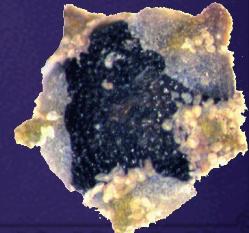


# Quinoa Phylogenetic Insights Based on Nuclear and Chloroplast DNA Sequences



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Department of Plant & Wildlife Sciences  
Brigham Young University, Provo, Utah, USA



# Nez Perce or Nimi'ipoo People, 1832: "Rabbit-Skin-Leggings"

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# Disease & Pest Resistance: Humans vs. Quinoa



New World  
isolation;  
restricted  
exposure to  
Old World  
diseases



Thousands of  
years of  
exposure to  
Old World  
diseases



Genetic resistance (their ancestors survived epidemics); vaccinations



Genetically resistant Eastern Hemisphere *C. album*, domesticated (l) and wild (r); vaccination impossible (⌚)



# Importance of Wild Species for Quinoa Breeding



## Wild & weedy *Chenopodium*

Biotic stress  
resistance alleles

Abiotic stress  
resistance alleles

Agronomic,  
quality alleles



## Domesticated quinoa

(Limited diversity of quinoa  
pests and pathogens at high  
elevation)

(Good cold, drought, salinity,  
N-efficiency; poor heat  
tolerance)



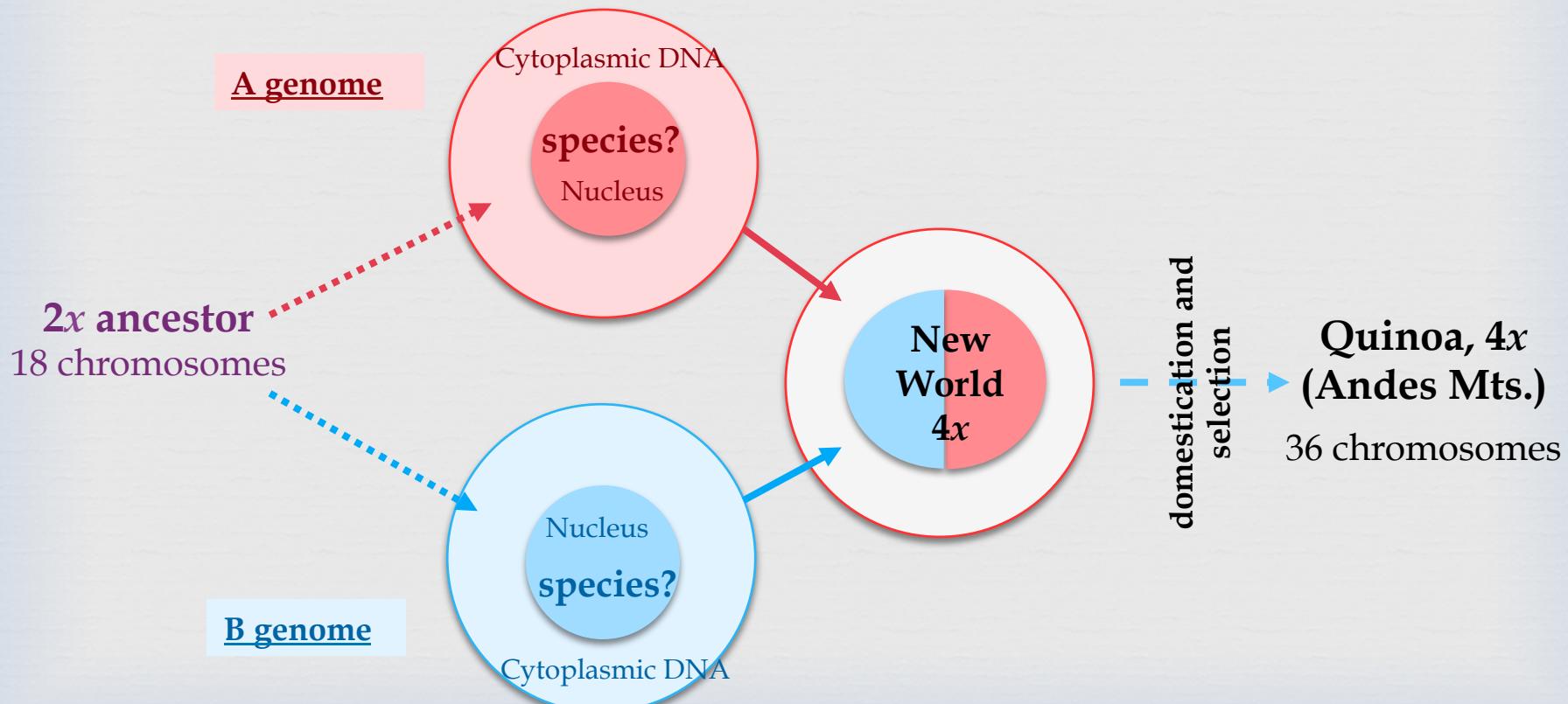
Extensive variation for  
agronomic and quality  
alleles

# Searching for Quinoa's Ancestors



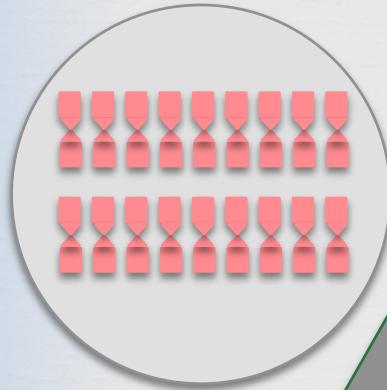
- Allopolyploid:

- Two diploid ( $2x$ ) ancestors crossed to make a sterile hybrid
- That hybrid's chromosomes then doubled to produce a new fertile species ( $4x$ )
- This wild/weedy  $4x$  ancestor was eventually domesticated as quinoa
  - (1000's to 100,000's of years later)



