

# Phylogenetic relationships of genus *Amaranthus*:

## A tool for evolutionary genetics studies

emerging phylogeny & evolutionary genetics studies

Department of Genetics & Biochemistry

Clemson University

[www.clemson.edu/lawtonrauhlab](http://www.clemson.edu/lawtonrauhlab)



# Genus Amaranthus

Locally adapted crop (?)

Weed: unintended during cultivation



<http://pizzagov.com/wp-content/uploads/2010/01/amaranth.jpg>



<http://permaculture.org.au/2016/08/17/gm-crops-facing-meltdown-in-the-usa/>

# Amaranthus (ἀμάραντος), 苋菜 :

~ 60-70 annual C4 species, five major 'types'

## Grain crops

*A. caudatus*, *A. hypochondriacus*, *A. hybridus*, *A. cruentus*

## Leaf-vegetable crops, potherbs (high lysine)

*A. cruentus*, *A. tricolor*, *A. dubius*, *A. blitum*, *A. gangeticus*,  
*A. lividus*, *A. spinosus*, (*A. palmeri*)

## Showy ornamental varieties

*A. caudatus*

## Herbaceous species with limited distribution

Several sp., *A. retroflexus*

## Weedy species

*A. palmeri*, *A. tuberculatus*

## Weed



## Grain

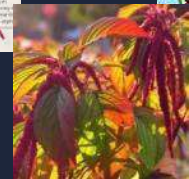


<http://www.all-creatures.org/recipes/-amaranth.html>

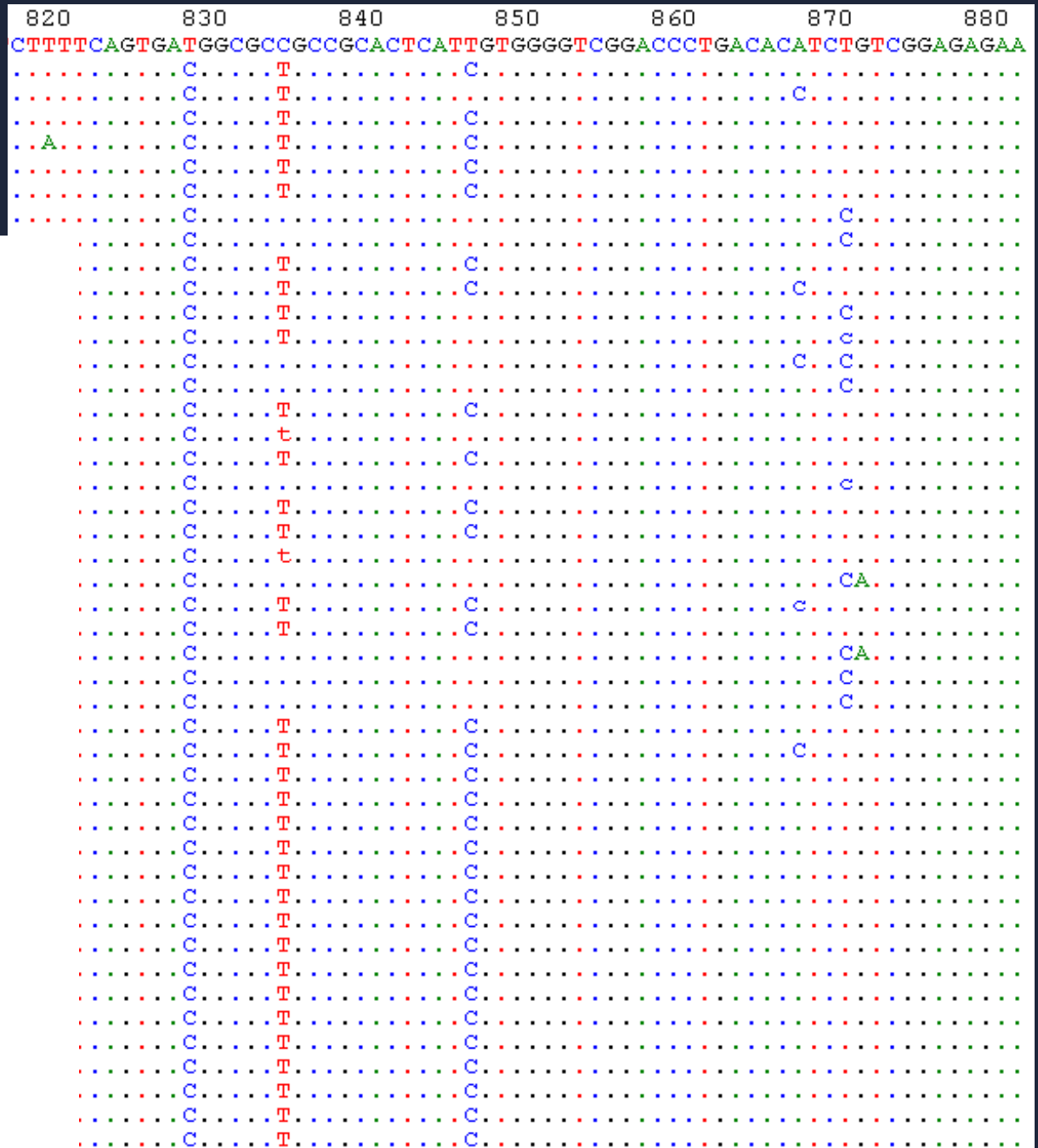
## Leaf-vegetable, Potherb



## Ornamental



adenine  
thymine  
cytosine  
guanine

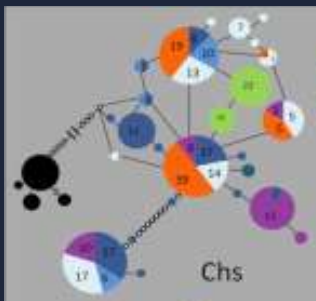
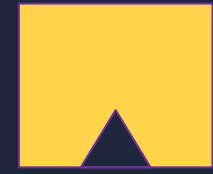
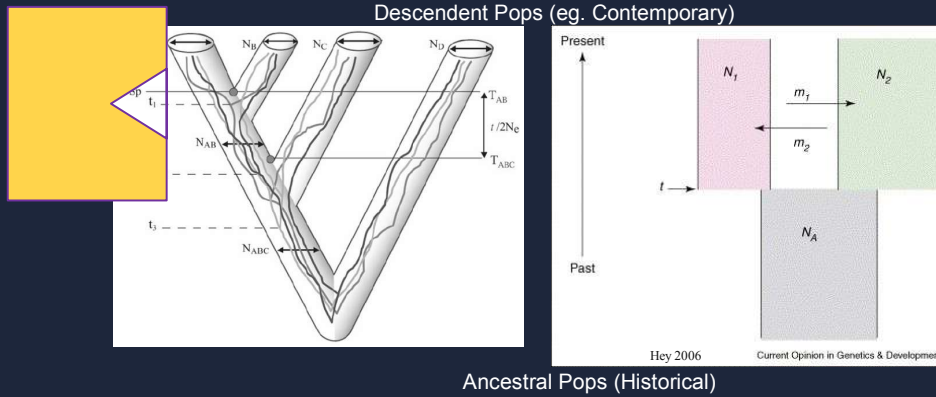




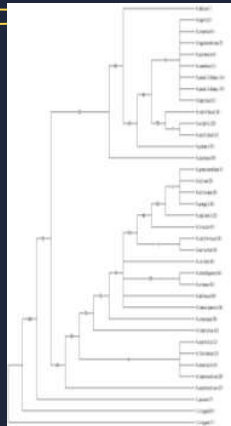
# Workflow...

## Explicit Models

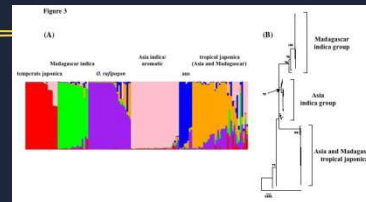
