



Determining genetic relationships among wild and domesticated pumpkin species

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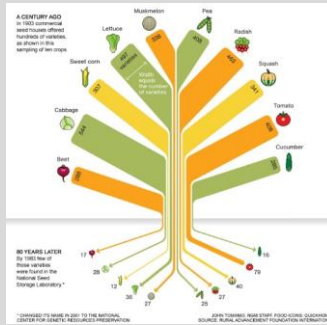
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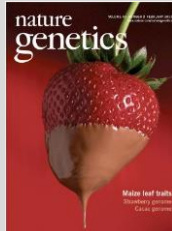
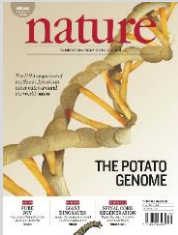
3 Univ Florida, Dept Biol, Gainesville, FL 32611 USA

Texas A&M Plant Breeding Symposium 2017

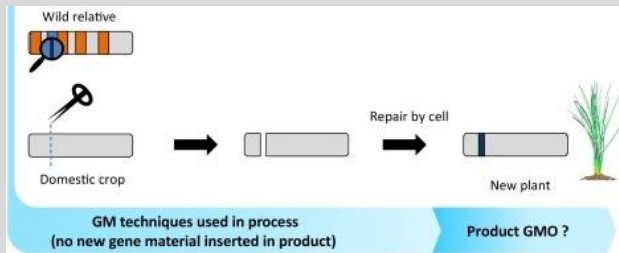
BOTANY, GENETICS, & CROP DIVERSITY



There is a growing need to reintroduce **traits from wild plants** into crops.



Genomic resources generated for plant breeding are used to study wild plants.



Advances in genetic engineering will enable us to **utilize crop wild relatives** in new ways.

PHYLOGENETIC APPROACH

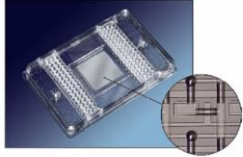
Incongruent evolutionary histories among genes have complicated *Cucurbita* phylogenetics

We sequenced a set of 44 nuclear introns for 96 wild and domesticated individuals from 19 *Cucurbita* taxa.

PCR primers designed in *conserved* sequence flanking **48 target introns variable** and single-copy across 4 *Cucurbita* genomes



Microfluidic PCR (Fluidigm Access Array™)



2x300 sequencing on Illumina MiSeq™



Amplicon-seq assembly with dbcAmplicons
(github.com/msettles/dbcAmplicons)