# How Does an Allopolyploid Arise?

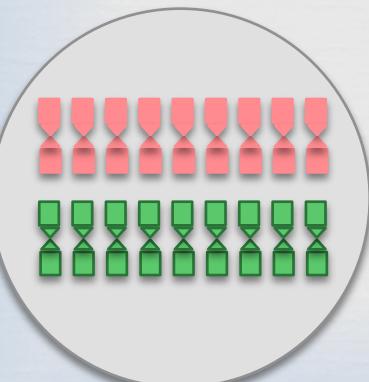
Species "X"



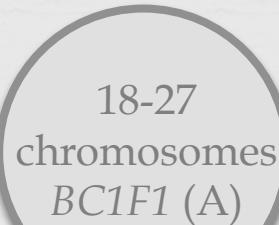
Species "Y"



Hybrid "XY":  
Self-sterile *as is*;  
Five possible  
fates



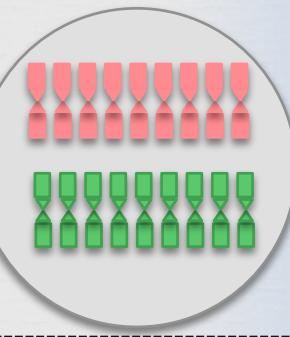
Fate #4:  
Egg is fertilized  
by pollen from  
"A"; backcross A



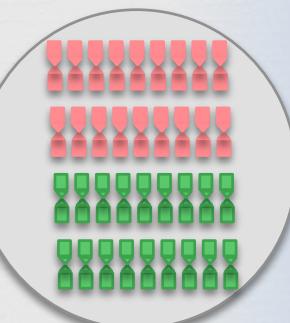
Fate #5:  
Egg is fertilized  
by pollen from  
"B"; backcross B



Fate #1:  
Meiotic failure  
Plant matures  
No seed set



Fate #2:  
Mitotic failure,  
chromosome  
doubling in a sector  
of the plant that  
becomes self-fertile



Fate #3:  
Meiotic failure,  
production of  
diploid pollen and/  
or eggs, fertility  
restored

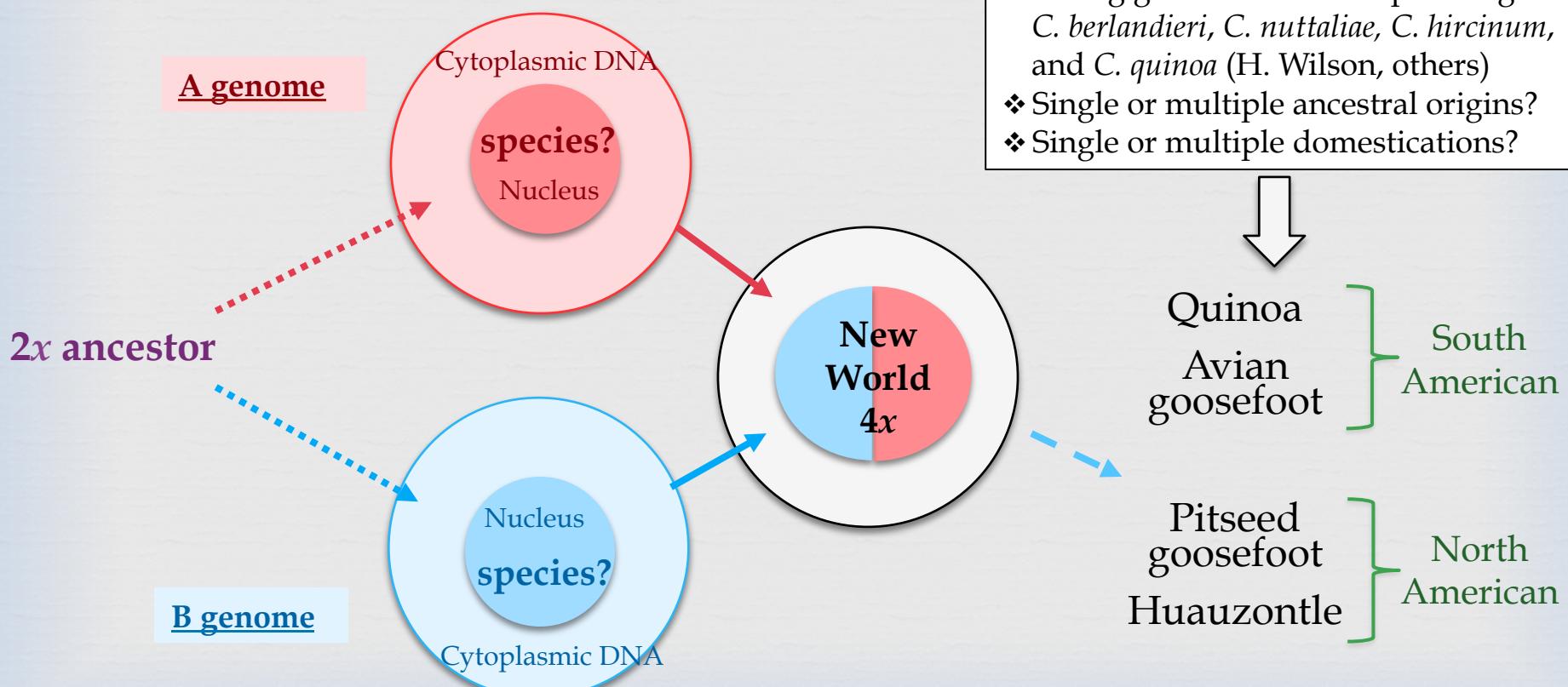


# Searching for Quinoa's Ancestors



- Ancestry Search strategy:

- Sequence nuclear genes → identify ancestors
- Sequence cytoplasmic (i.e., chloroplast) DNA → identify cytoplasm donor



# New Classifications of *Chenopodium*

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Source: Fuentes-Bazan et al. 2012,  
*Willdenowia* 42:5-24

