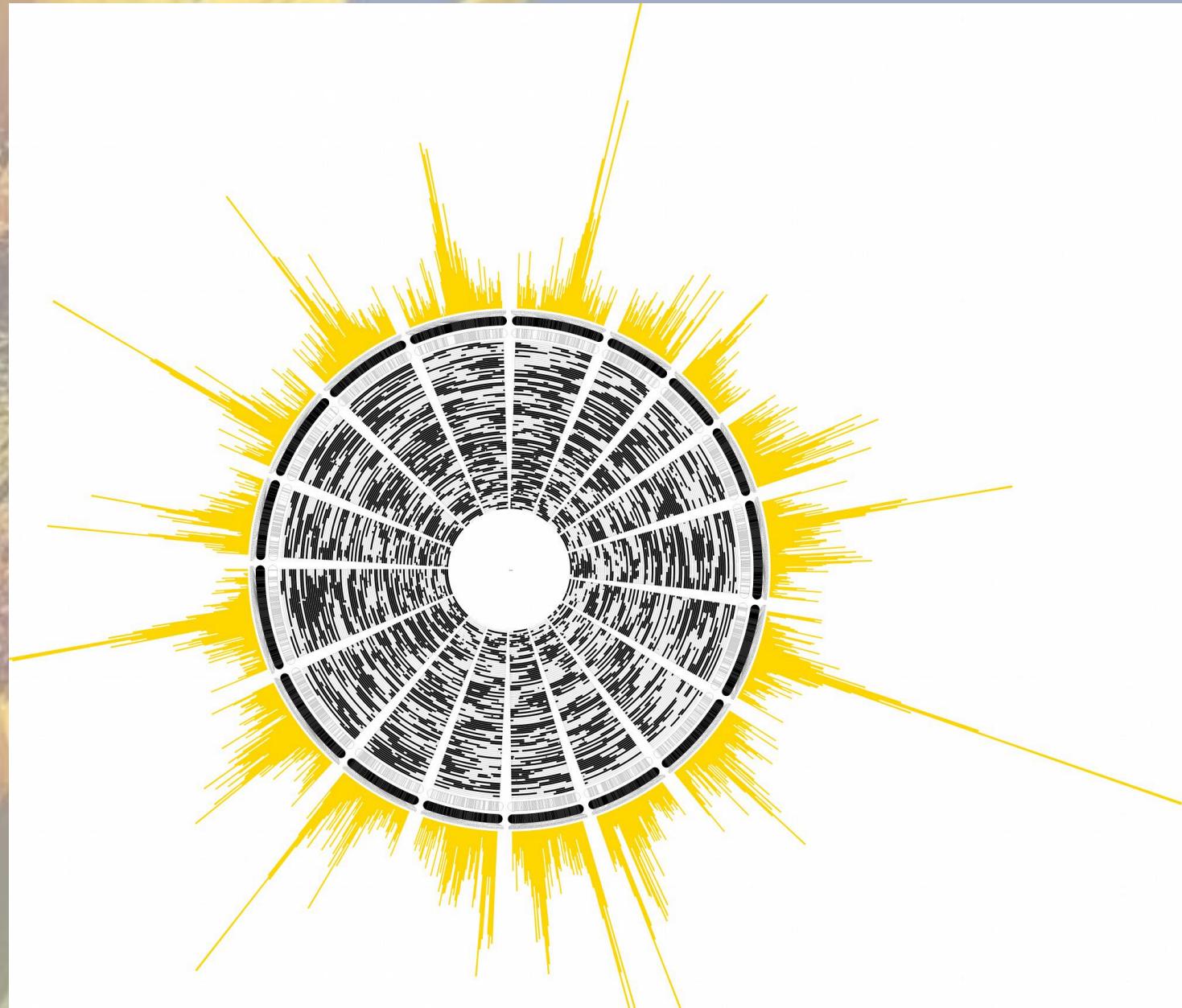
# Strategy





# Genetic Map - completed

- 96 RILs sequenced to 1x depth
- 2.6 million SNPs
- C. Grass, J. Bowers et al.