Gene concatenation +
ML

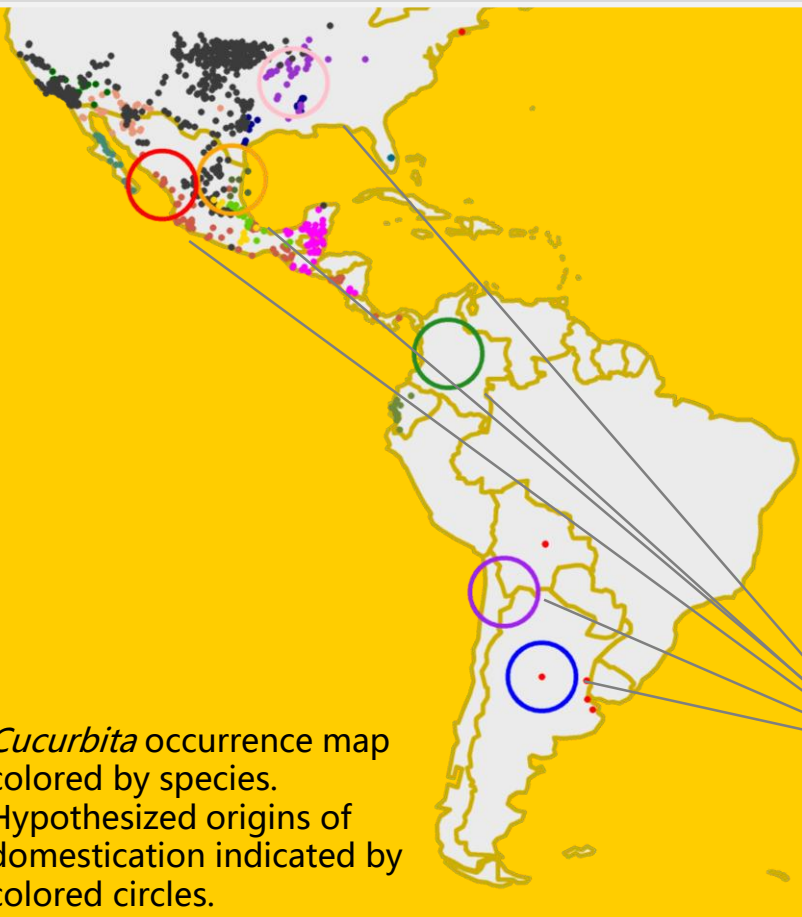
Gene trees →
Species Tree

Cucurbita (Cucurbitaceae, $2n=40$)

14 wild species are distributed in North and South America. Most species are native to Mexico and the southwestern U.S.

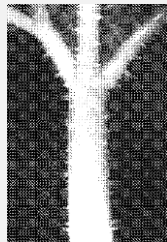
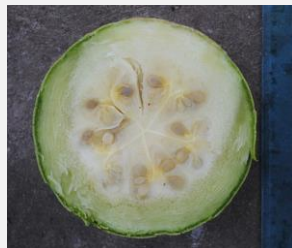
The six *Cucurbita* crop species were likely domesticated from different wild species.

Cucurbita occurrence map colored by species. Hypothesized origins of domestication indicated by colored circles.

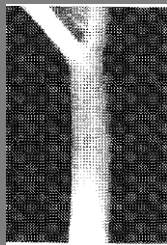


DOMESTICATION TRAITS AND BREEDING AIMS

Wild



Domesticated



Bush habit

Larger seeds

Fewer
trichomes

Larger fruit

Diverse fruit
morphology

DOMESTICATION TRAITS AND BREEDING AIMS

Domesticated *Cucurbita* exhibit a decreased resistance to disease.



DOMESTICATION TRAITS AND BREEDING AIMS

Domesticated *Cucurbita* water requirements are among the highest of all vegetable crops.



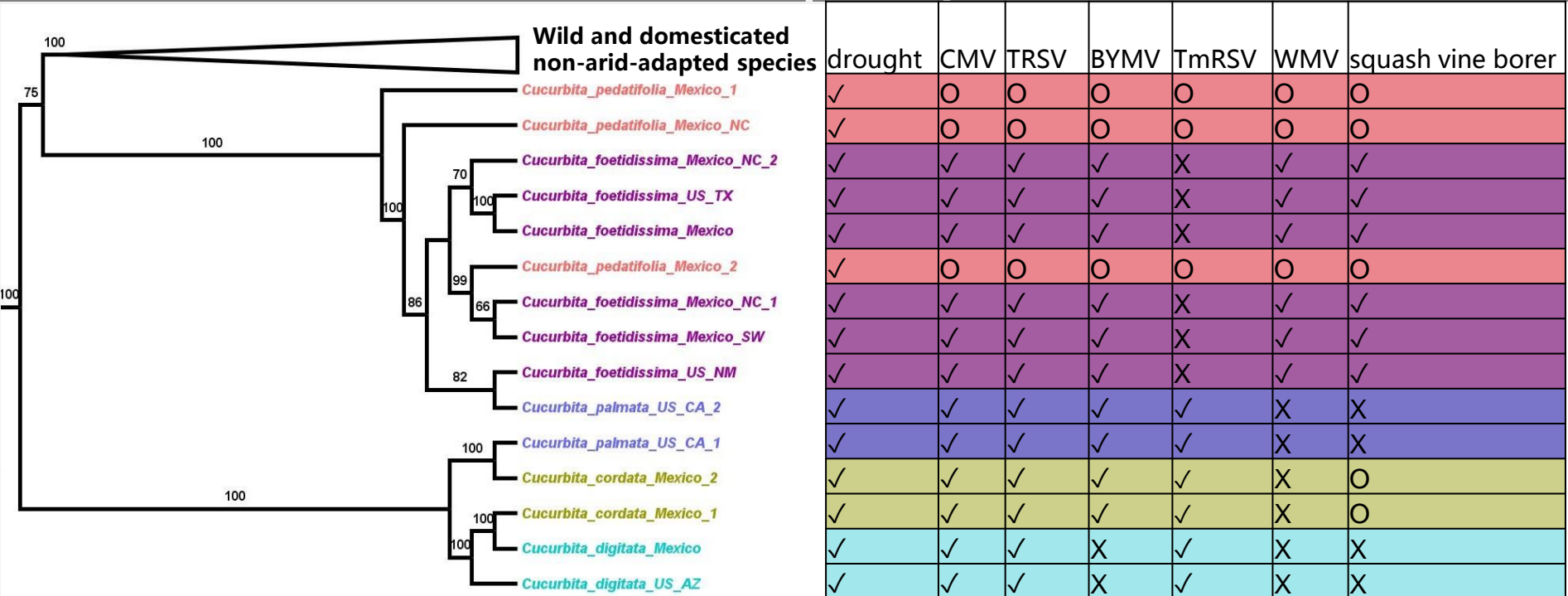
Wild buffalo gourd (*C. foetidissima*), Mojave Desert, CA ~6 in. rain/year