(eg. Isolation-Migration, Substitution Models, Phylogeny Estimation)



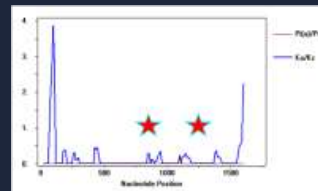
limer et al. 2010  
and submitted



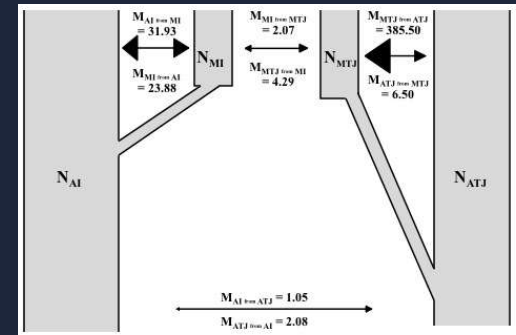
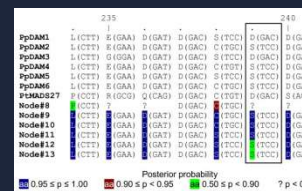
Jimenez et al. 2009



Mather et al. 2010



Jimenez et al. 2009



Mather et al. 2010

# Differences in genus *Amaranthus*: Phylogenetic history and adaptation

- **Contemporary Processes**  
*A. palmeri* (*A. tuberculatus*, *A. spinosus*)
  - resistant vs. sensitive populations
  - shared ancestral vs. independent variants (gene flow, or private)
    - demography vs. selection
- **Molecular Evolution (candidate genes)**
  - *Amaranthus palmeri*
  - genus-wide 'weeds', 'types'
  - phylogeny (ancestral vs. derived adaptations, crop origins)
  - among species: crop history (support cultural records?)
- **Model Testing and Hypotheses**
  - cultivation practices, management strategies, geography, expanded species distributions

# Overview of sampling

Obtain seeds, grow in greenhouse

Phylogenetic sampling

Glyphosate-applied and non-applied localities

Historical and contemporary populations

Intensive sampling: *A. tuberculatus*, *A. palmeri*, *A. spinosus*



# Non-candidate Genes: Phylogenetically informative

Normalized Roche 454 pyrosequencing data

*Amaranthus palmeri* (Burton Lab, NCSU)

pooled resistant biotypes

pooled sensitive biotypes

*Amaranthus tuberculatus* (Tranel Lab, Uni. Illinois)

