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6. Colorado Department of Agriculture
7. Colorado Plateau Research Station,
Northern Arizona University

Historically isolated tamarisk beetle species meet again on the Rio Grande & Pecos River

2019 Riparian Restoration Conference
February 6

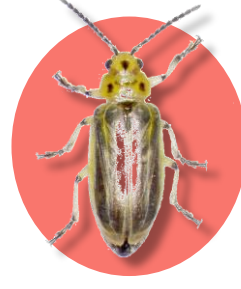
Six source populations of *Diorhabda* spp. have been introduced since 2001



D. sublineata



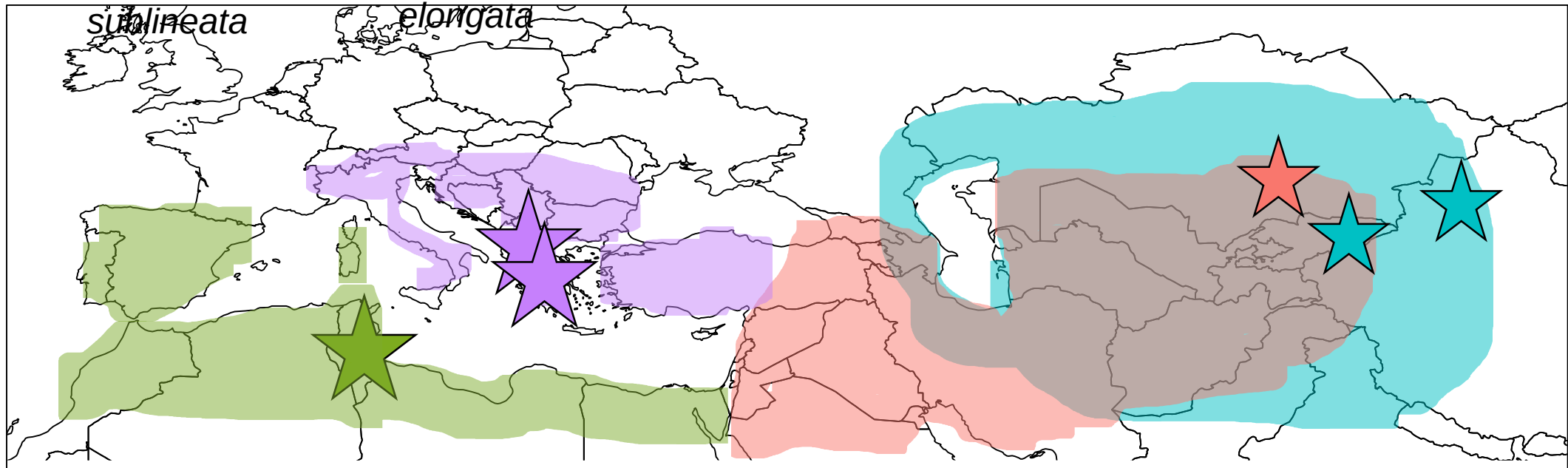
D. elongata



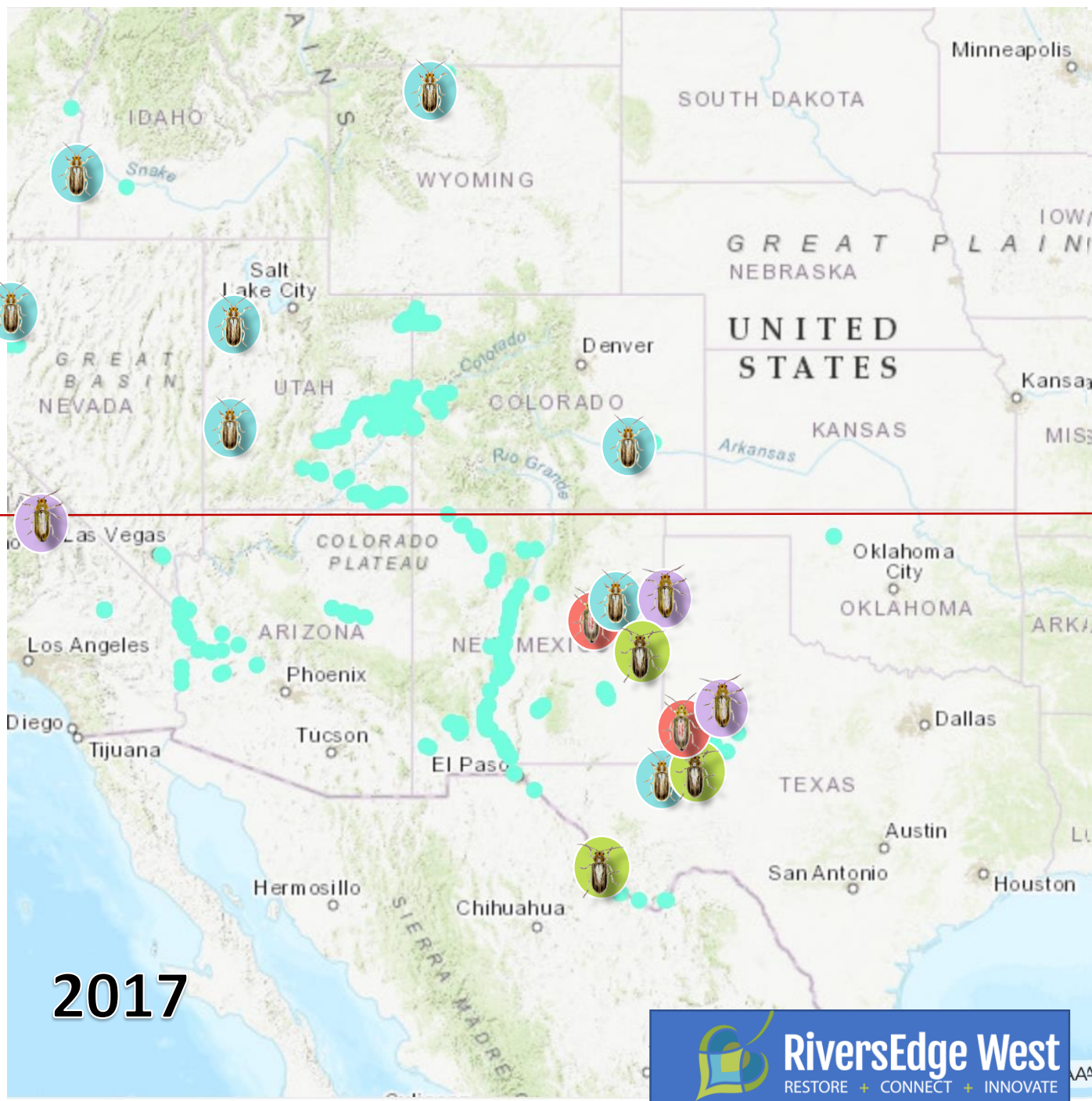
D. carinata



D. carinulata



Adapted from Bean, Kazmer, Garndner, Thompson, Reynolds, Keller, Gaskin (2013).