- Reclassification based on DNA sequence data (T. Borsch's group):
  - *Chenopodium* retains *C. album* group, *C. berlandieri-quinoa*, and their relatives
  - *Chenopodiastrum* includes *C. murale*, *C. hybridum*, and their relatives
  - *Blitum* includes *C. capitatum*, *C. bonus-henricus*, and their relatives
  - *Dysphania* includes *C. ambrosioides*, *C. botrys*, and their relatives
  - *Lipandra* contains *C. polyspermum*
  - *Oxybasis* includes *C. rubrum*, *C. glaucum*, and their relatives
  - *Teloxys* contains *C. aristatum*



*Oxybasis macrosperma*,  
formerly *C. macrospermum*

*Blitum californicum*,  
formerly *C. californicum*



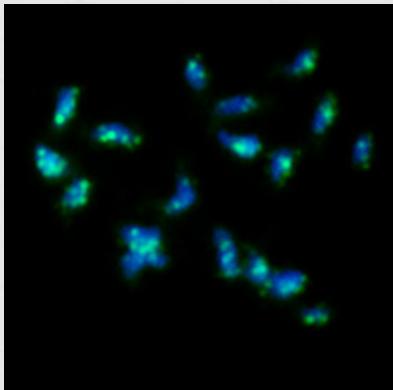
# Quinoa's Genetic Structure



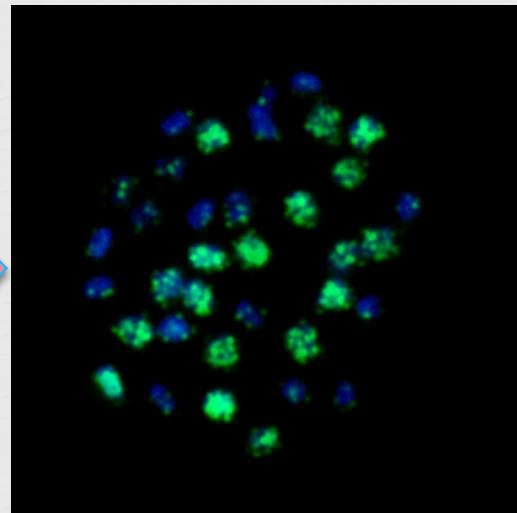
Source: Kolano et al.  
2011, *Genome* 54:710-717

*In situ* hybridization patterns of quinoa repeat 18-24J

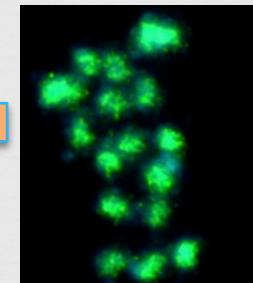
dispersed



18 dispersed, 18 concentrated



concentrated



Multiple species, same pattern:

*C. pallidicaule* (crop, Andes)

*C. neomexicanum* (wild)

$2n = 18$

**AA GENOME**

**ORIGINATED IN  
NORTH OR SOUTH  
AMERICA**

Multiple species, same pattern:

Huauzontle

Quinoa

Pitseed goosefoot (weed)

$2n = 4x = 36$ , **AABB**

*C. album* variant (weed)

$2n = 18$

**BB GENOME**

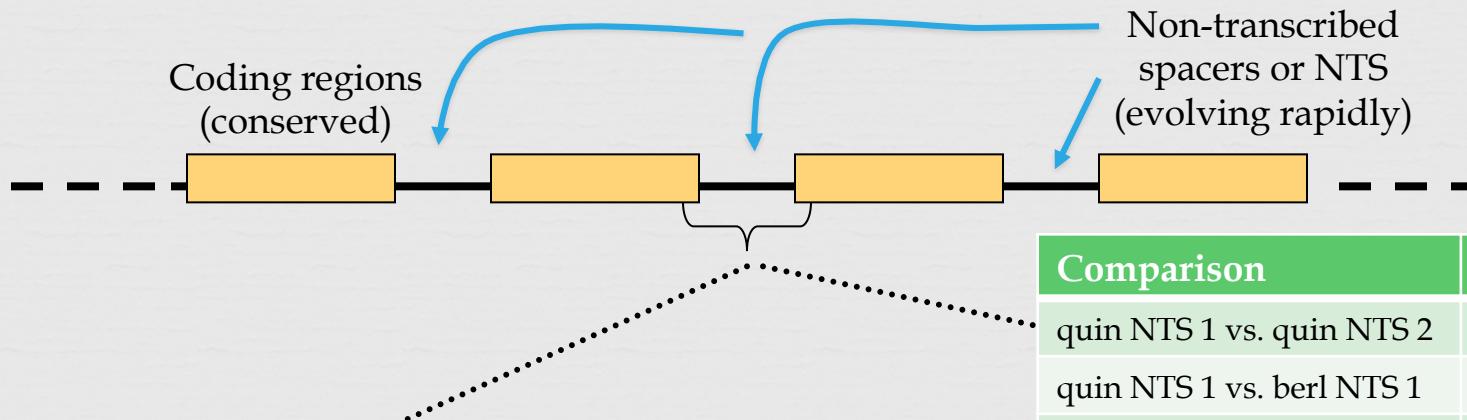
**ORIGINATED IN  
EURASIA???**

# 5S rDNA Comparative Sequencing:

Maughan et al. (2006) *Genome* 49:825-839

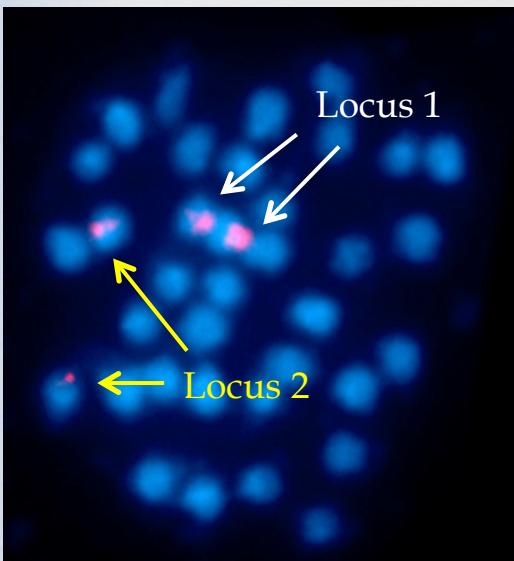


- Tandem arrays of 100's – 1000's of gene copies
- QUINOA, PITSEED GOOSEFOOT HAVE SHARED ORIGINS