pooled genome-wide representation

Local BLAST- compare assemblies

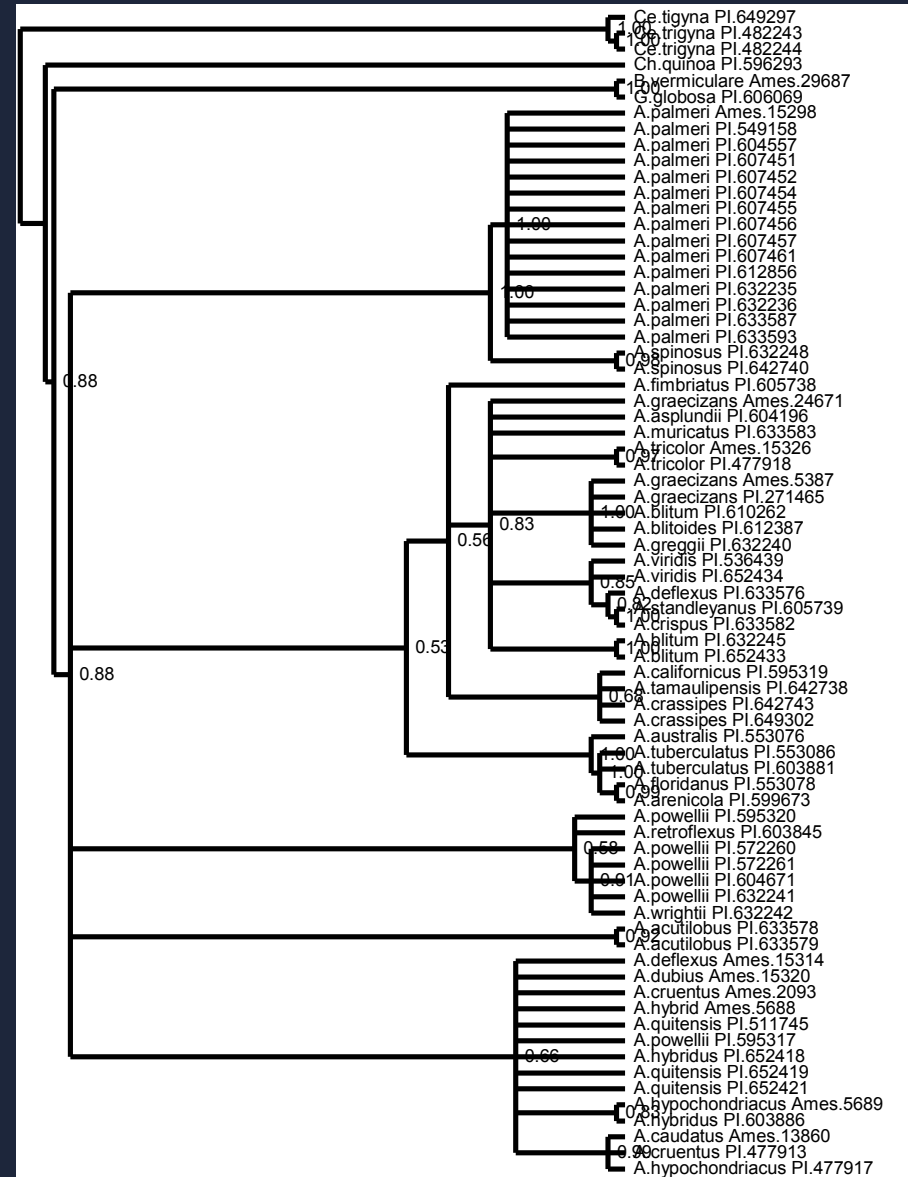
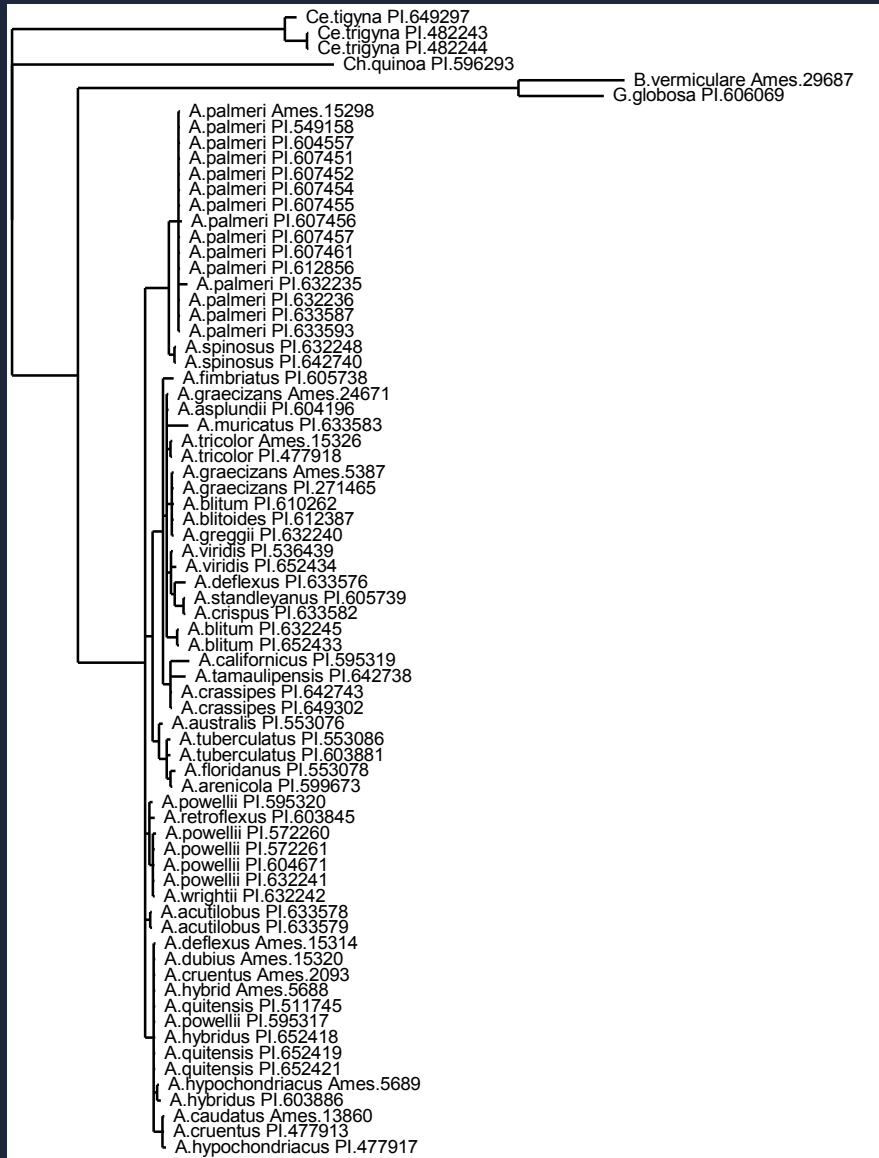
Sanger sequenced common 'non-candidate' genes