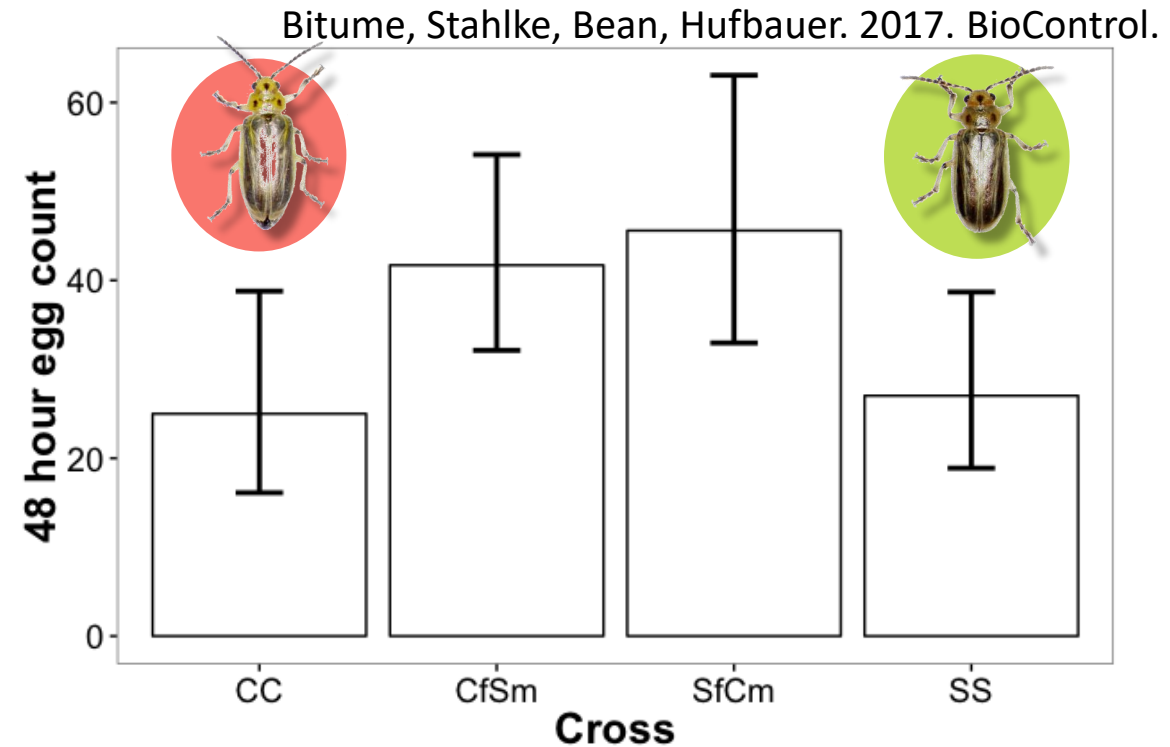
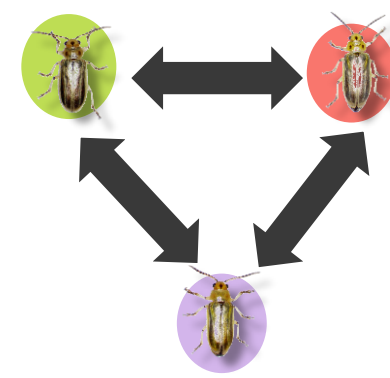
Where are these historically isolated lineages now?

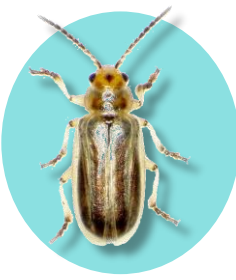
Will range expansion and species interactions impact safety or efficacy?

How can we use genetics/genomics to track and predict *Diorhabda* spp?