Domesticated pumpkin (*C. pepo* ssp. *pepo*), Verde Valley, AZ ~1 in. water/week

DOMESTICATION TRAITS AND BREEDING AIMS

Arid-adapted *Cucurbita* species exhibit more disease resistance than non-arid-adapted species

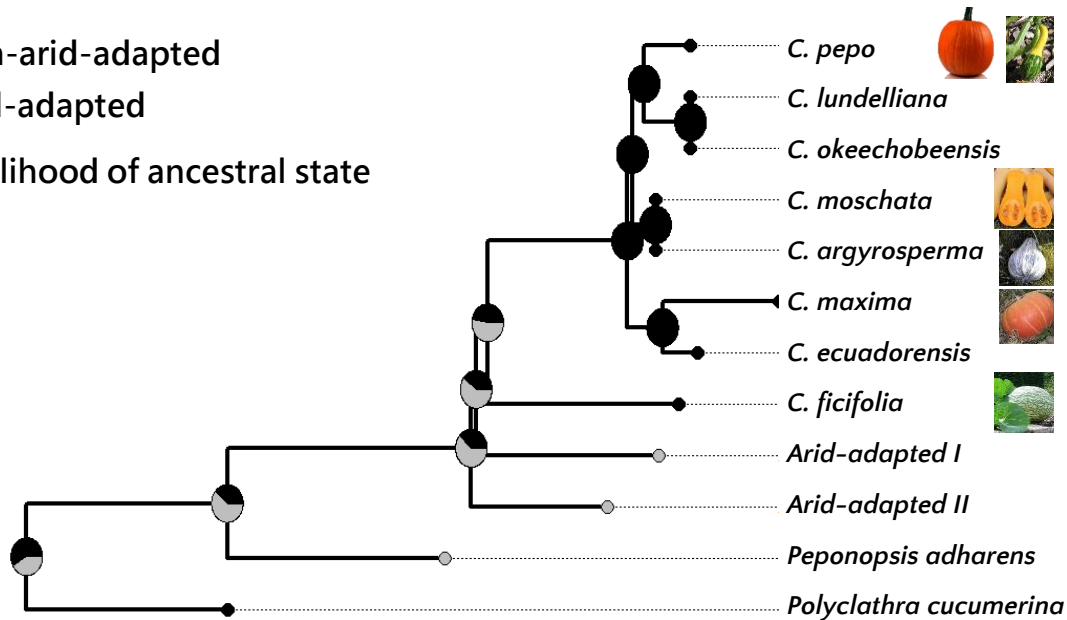


Disease resistance data from Providenti et al. 1978. ✓ resistant; X susceptible; O no data