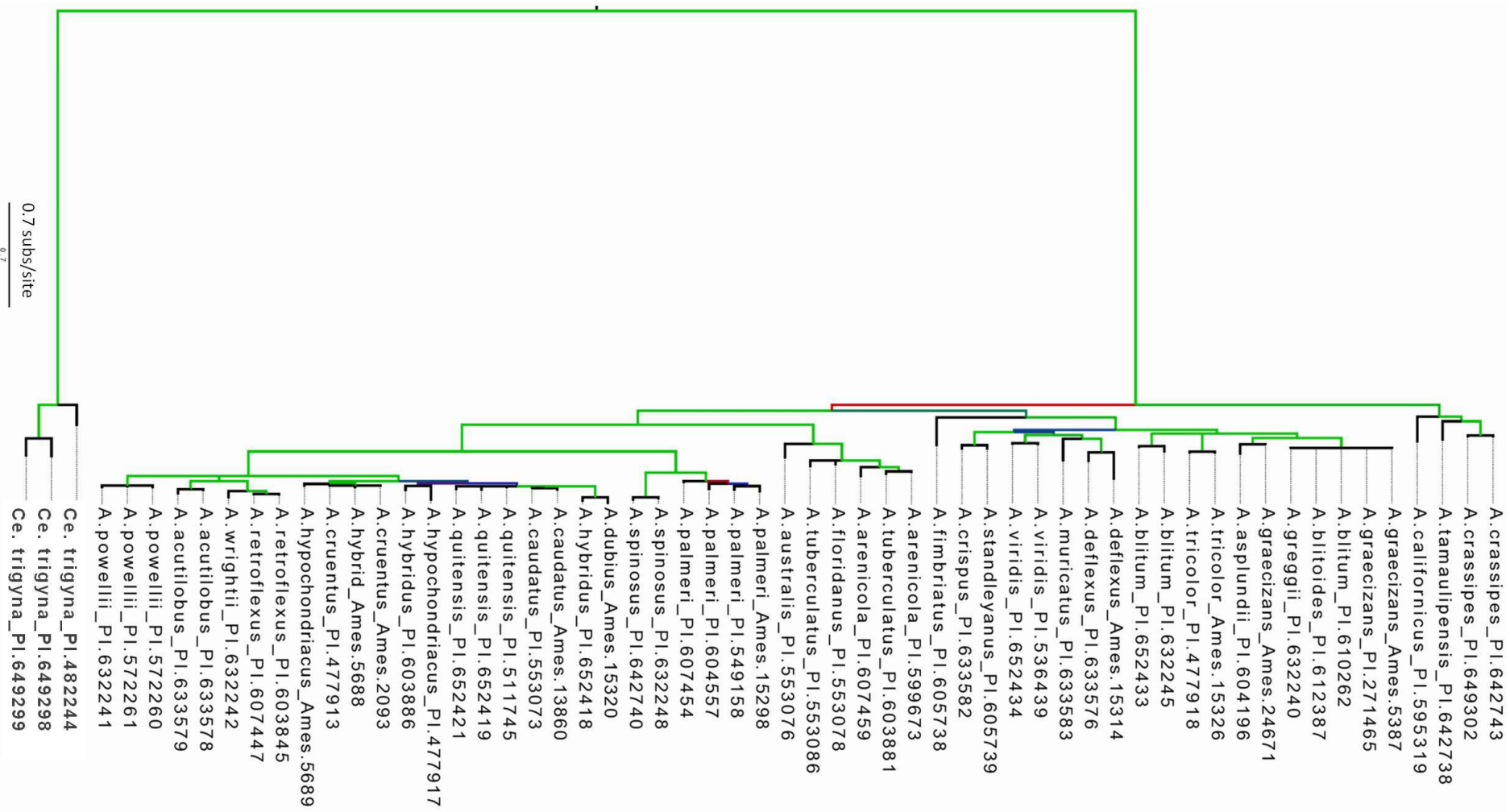
Gene	Putative function	# inf. sites / total # sites (# of indivs)
MatK	maturase K	86 / 819 (75)
ITS	5.8S rRNA gene and internal transcribed spacers 1 and 2	67 / 660 (69)
Trn	tRNA-Leu (trnL) gene	35 / 830 (64)
A7	Endosomal P24A protein precursor, putative	92 / 610 (64)
	dead box ATP-dependent RNA	