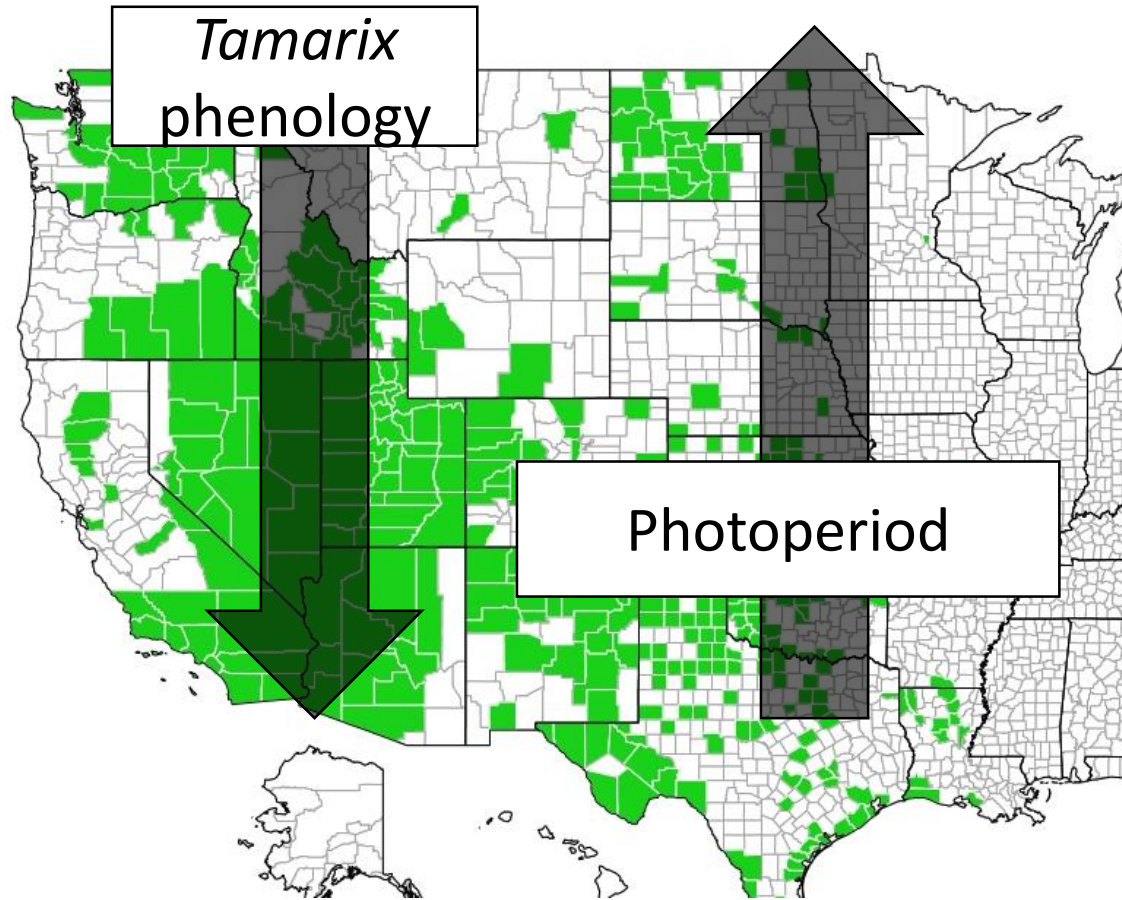
Hybridization may affect **safety** and **efficacy** in biological control

- Big boost in genetic diversity may increase likelihood of establishment.
- Novel genetic combinations in a novel environment could yield transgressive phenotypes.
- Hybrids could be sterile, ineffective

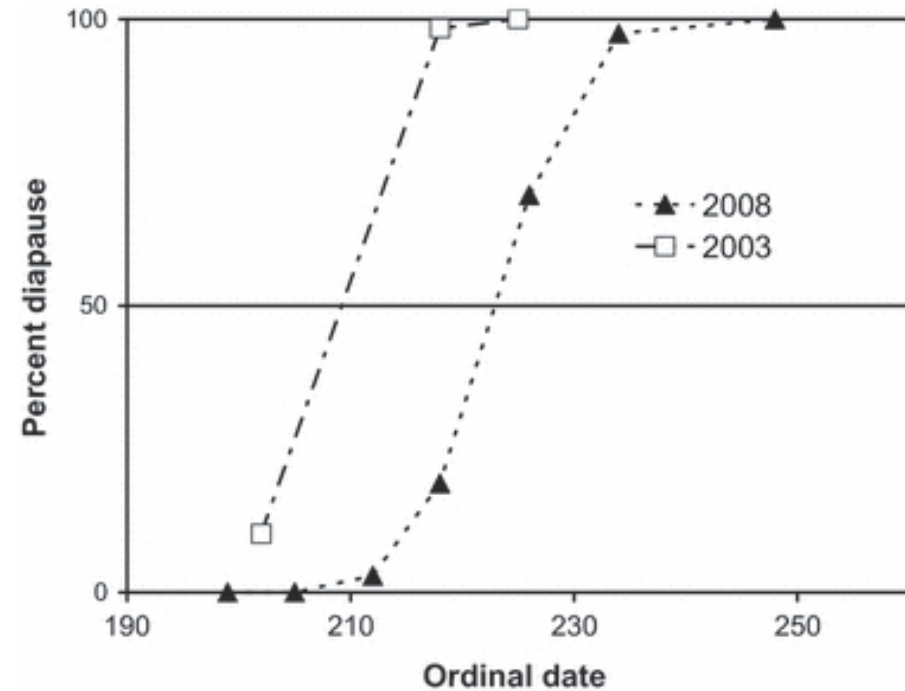




Range expansion enabled in part by evolution in diapause response.