Quinoa NTS 1  $\cong$  *C. berlandieri*

Comparison	# changes
quin NTS 1 vs. quin NTS 2	44
quin NTS 1 vs. berl NTS 1	5
quin NTS 2 vs. berl NTS 1	42



<i>C. quinoa</i> class1	(1)	CTTTTTT-GCCGAATTCGTAATTTTATTGTTTGGTTATTTGAGAT
<i>C. quinoa</i> class2	(1)	CTTTTTCCGCCAAATTGAAATTTTTCGTTTTGGTTATTTGAGCT
<i>C. berlandieri</i>	(1)	CTTTTTT-GCCGAATTCGTAATTTTATTGTTTGGTTATTTGAGAT
<i>C. quinoa</i> class1	(50)	GGGAAATGGTTGGGACCGCGTAGATCTCGAAAAAGAACCAATTAAAAA
<i>C. quinoa</i> class2	(51)	GGGACATGGTGTGCAAAGCGTACATCTCGAAAAATACTCGCTTGGAAA
<i>C. berlandieri</i>	(50)	GGGAAATGGTTGGGACCGCGTAGATCTCGAAAAATAACCAATTGGAAA
<i>C. quinoa</i> class1	(100)	AAAAAAATGTTAATTGGACGATCGGGCACAAAGTTACGGCATTTCGA
<i>C. quinoa</i> class2	(101)	AAACAAAT-TGCTCAATTAGGACGACCGGGCGCAAAGTTACGGACTTCGA
<i>C. berlandieri</i>	(100)	AAAAAAATGTTAATTGGACGATCGGGCACAAAGTTACGGCATTTCGA
<i>C. quinoa</i> class1	(150)	AGGAAATAGGGGCTGGAAGGGTATAATACAAAATAGCGCAATAAGTG
<i>C. quinoa</i> class2	(150)	AGGAAATG-GGGGCTGGGAAGGGGTATAATACAAAATGAGAGCGATAAGTA
<i>C. berlandieri</i>	(149)	AGGAAATCAGGGCTGGAAGGGTATAATACAAAATAGCGCAATAAGTA

# Chloroplast *trnH-psbA* Spacer Sequence

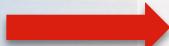
QUINOA'S NEW WORLD ANCESTOR  
WAS THE CYTOPLASM DONOR



<i>C. berlandieri</i> (4x)	ATTCGCTCC	TTTACTAATTAGAAAAATTCT	TTGTAACTAAATT	AAGAA
<i>C. berlandieri</i> (4x)	ATTCGCTCC	TTTACTAATTAGAAAAATTCT	TTGTAACTAAATT	AAGAA
<i>C. berlandieri</i> sin. (4x)	ATTCGCTCC	TTTACTAATTAGAAAAATTCT	TTGTAACTAAATT	AAGAA
<i>C. berlandieri</i> zsch. (4x)	ATTCGCTCC	TTTACTAATTAGAAAAATTCT	TTGTAACTAAATT	AAGAA
<i>C. berlandieri</i> zsch. (4x)	ATTCGCTCC	TTTACTAATTAGAAAAATTCT	TTGTAACTAAATT	AAGAA
<i>C. berlandieri</i> (4x)	ATTCGCTCC	TTTACTAATTAGAAAAATTCT	TTGTAACTAAATT	AAGAA
<i>C. desiccatum</i> (2x)	ATTCGCTCC	TTTACTAATTAGAAAAATTCT	TTGTAACTAAATT	AAGAA
<i>C. fremontii</i> (2x)	ATTCGCTCC	TTTACTAATTAGAAAAATTCT	TTGTAACTAAATT	AAGAA
<i>C. leptophyllum</i> (2x)	ATTCGCTCC	TTTACTAATTAGAAAAATTCT	TTGTAACTAAATT	AAGAA
<i>C. neomexicanum</i> (2x)	ATTCGCTCC	TTTACTAATTAGAAAAATTCT	TTGTAACTAAATT	AAGAA
<i>C. pratericola</i> (2x)	ATTCGCTCC	TTTACTAATTAGAAAAATTCT	TTGTAACTAAATT	AAGAA
<i>C. pratericola</i> (2x)	ATTCGCTCC	TTTACTAATTAGAAAAATTCT	TTGTAACTAAATT	AAGAA
<i>C. watsonii</i> (2x)	ATTCGCTCC	TTTACTAATTAGAAAAATTCT	TTGTAACTAAATT	AAGAA
<i>C. hircinum</i> (4x)	ATTCGCTCC	TTTACTAATTAGAAAAATTCT	TTGTAACTAAATT	AAGAA
<i>C. pallidicaule</i> (2x)	ATTCGCTCC	TTTACTAATTAGAAAAATTCT	TTGTAACTAAATT	AAGAA
<i>C. petiolare</i> (2x)	ATTCGCTCC	TTCGCTATTAGAAAATTCT	TTGTAACTAAATT	AAGAA
<i>C. quinoa</i> (KU-2) (4x)	ATTCGCTCC	TTTACTAATTAGAAAAATTCT	TTGTAACTAAATT	AAGAA
<i>C. berlandieri</i> (4x)	ATTCGCTCC	TTTACTAATTAGAAAAATTCT	TTGTAACTAAATT	AAGAA
<i>C. album</i> (6x)	ATTCGCTCC	TTCGCTATTAGAAAATTCT	TTGTAC-TAAATT	AAGAA
<i>C. album</i> (6x)	ATTCGCTCC	TTCGCTATTAGAAAATTCT	TTGTAC-TAAATT	AAGAA
<i>C. album</i> (6x)	ATTCGCTCC	TTCGCTATTAGAAAATTCT	TTGTAC-TAAATT	AAGAA
<i>C. strictum</i> (4x)	ATTCGCTCC	TTCGCTATTAGAAAATTCT	TTGTAC-TAAATT	AAGAA
<i>C. giganteum</i> (6x)	ATTCGCTCC	TTCGCTATTAGAAAATTCT	TTGTAC-TAAATT	AAGAA
<i>Oxybasis macroisperma</i>	ATTCGCTCC	TTTACCAATTAGAAAAATTCT	TTGTAACTAAATT	AAGAA
<i>Chenopodiastrum murale</i>	ATTCGCTCC	TTTACCAATTAGAAAAATTCT	TTGTAACTAAATT	AAGAA
<i>Oxybasis rubra</i>	ATTCGCTCC	CTTACCAATTAGAAAAATTCT	TTGTAACTAAATT	AAGAA
<i>Oxybasis glauca</i>	ATTCGCTCC	CTTACCAATTAGAAAAATTCT	TTGTAACTAAATT	AAGAA