# Physical Map – Completed

(iterative FPC assembly; N. Gill et al. unpublished)

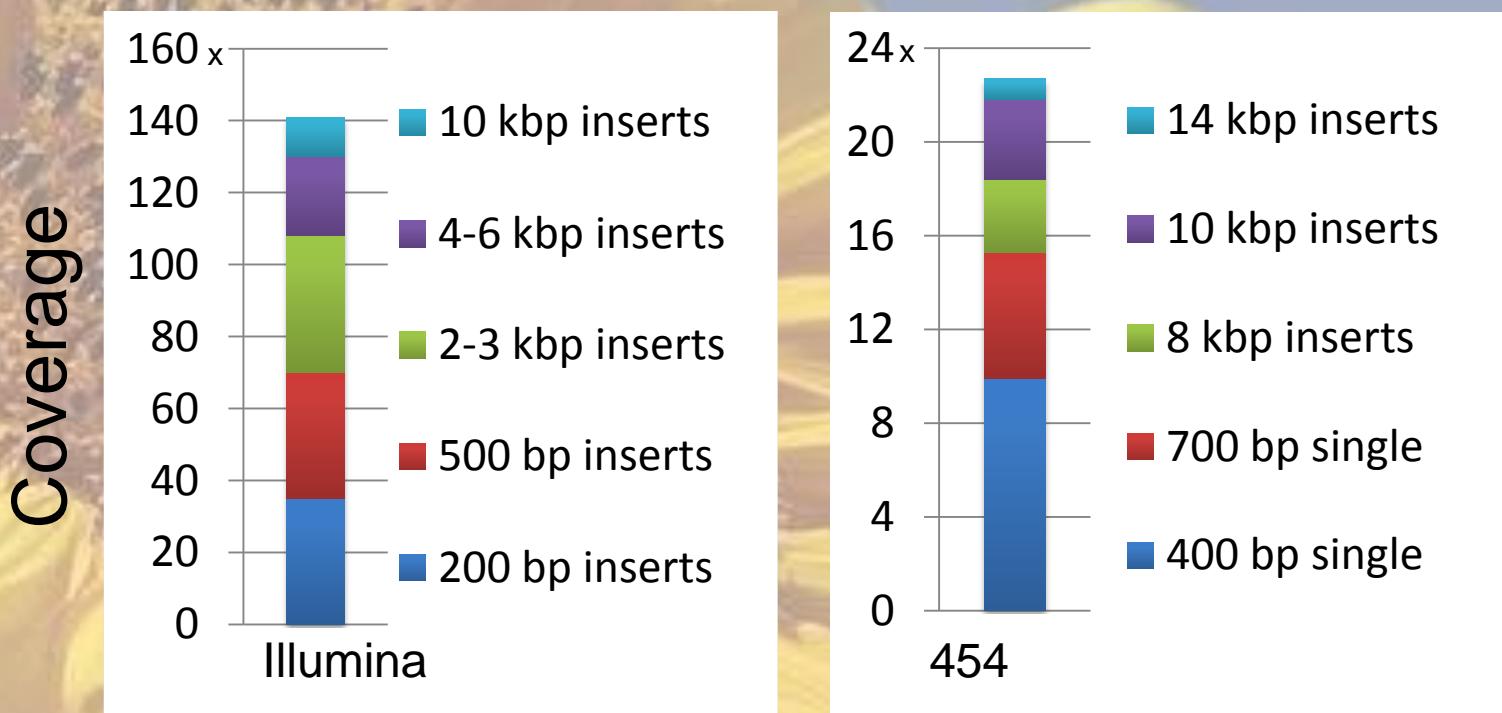


| Parameter                         | Value                  |
|-----------------------------------|------------------------|
| Genome size                       | 3,600 Mbp              |
| Genome equivalents of BACs tested | 12.5x                  |
| Total No. unique tags             | 483,758                |
| Average tags per BAC              | 20                     |
| Total # of BACs in FPC            | 335,201                |
| Number and % BACs in contigs      | 287,344 (86%)          |
| <b>Coverage of the genome</b>     | <b>3,500 Mbp (97%)</b> |
| <b>Total number of contigs</b>    | <b>3,332</b>           |
| N50 contig size (BACs)            | 57                     |
| N50 contig size (Mbp)             | 1,783 Kb               |

# Sequencing and Assembly



Nolan Kane



- Sequencing is finished and latest assembly covers 94% of genome

# Integration and Annotation



- 63% of genome placed on chromosomes and annotated

# Questions:

- When will we have a reference sequence for sunflower?
- Can we develop sunflower cultivars with favorable cellulosic biomass traits?