# Bayesian-based Phylogeny Estimation, Preliminary ITS Only

MrBayes, Generations = 2,000,000; final std dev split freq = 0.043512

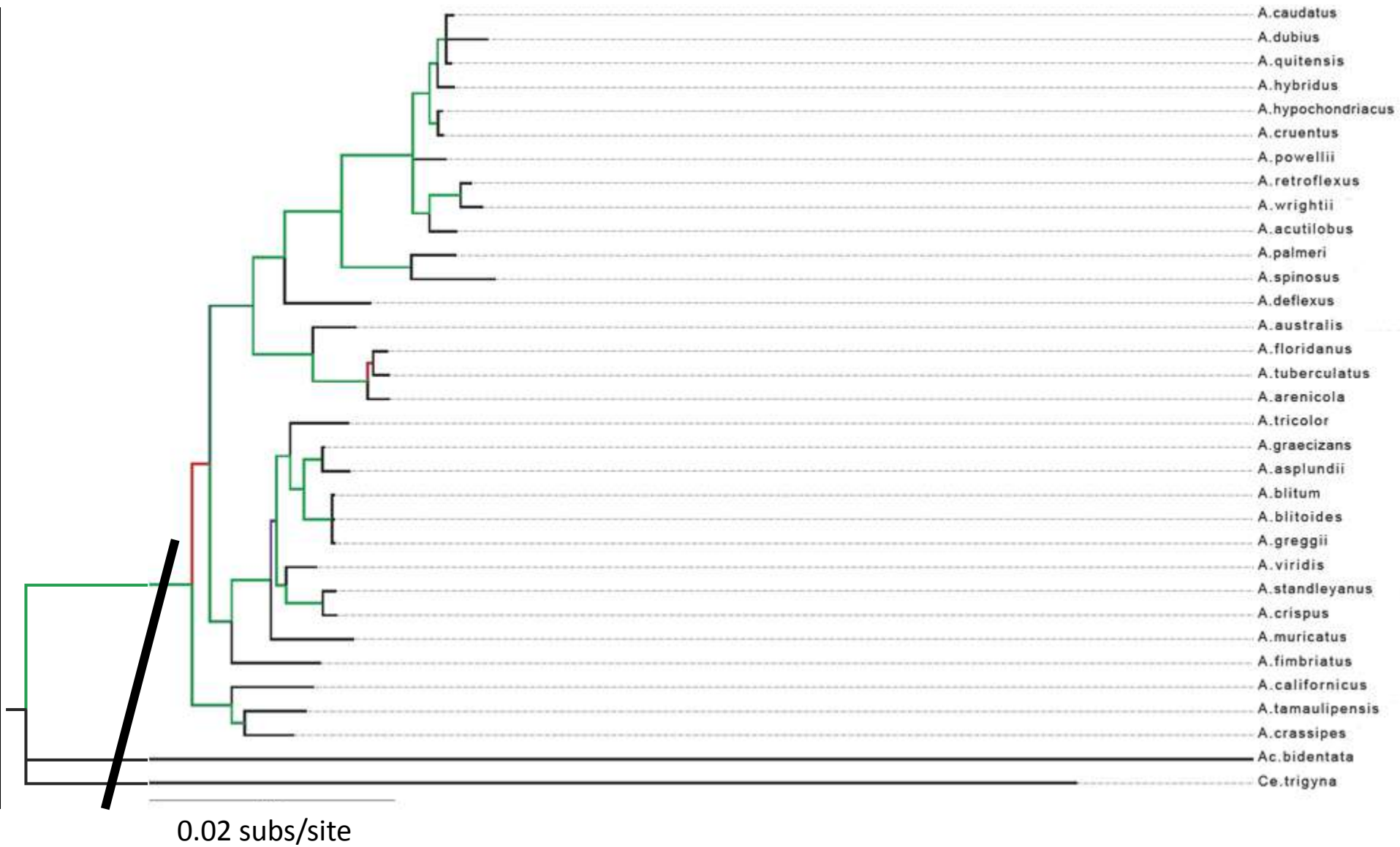




Posterior probabilities:



...in progress



Posterior p

1.0 0.9

0.7

0.6

*...in progress*

# Best fit phylogeny, 32 species

MrBayes

Mutation model: GTR with G

Generations = 2,000,000

6 Concatenated genes

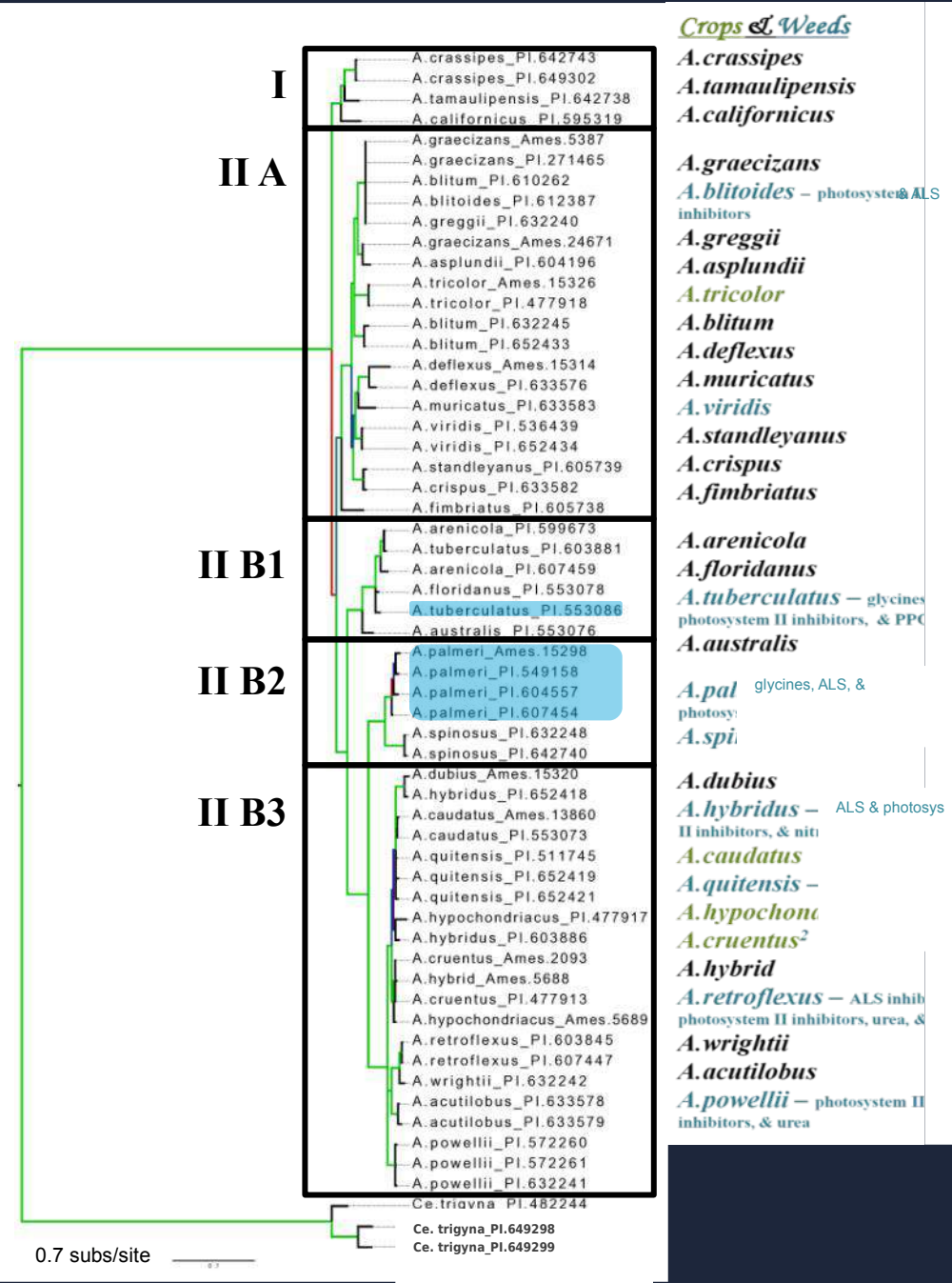
Scale bar = 0.7 substitutions/site

SNPs Phased or assigned ambiguity codes

InDel Variation resolved via sequenced clones

GTR with gamma distribution

...in progress



Posterior



# Genus Amaranthus

Locally adapted crop (?)

Weed: unintended during cultivation



<http://pizzagov.com/wp-content/uploads/2010/01/amaranth.jpg>



<http://permaculture.org.au/2016/08/17/gm-crops-facing-meltdown-in-the-usa/>



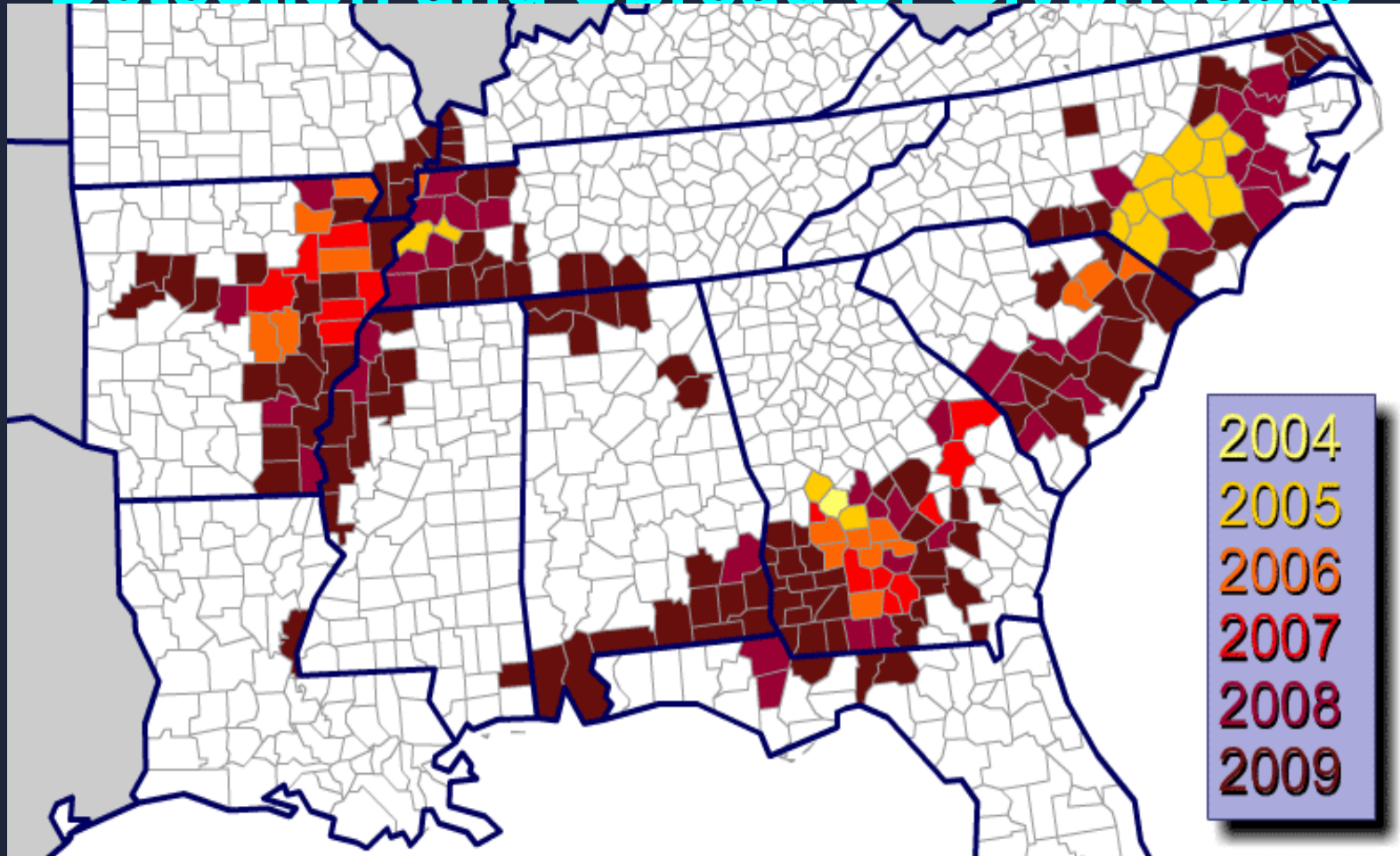


# (Glyphosate) Herbicide SENSITIVE Palmeri

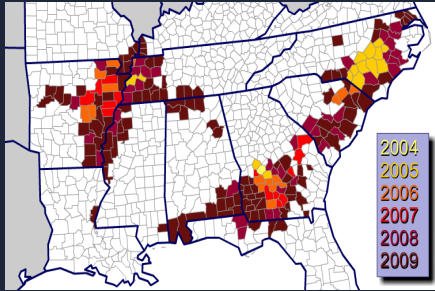


<http://vernon.tamu.edu/HOMEPAGE/TBAUGHMA/images/CottonField-01.jpg>

# Detection and Spread of Glyphosate







# Strategies for weed control

preventative measures  
(seed laws, equipment sanitation)

deplete the seedbank

reduce emergence by water management

targeted removal of individuals from the crop

crop rotation or fallowing

herbicide resistant crop systems  
(eg. RoundUp-Ready, glyphosate)