New  
World  
natives

Old  
World  
natives



# Searching for Quinoa's Ancestors



- Polyploidization event: N. America or S. America? Would have required the B-genome diploid to be in the Americas pre-1492
- Eurasian event would have required an unlikely “return trip” to Americas

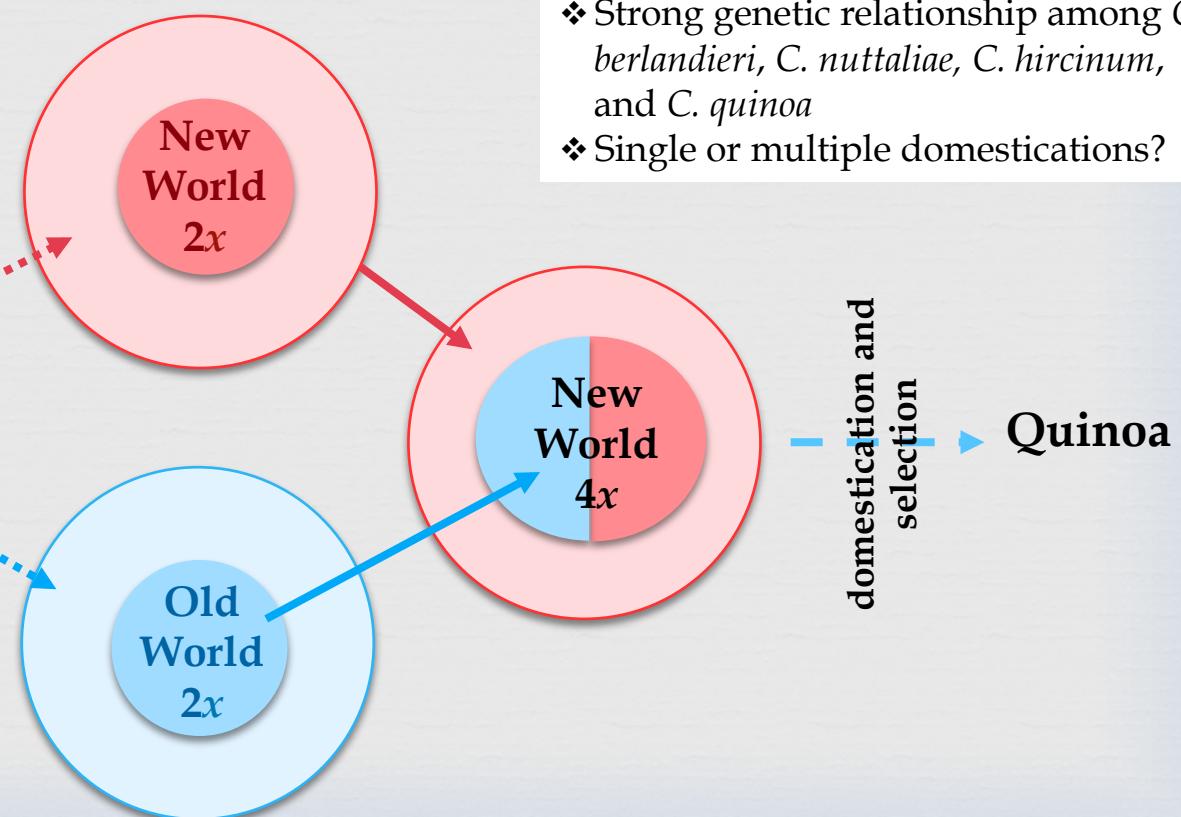
## A-genome candidates:

- ❖ North or South America?
  - ❖ Cultivated (kañiwa) or wild-weedy?
- ❖ Long list of candidates
- ❖ Restricted species access
- ❖ Limited existing collections

***2x* ancestor**

## Tetraploid evolution:

- ❖ Strong genetic relationship among *C. berlandieri*, *C. nuttalliae*, *C. hircinum*, and *C. quinoa*
- ❖ Single or multiple domestications?



## B-genome candidates:

- ❖ “*C. album* diploids”
  - ❖ *C. ficiifolium*, *suecicum*
- ❖ Poor taxonomy
- ❖ Limited existing collections