# What makes an ideal biofuels feedstock?



Jessica Barb

**ideal feedstock**  
readily available    high sugar release  
resistant to insects high S/G ratio low hemicellulose  
flexible dual purpose high cellulose storables  
low lignin adaptable drought tolerant  
use available farm infrastructure stress tolerant  
    no toxic by-products

high sugar release  
low hemicellulose  
fast growth  
low inputs  
optimal ratio  
storables  
drought tolerant  
stress tolerant  
no toxic by-products  
easy to harvest  
limited adverse effects  
adaptable  
fast establishment  
high cellulose



# Population Development

*H. annuus* x (*H. annuus* x *H. argophyllus*) BC<sub>1</sub>

NMS/RHA377 (PI560145)

- Elite apically branched oilseed inbred B-line



ARG1820 (PI49480)

- Tall, late flowering, drought resistant





# Phenotypic Data - Pearson's Correlations

|                         | Lignin |       |       |       | S-lignin |       |       |       | G-lignin |       |       |       | S/G-lignin |       |       |       |
|-------------------------|--------|-------|-------|-------|----------|-------|-------|-------|----------|-------|-------|-------|------------|-------|-------|-------|
|                         | HOPI   | GA    | BC    | IA    | HOPI     | GA    | BC    | IA    | HOPI     | GA    | BC    | IA    | HOPI       | GA    | BC    | IA    |
| S-lignin                | 0.86   | 0.88  | 0.82  | 0.77  |          |       |       |       |          |       |       |       |            |       |       |       |
| G-lignin                | 0.47   | 0.62  | 0.59  | 0.69  |          | 0.19  |       |       |          |       |       |       |            |       |       |       |
| S/G-lignin              | 0.32   | 0.44  | 0.28  |       | 0.75     | 0.80  | 0.78  | 0.67  | -0.68    | -0.43 | -0.60 | -0.68 |            |       |       |       |
| Hemicelluloses          | -0.69  | -0.68 | -0.44 | -0.86 | -0.37    | -0.43 | -0.17 | -0.48 | -0.65    | -0.65 | -0.46 | -0.79 |            | 0.16  | 0.24  |       |
| Celluloses              | -0.76  | -0.69 | -0.42 | -0.86 | -0.46    | -0.54 | -0.26 | -0.55 | -0.64    | -0.50 | -0.32 | -0.72 |            | -0.20 |       |       |
| Cellulose/Hemicellulose |        | 0.51  | 0.24  | 0.48  | -0.16    | 0.25  |       | 0.21  | 0.32     | 0.59  | 0.35  | 0.50  | -0.32      |       | -0.23 | -0.21 |
| C6overLig               | -0.88  | -0.93 | -0.79 | -0.93 | -0.61    | -0.78 | -0.58 | -0.63 | -0.63    | -0.62 | -0.53 | -0.74 |            | -0.35 |       |       |
| Height                  | 0.32   | 0.47  | 0.32  | 0.45  | 0.36     | 0.53  | 0.25  | 0.52  |          | 0.23  |       | 0.25  | 0.43       |       | 0.30  |       |
| Biomass                 |        | 0.34  | 0.20  |       |          | 0.39  |       |       | -0.20    |       | 0.19  |       | 0.19       | 0.31  |       |       |
| % Main Stem             | N/A    |       | 0.16  |       | N/A      |       | 0.21  | 0.19  | N/A      | -0.16 |       |       | N/A        |       | 0.17  | 0.20  |
| Flower                  |        |       | 0.20  |       |          |       | 0.21  |       |          |       |       |       |            | 0.16  |       |       |
| Stem Diameter           |        | 0.20  | -0.17 | 0.32  |          | 0.22  |       | 0.28  | -0.23    |       |       |       | 0.20       | 0.18  |       |       |
| Stem Density            | 0.27   |       |       |       | 0.26     |       | 0.20  |       |          |       |       |       |            |       | 0.24  |       |