# Other glyphosate-insensitive species

October 2010

Resistant = Insensitive

***Ambrosia artemisiifolia*** (USA)

***Ambrosia trifida*** (USA)

***Conyza bonariensis*** (South Africa, Spain, Brazil, Colombia, USA)

***Conyza canadensis*** (Brazil, USA, China, Spain, Czech Repl.)

***Conyza sumatrensis*** (Spain)

***Digitaria insularis*** (Paraguay, Brazil)

***Echinochloa colona*** (Australia)

***Eleusine indica*** (Malaysia, Colombia, Philippines)

***Euphorbia heterophylla*** (Brazil)

***Kochia scoparia*** (USA)

***Lolium multiflorum*** (Chile, Brazil, Spain, Argentina, USA)

***Lolium perenne*** (Argentina)

***Lolium rigidum*** (Australia, South Africa, France, Spain, Italy, USA)

***Parthenium hysterophorus*** (Colombia)

***Plantago lanceolata*** (South Africa)

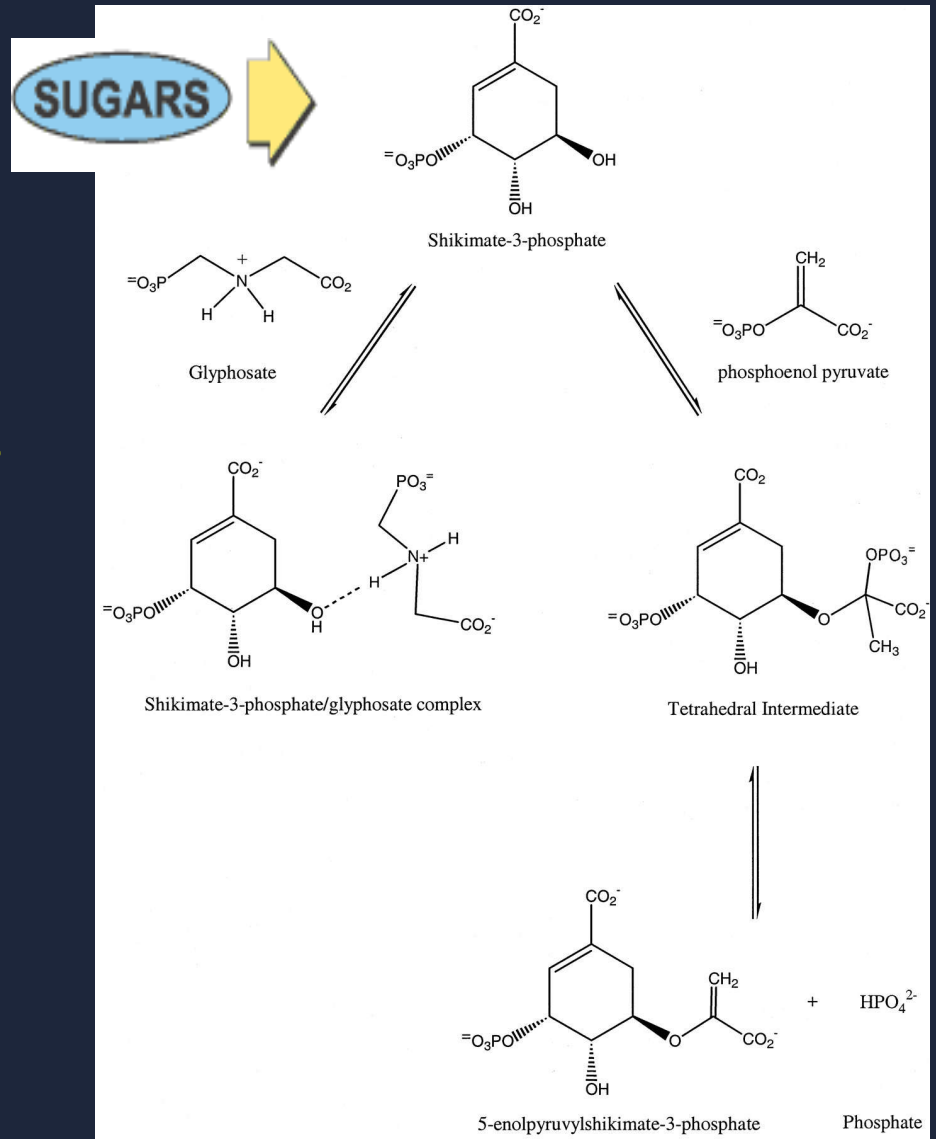
***Sorghum halepense*** (Argentina, USA)

***Urochloa panicoides*** (Australia)

# EPSPS:

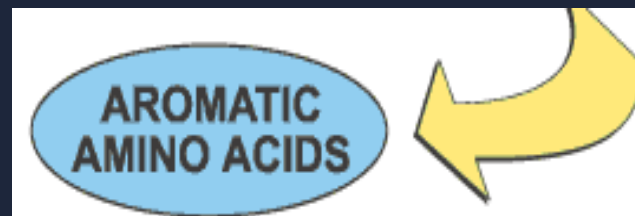
## 5-enol pyruvylshikimate-3-phosphate synthase