# BYU *Chenopodium* Collections: 2004-2013

## ► >370 accessions: Western USA, Mexico, Chile

• <i>C. album</i>	EXOTIC	Urban, agricultural (exotic, all N. and S. America)
• <i>C. atrovirens</i>		Rocky Mountains, steppe
• <i>C. berlandieri</i> var. <i>boscianum</i>		Gulf of Mexico Coast (saline)
• <i>C. berlandieri</i> var. <i>sinuatum</i>		Sonoran Desert, California (arroyos, pastures)
• <i>C. berlandieri</i> var. <i>zschackei</i>		all of N. America
• <i>C. desiccatum</i>		Steppe, desert (Great Basin, Colorado Plateau)
• <i>C. fremontii</i>		Montane
• <i>C. hians</i>		California Peninsular & Transverse Ranges
• <i>C. hircinum</i>		Urban, agricultural (S. America)
• <i>C. incanum</i> var. <i>elatum</i>		Chihuahuan Desert (arroyos, pastures)
• <i>C. incanum</i> var. <i>incanum</i>		Intermountain & High Plains
• <i>C. incanum</i> var. <i>occidentale</i>		Great Basin (arroyos, burn areas)
• <i>C. leptophyllum</i>		Steppe, desert (Great Basin, Colorado Plateau)
• <i>C. neomexicanum</i>		Mogollon Rim, Gila Mountains
• <i>C. neomexicanum</i> var. <i>palmeri</i>		Sonoran Desert (arroyos)
• <i>C. nevadense</i>		W. Great Basin (alkaline playas)
• <i>C. petiolare</i>		High Atacama, Altiplano (saline)
• <i>C. pratericola</i>		Steppe, desert (all N. America)
• <i>C. strictum</i>	EXOTIC	Urban, agricultural (all N. and S. America)
• <i>C. subglabrum</i>		High Plains (sand hills, wallows)
• <i>C. watsonii</i>		Sonoran & Chihuahuan Deserts (farms, arroyos)

# *Chenopodium berlandieri*



## Comparison of nuclear GLN-1 sequences (N metabolism)



(I. Arano, BYU undergrad and  
H. Munir, UAF-Pakistan)

Consensus

20 30 40 50 60 70 80 90 100 110

1. *atrovir*  
2. *standley*  
3. G205  
4. 0654  
5. *hircinum*  
6. KU-2  
7. *suecicum*

GGAAATCTCTGCT GGAGATGAGTTGGGTAGCTCGTTACATTTGGAGGTATTT ACAACACATATGCATTTCATCTTAAGTGAA-TGCAATTTC-TTAA  
GGAAATCTCTGCT GGAGATGAGTTGGGTAGCTCGTTACATTTGGAGGTATTT ACAACACATATGCATTTCATCTTAAGTGAA-TGCAATTTC-TTAA

New World 2x

New World 4x

Old World species

G205, 0654, and KU-2 are quinoas

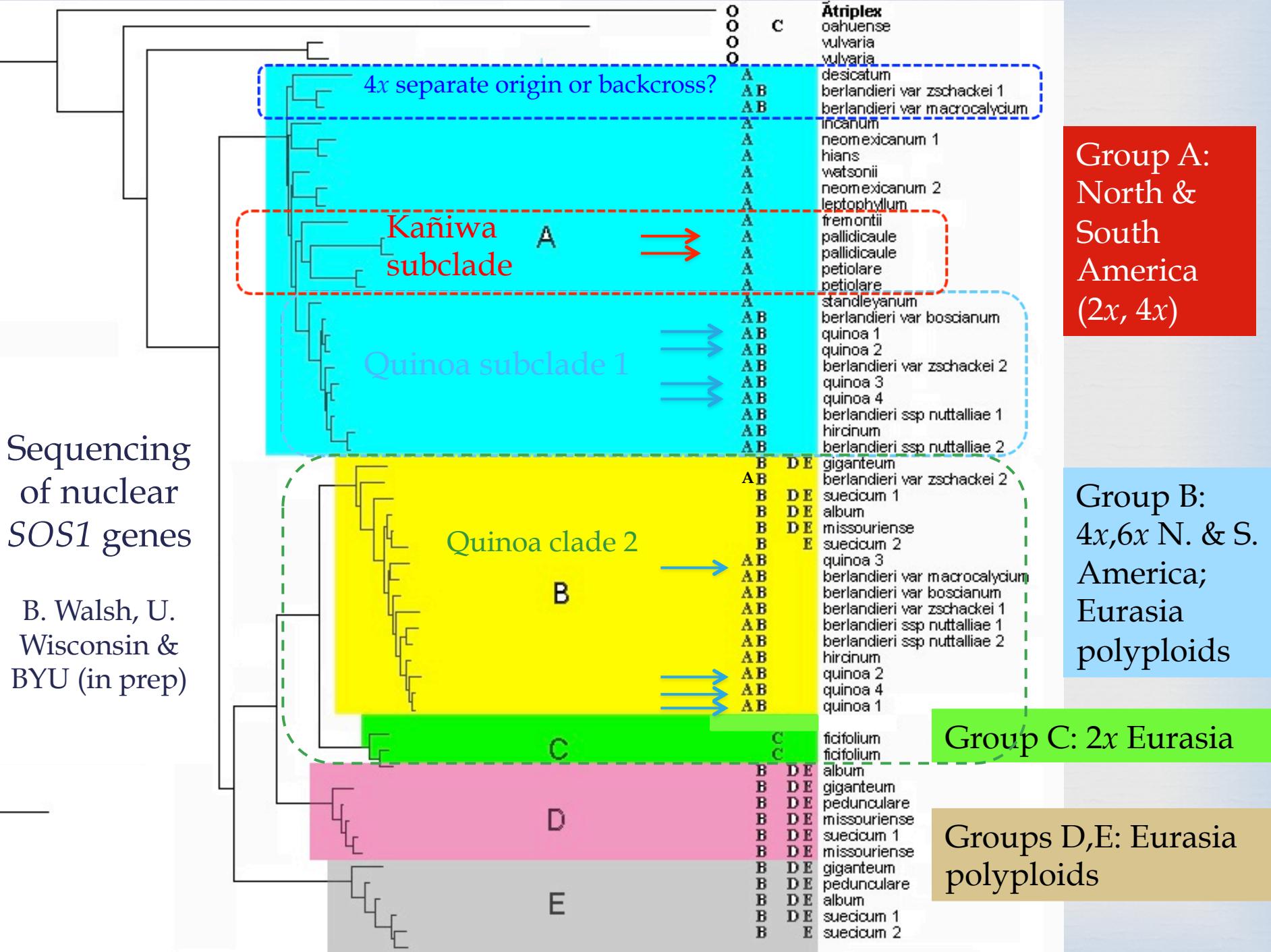
Consensus	610	620	630	640	650	660	670	680	690	700	710	720	730
1. atrovir	T	G	F	T	T	A	C	T	T	C	T	G	A
2. standley	T	T	G	T	T	A	G	T	T	C	T	G	C
3. G205	T	T	G	T	T	A	G	T	T	C	T	G	C
4. 0654	T	T	G	T	T	A	G	T	T	C	T	G	C
5. hircinum	T	T	G	T	T	A	G	T	T	C	T	G	C
6. KU-2	T	T	G	T	T	A	G	T	T	C	T	G	C
7. suecicum	T	T	G	T	T	A	G	T	T	C	T	G	C