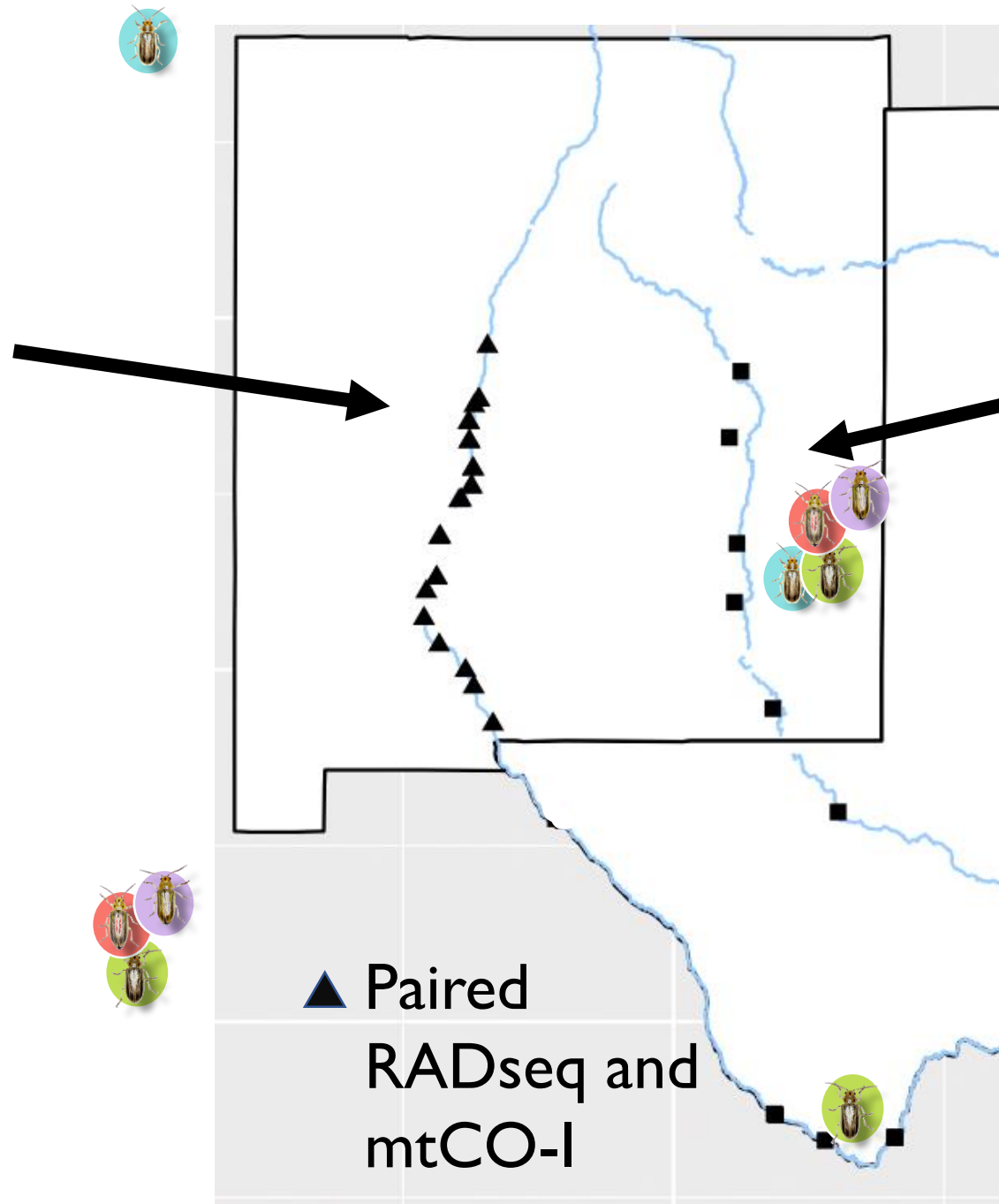
Rapid evolution of phenology



Bean, Dalin, Dudley. 2012. Evo. App.