### Applied glyphosate competes with EPSPS



Alibhai M F , Stallings W C PNAS 2001;98:2944-2946

Phenylalanine  
Tyrosine  
Tryptophan

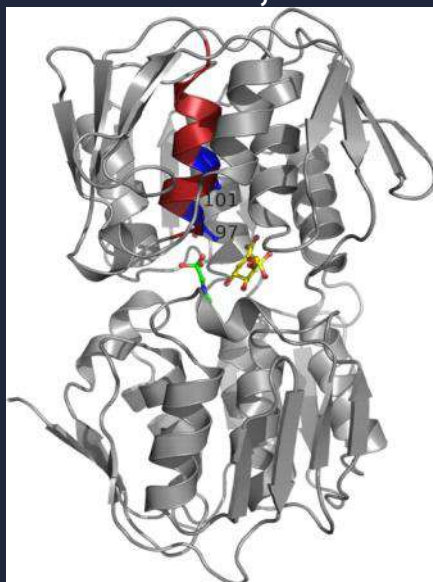




# Glyphosate resistance, possible mechanisms

- 1.) **EPSPS GM sites for 'RoundUp-Ready' (Thr97, Pro101)**  
5-enol pyruvylshikimate-3-phosphate synthase
- 2.) **Proliferation of EPSPS gene copy number**  
Gaines T A et al. PNAS 2010;107:1029-1034
- 3.) **Independent mechanism(s), other genes...?**

Thr97 and Pro101 **Other herbicides commonly applied**  
WT EPSPS, *E. coli*



Funke T et al. J. Biol. Chem. 2009

Foliar herbicides  
PS II inhibitors  
Shoot growth inhibitors  
Soil-applied herbicides  
Root growth inhibitors

# Resistant and Sensitive Pilot Populations: Genetic Variation, EPSPS

7 plants sampled from each population (*Amaranthus palmeri*)

Accession#	Location
PI549158	Mali, Africa
PI604557	Puebla, Mexico
PI633593	Colima, Mexico
(PI633587	Senegal, Africa)
Ames15298	Arizona, United States
PI607451	Kansas, United States
PI607452	Kansas, United States
PI607454	Kansas, United States
PI607455	Kansas, United States
PI607456	Kansas, United States
PI607457	Kansas, United States
PI607461	Kansas, United States
PI612856	Arizona, United States
PI632235	Arizona, United States
PI632236	Arizona, United States



Resistant (GR)  
Susceptible  
(CL)

# Resistant and Sensitive Pilot Populations: Genetic Variation, EPSPS

7 plants sampled from each population (*Amaranthus palmeri*)

Genetic diversity:  
in susceptible popn

Sampling sites:  
in susceptible popn

Relative mutation rates:  
(0.20)



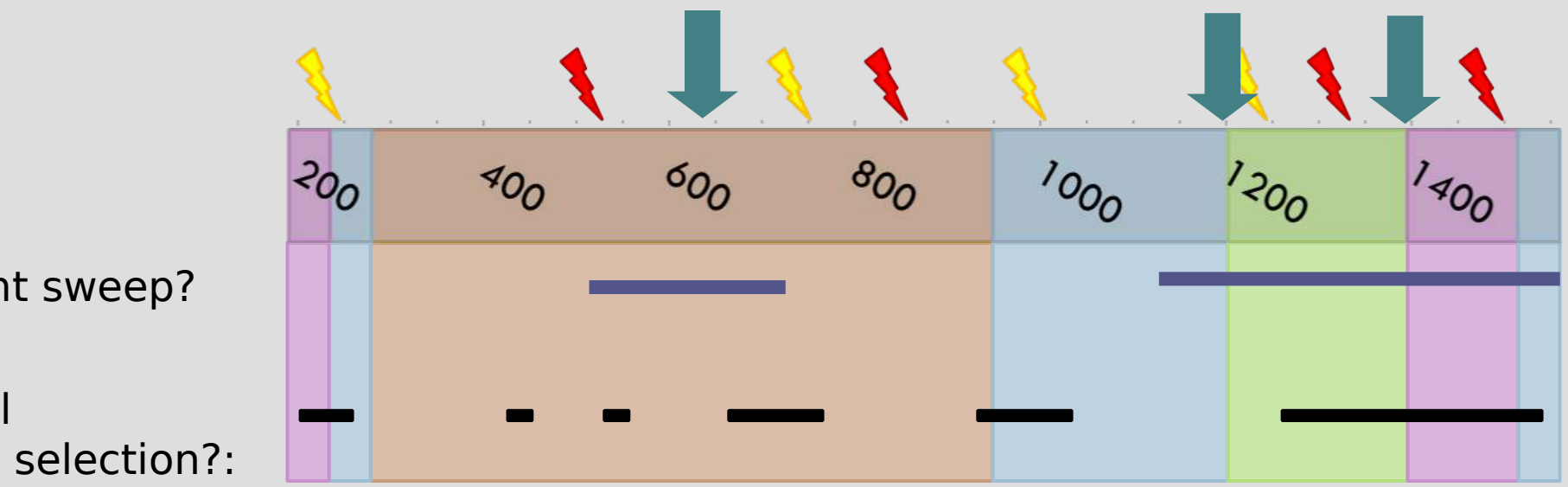
Resistant (GR)  
Susceptible  
(CL)

# EPSPS: Purifying Selection 'Baseline' with Recent Selective

GR  $\pi_A/\pi_S = 0$

## Sweep in GR?

High  $K_a/K_s$





# Differences in genus *Amaranthus*: Phylogenetic history and adaptation

- **Contemporary Processes**  
*A. palmeri* (*A. tuberculatus*, *A. spinosus*)
  - resistant vs. sensitive populations
  - shared ancestral vs. independent variants (gene flow, or private)
    - demography vs. selection
- **Molecular Evolution (candidate genes)**
  - *Amaranthus palmeri*
  - genus-wide 'weeds', 'types'
  - phylogeny (ancestral vs. derived adaptations, crop origins)
  - among species: crop history (support cultural records?)
- **Model Testing and Hypotheses**
  - cultivation practices, management strategies, geography, expanded species distributions

# of Amaranthus research in the lab

- 
- 
- 
- 
- 
- 
- 
- 
- 
- 
- 
- 
- 
- 



of Amaranthus research in the lab

# International Fellowship 2012..... Host? Dormancy

- 
- 
- 