THE ARID ORIGINS OF *CUCURBITA*

All *Cucurbita* species likely evolved from an arid-adapted ancestor

- Non-arid-adapted
- Arid-adapted
- Likelihood of ancestral state



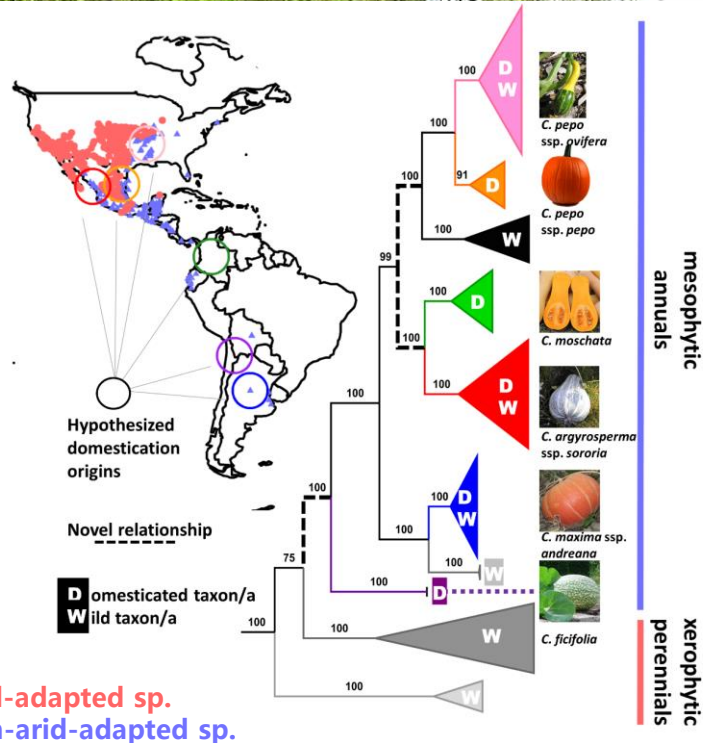
**Non-arid-adapted
(wild and
domesticated)**

Arid-adapted (wild)

Ancestral character state reconstruction for arid-adaptation

THE ARID ORIGINS OF *CUCURBITA*

The wild ancestors of *Cucurbita* crops grow in moist or dry forests and plains



Arid-adapted sp.
Non-arid-adapted sp.

RELATIONSHIPS BETWEEN WILD AND DOMESTICATED *CUCURBITA*

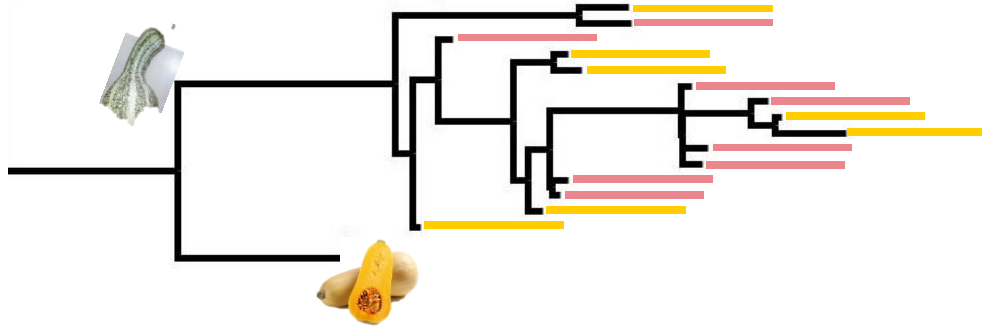


Cushaw (*C. argyrosperma*)

Giant pumpkin (*C. maxima*)