$4x$  quinoa has one *GLN-1* gene like the New World diploids and one like the Old World species

## Characteristic Old World vs. New World INDEL

## Sequencing of nuclear *SOS1* genes

B. Walsh, U.  
Wisconsin &  
BYU (in prep)



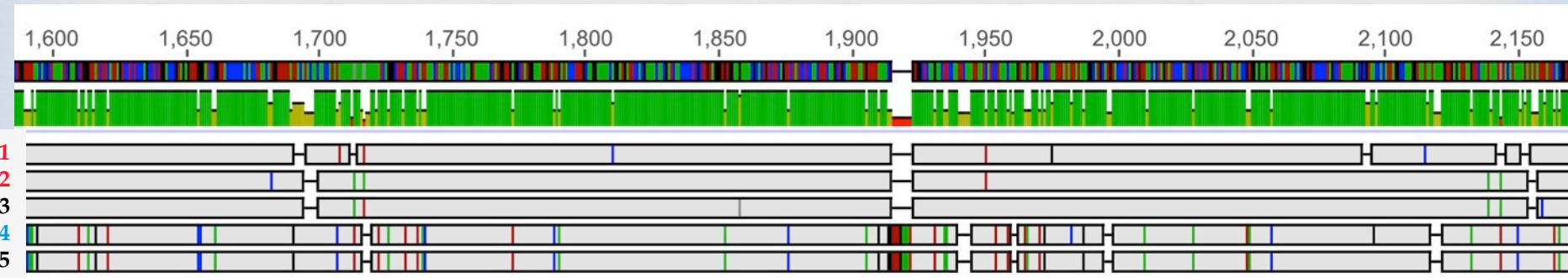
# Sequencing of *GBSSI* gene (starch production)

Doug Brown et al., BYU (M.S.)



## Lanes:

- 1 - *C. pallidicaule* (kañiwa), S. America, Andean crop (2x)
- 2 - *C. standleyanum*, N. America, Mississippi Valley weed (2x)
- 3 - Quinoa cv. 'Ollague', S. America, cloned A-genome locus (4x)



- 4 - *C. ficifolium*, Eurasia, weed from *C. album* complex (2x)
- 5 - Quinoa cv. 'Ollague', S. America, cloned B-genome locus (4x)

## % Identity:

- C. pallidicaule* vs. 'Ollague' = 97.7%  
*C. standleyanum* vs. 'Ollague' = 98.1%