Rio Grande

Tracking
population
dynamics over
a full season



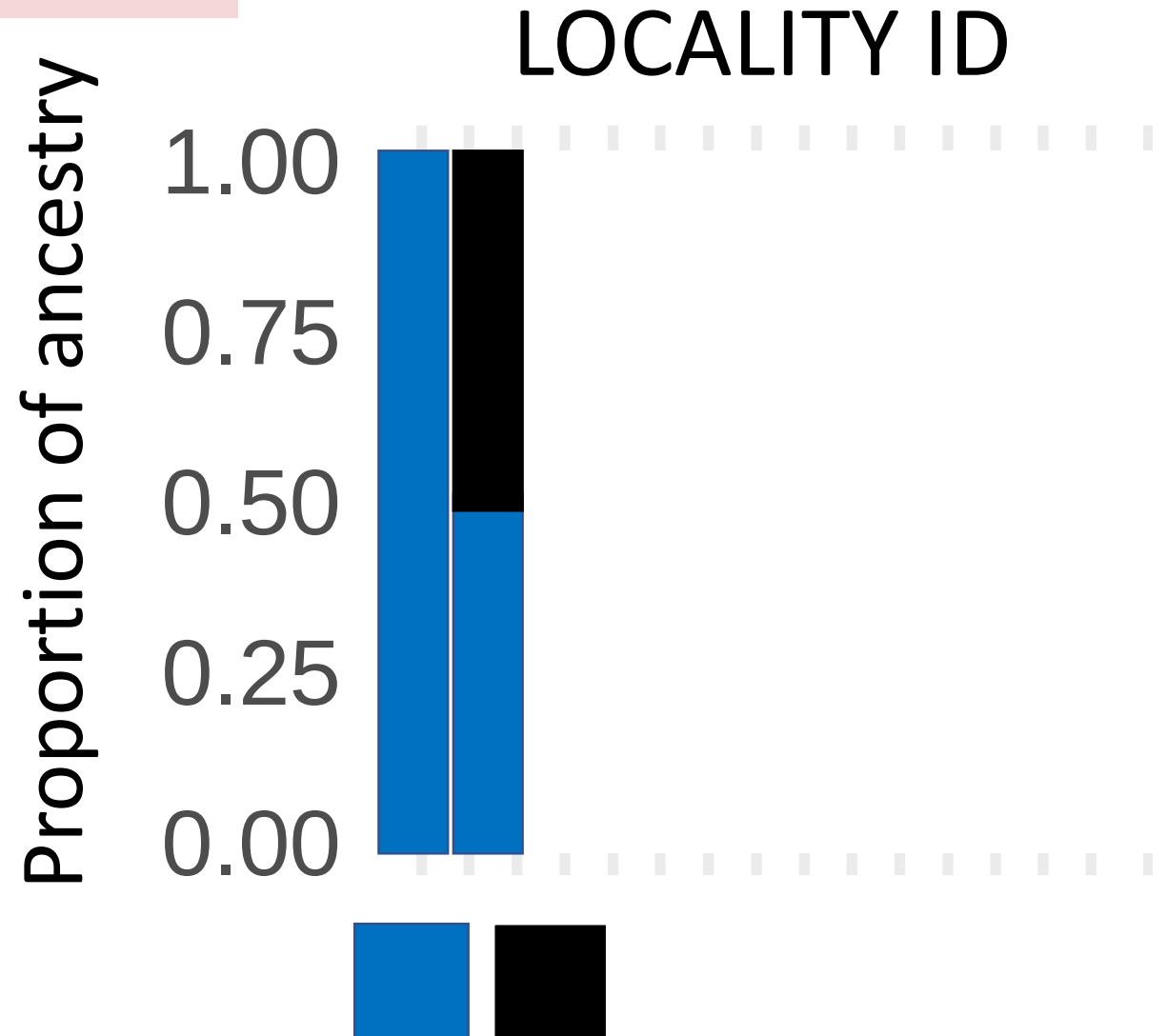
Pecos River

Part of a large
project to
broadly
characterize
hybridization
and population
structure