Lignin and carbohydrate content are **negatively** correlated

# Phenotypic Data - Pearson's Correlations

|                         | Lignin |       |       |       | S-lignin |       |       |       | G-lignin |       |       |       | S/G-lignin |       |       |       |
|-------------------------|--------|-------|-------|-------|----------|-------|-------|-------|----------|-------|-------|-------|------------|-------|-------|-------|
|                         | HOPI   | GA    | BC    | IA    | HOPI     | GA    | BC    | IA    | HOPI     | GA    | BC    | IA    | HOPI       | GA    | BC    | IA    |
| S-lignin                | 0.86   | 0.88  | 0.82  | 0.77  |          |       |       |       |          |       |       |       |            |       |       |       |
| G-lignin                | 0.47   | 0.62  | 0.59  | 0.69  |          | 0.19  |       |       |          |       |       |       |            |       |       |       |
| S/G-lignin              | 0.32   | 0.44  | 0.28  |       | 0.75     | 0.80  | 0.78  | 0.67  | -0.68    | -0.43 | -0.60 | -0.68 |            |       |       |       |
| Hemicelluloses          | -0.69  | -0.68 | -0.44 | -0.86 | -0.37    | -0.43 | -0.17 | -0.48 | -0.65    | -0.65 | -0.46 | -0.79 |            | 0.16  | 0.24  |       |
| Celluloses              | -0.76  | -0.69 | -0.42 | -0.86 | -0.46    | -0.54 | -0.26 | -0.55 | -0.64    | -0.50 | -0.32 | -0.72 |            | -0.20 |       |       |
| Cellulose/Hemicellulose |        | 0.51  | 0.24  | 0.48  | -0.16    | 0.25  |       | 0.21  | 0.32     | 0.59  | 0.35  | 0.50  | -0.32      |       | -0.23 | -0.21 |
| C6overLig               | -0.88  | -0.93 | -0.79 | -0.93 | -0.61    | -0.78 | -0.58 | -0.63 | -0.63    | -0.62 | -0.53 | -0.74 |            | -0.35 |       |       |
| Height                  | 0.32   | 0.47  | 0.32  | 0.45  | 0.36     | 0.53  | 0.25  | 0.52  |          | 0.23  |       | 0.25  | 0.43       |       | 0.30  |       |
| Biomass                 |        | 0.34  | 0.20  |       |          | 0.39  |       |       | -0.20    |       | 0.19  |       | 0.19       | 0.31  |       |       |
| % Main Stem             | N/A    |       | 0.16  |       | N/A      |       | 0.21  | 0.19  | N/A      | -0.16 |       |       | N/A        |       | 0.17  | 0.20  |
| Flower                  |        |       | 0.20  |       |          |       | 0.21  |       |          |       |       |       |            | 0.16  |       |       |
| Stem Diameter           |        | 0.20  | -0.17 | 0.32  |          | 0.22  |       | 0.28  | -0.23    |       |       |       | 0.20       | 0.18  |       |       |
| Stem Density            | 0.27   |       |       |       | 0.26     |       | 0.20  |       |          |       |       |       |            | 0.24  |       |       |

S-lignin and  
G-lignin  
contents are  
**NOT**  
correlated

It should be possible to develop sunflower cultivars with favourable cellulosic biomass traits



# Conclusions:

- Assembly of sunflower genome challenging because of its large size, past polyploidization events, and recent amplification of repetitive elements.
- Nonetheless, 63% of genome placed on chromosomes and annotated.
- Transgressive segregation of key cellulosic biomass traits and favorable genetic correlations should permit breeding of a sunflower biofuel feedstock.
- Dual use sunflower has promise for subsistence agriculture.

# Acknowledgements

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