RELATIONSHIPS BETWEEN WILD AND DOMESTICATED CUSHAW

C. argyrosperma (cushaw)



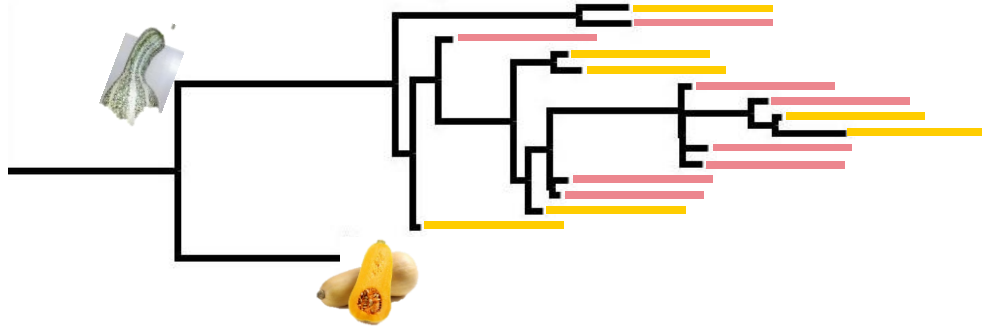
Domesticated

Wild

Patterns of relatedness within *C. argyrosperma* are consistent with a broad domestication bottleneck(s) and continued introgression

RELATIONSHIPS BETWEEN WILD AND DOMESTICATED GIANT PUMPKIN

C. argyrosperma (cushaw)



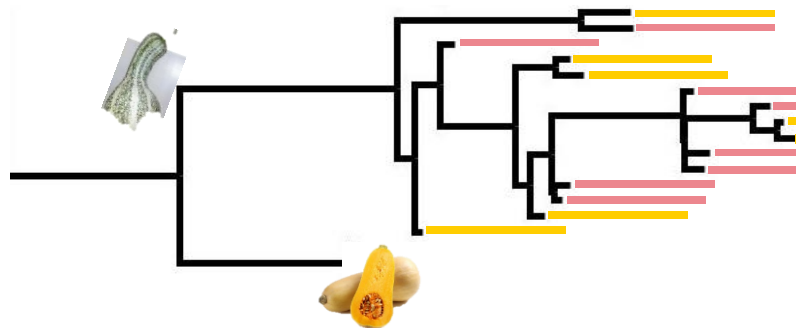
C. maxima (giant pumpkin)



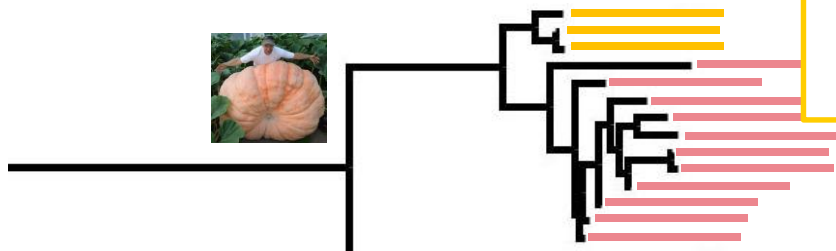
Patterns of relatedness within *C. maxima* are consistent with a narrow domestication bottleneck and limited introgression







GENETIC DIVERSITY WITHIN DOMESTICATED CUSHAW AND DOMESTICATED GIANT PUMPKIN

C. argyrosperma (cushaw)



C. maxima (giant pumpkin)



Crop species	Heterozygosity ¹
 <i>C. pepo</i> ssp. <i>pepo</i>	18%
 <i>C. pepo</i> ssp. <i>ovifera</i>	15%
 <i>C. maxima</i> ssp. <i>maxima</i>	13%
 <i>C. argyrosperma</i> ssp. <i>argyrosperma</i>	19%
 <i>C. moschata</i>	24%
 <i>C. ficifolia</i>	15%

Proportion of biallelic individuals per locus averaged over all loci

Domesticated

Wild

Domesticated *Cucurbita argyrosperma* and *C. maxima* have contrasting levels of observed heterozygosity