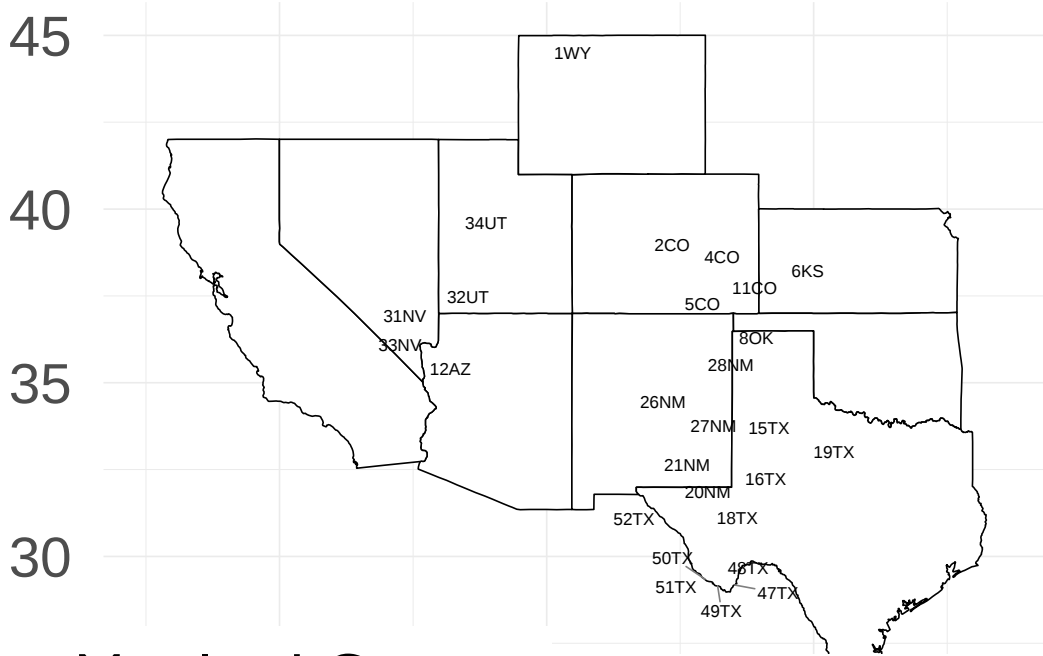
Genomic ancestry assignment

RADseq: Fast, relatively cheap, flexible, molecular method to assess **genomic variation**
9,125 SNPs

STRUCTURE, a Bayesian clustering algorithm
Probability($X|K$)