**CONCLUSION: Quinoa's (wild) ancestry traces back to North America.**

# Quinoa's Putative Ancestors

New World 2x (i.e., *C. standleyanum*); Mississippi Valley



New World 4x *C. hircinum*; Pampas

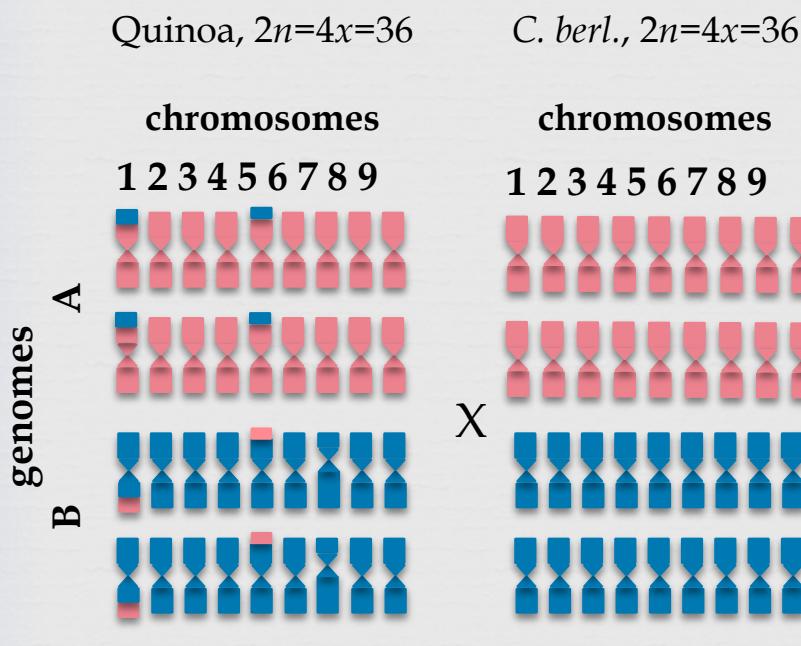


Old World 2x (*C. ficifolium*); Eurasia



# Breeding Strategies for Quinoa:

## #1: Primary Gene Pool – *berlandieri* 4x Complex



### Benefits:

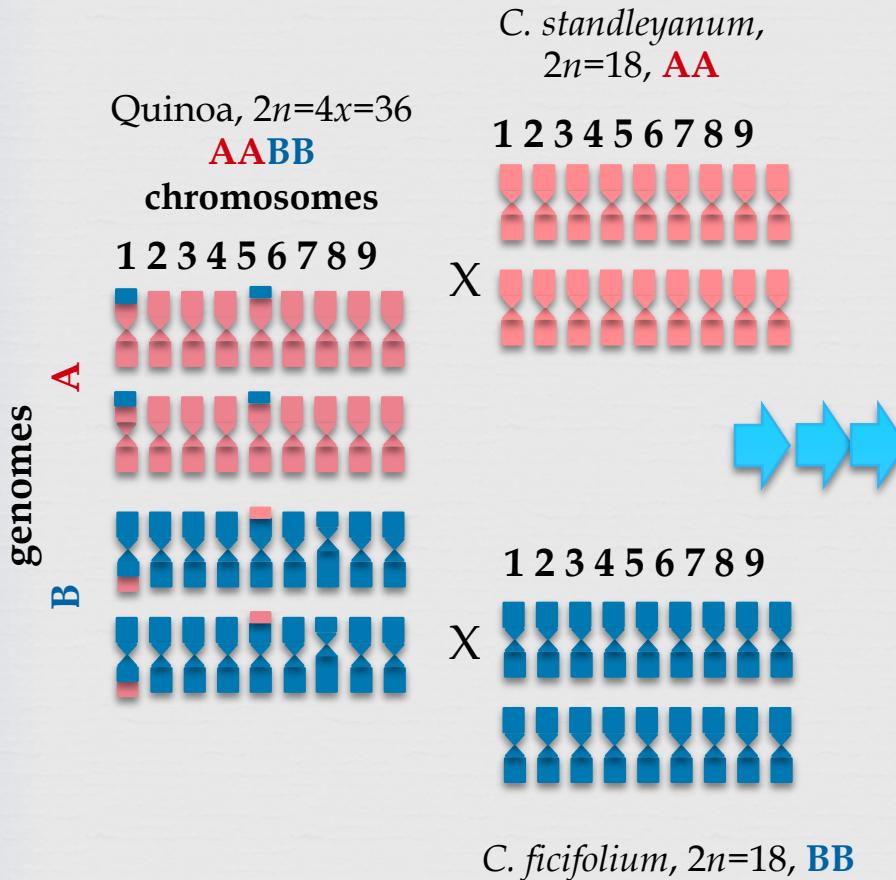
- ✓ Adapted to wide range of environments
- ✓ Novel traits for quinoa improvement

### Challenges (hypothetical):

- ✓ Elimination of exotic alleles on all 18 chromosome pairs
- ✓ Translocations → **quadrivalents**
- ✓ Inversions → **reduced recombination**
- ✓ Aneuploidy

# Breeding Strategies for Quinoa:

## #2: Secondary Gene Pool – Diploid Ancestors



### Benefits:

- ✓ Only one genome recombines
- ✓ Faster recovery of recurrent parent
- ✓ *C. ficiifolium* ranges across Eurasian environments (probably highly variable)
- ✓ Multiple New World diploids

### Challenges (hypothetical):

- ✓ Same issues with inversions, translocations, aneuploidy
- ✓ More distantly related so more crossing barriers (embryo rescue?)
- ✓ Seed dormancy in diploids

# Hybridization in the Wild



Arroyo in Gila Mts., New Mexico, with  $2x$  *C. watsonii*;  $2x$  *C. fremontii*;  $2x$  *C. neomexicanum*;  $4x$  *C. berlandieri*; and hybrids in a swarm. One *C. berlandieri* collected (BYU 841) had triangular leaf morphology similar to *C. watsonii*. Summer, 2008.

# Hybridization in the Wild

---

Extreme variation in plant morphology from hybrid swarm along the Verde River arroyo, Yavapai County, Arizona, summer 2005.



# Conclusions

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- Gene Pools:
  - Primary: quinoa, huauzontle, pitseed goosefoot, avian goosefoot
  - Secondary: A/B diploids, *C. album* complex
- Traits of primary importance in these gene pools include disease and insect resistance, heat tolerance, seed quality, etc.
- These resources should be collected, curated, and evaluated
- Collections we (BYU) make within U.S. borders will be made available internationally to geneticists according to the principles of germplasm exchange of the U.S. government (USDA-NPGS)
- Reclassification ???:
  - *C. quinoa* ssp. *ancestrale* = *C. berlandieri* ssp. *berlandieri*
  - *C. quinoa* ssp. *quinoa* = *C. quinoa*
  - *C. quinoa* ssp. *mexicana* = *C. berlandieri* ssp. *nuttalliae*
  - *C. quinoa* ssp. *foetida* = *C. hircinum*
  - *C. quinoa* ssp. *jonesiana* = *C. berlandieri* ssp. *jonesianum* (extinct)

# Acknowledgments

- 
- (A green maple leaf is centered above the horizontal line.)
- Huauzontle starch project and germplasm (Mexico):
    - Drs. Eulogio de la Cruz (ININ, Ocoyoacac) & Lupe Palomino (UNAM, Mexico City)
    - Dr. Veronica Cepeda (BYU & UNAM)
  - SOS1 sequencing project:
    - Brian Walsh & Dr. Eve Emshwiller (Univ. Wisconsin, Madison)
  - FISH projects:
    - Dr. Bozena Kolano (Univ. Silesia, Katowice, Poland); Brian Gardunia, Maria Sederberg (M.S., BYU)
  - Germplasm:
    - David Brenner, USDA-NPGS & Iowa State Univ.
    - Drs. Francisco Fuentes (Univ. Arturo Prat, Iquique), Daniel Bertero (Univ. Buenos Aires), Angel Mujica (Univ. Altiplano, Puno), Luz Gomez (UNALM, Lima)
  - BYU Lab Group:
    - 5S rDNA: Nate Coles, Susan Parkinson (M.S.)
    - cpDNA: Marcus Soliai, Ben King, Brad Petty, Austin Hackett, Jonathan Hintze, Matt Wells
    - SOS1: Dinesh Adhikary, Kendra Patton, Orrin Probst
    - GLN1: Ivan Arano, Scott Pew, Kierstyn Edwards
    - GBSSI: Doug Brown (M.S.)
    - Collections: Jenna Miller, Breanna Meldrum, Elisabeth Svedin
    - Amalia Vargas (M.S., Bolivia), Felix Jimenez (M.S., Peru)
    - Gabi Amado (Peru), Manal Mhada (Morocco)