INVESTIGATING THE DOMESTICATION PROCESS AND DOMESTICATION GENES IN *CUCURBITA*

Wild



Landrace



Improved



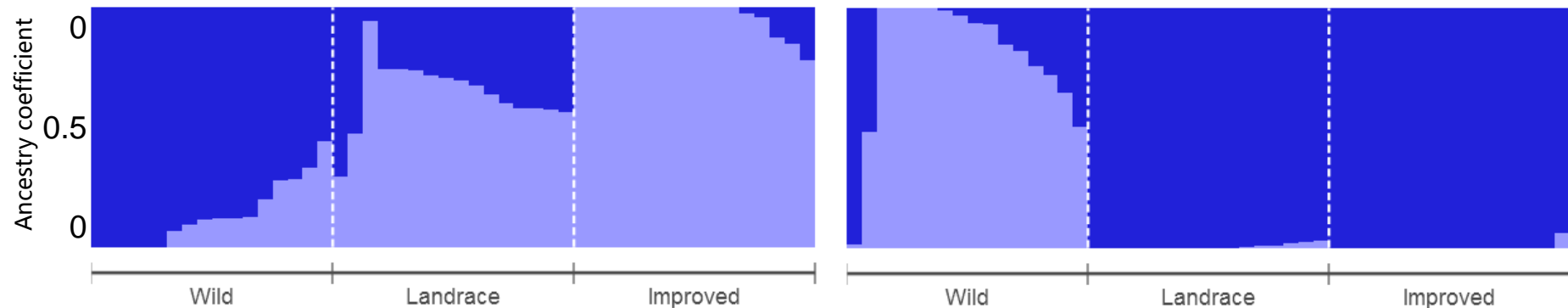
10,000 genomic loci targeted and sequenced for 96 individuals



Cushaw (*C. argyrosperma*)

Giant pumpkin (*C. maxima*)

INVESTIGATING THE DOMESTICATION PROCESSES AND DOMESTICATION GENES IN *CUCURBITA*



Cushaw (*C. argyrosperma*)

Giant pumpkin (*C. maxima*)

Structure plots for K=2 based on 30,264 (*C. argyrosperma*) and 25,718 (*C. maxima*) SNP loci for 48 individuals per species

Preliminary population genomics data suggests genetic structure consistent with phylogeny-based domestication hypotheses

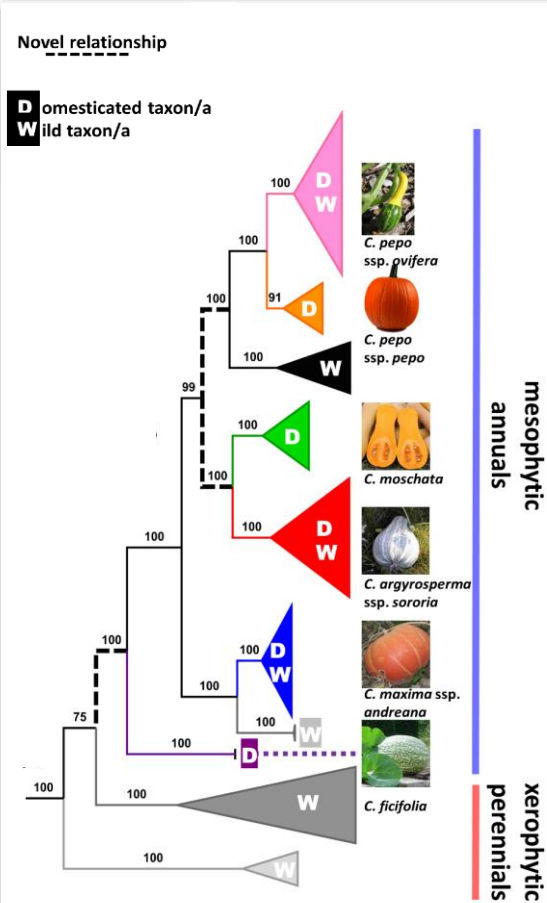
CONCLUSIONS

1. Most comprehensive and robust molecular phylogeny for *Cucurbita* to date.

2. Single loss of arid-adaptation suggests traits for robustness lost before primary genepool CWR arose.

3. Phylogeny provides framework for domestication-genetics studies.

4. Independently domesticated *Cucurbita* species exhibit different patterns of genetic structure.



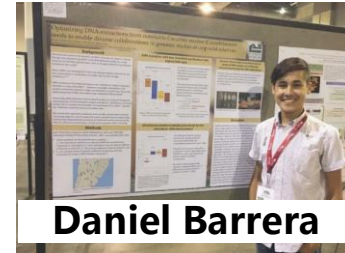
THANK YOU! QUESTIONS?

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Germplasm and sample preparation



Daniel Barrera

Research funding and travel scholarship



Texas A&M Plant Breeding Symposium Committee

University of Florida, Gainesville