Southwest United States

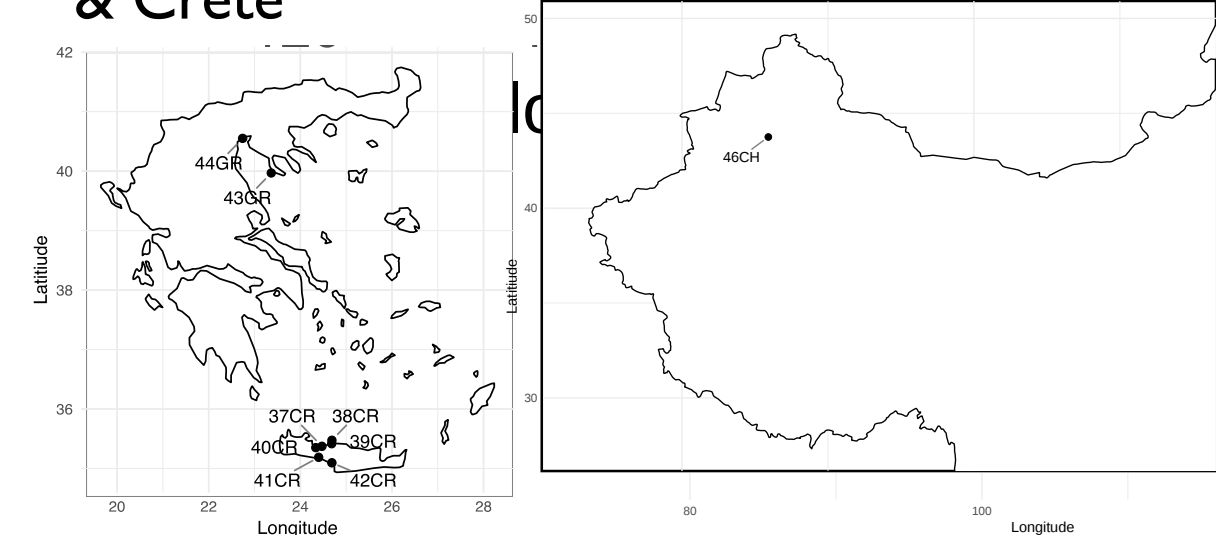


Collected field samples from lab cultures and over 50 sites within introduced and native range



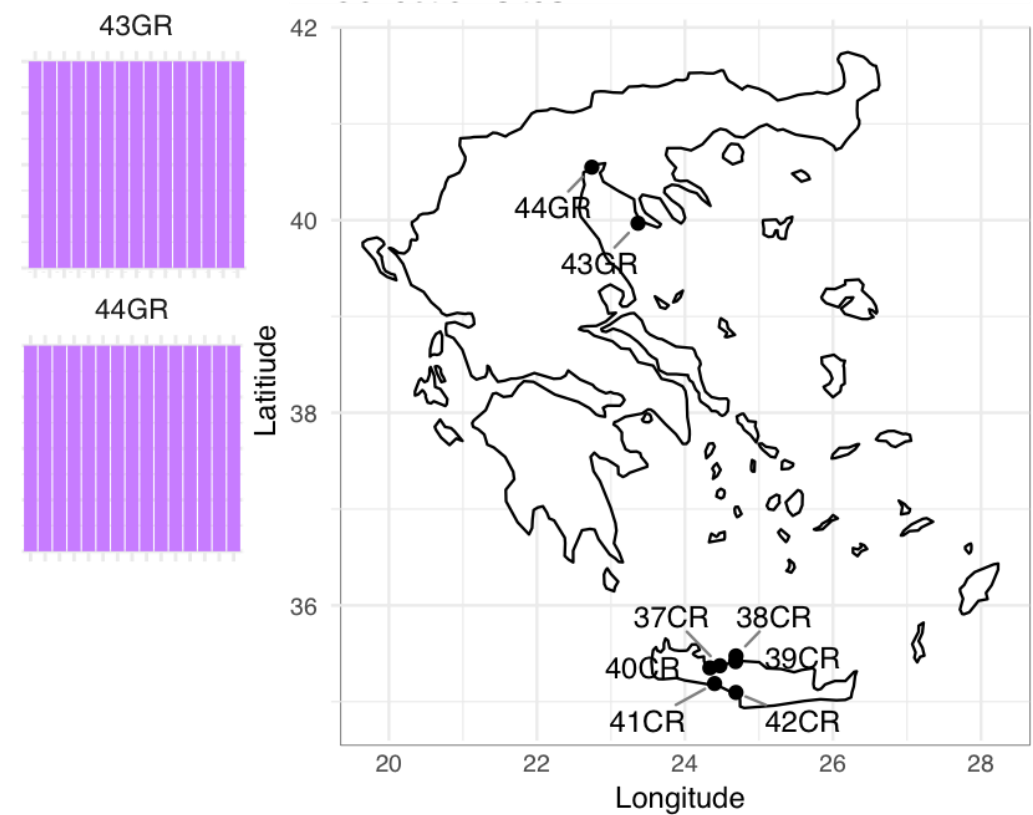
Mainland Greece & Crete

Xinjiang, China

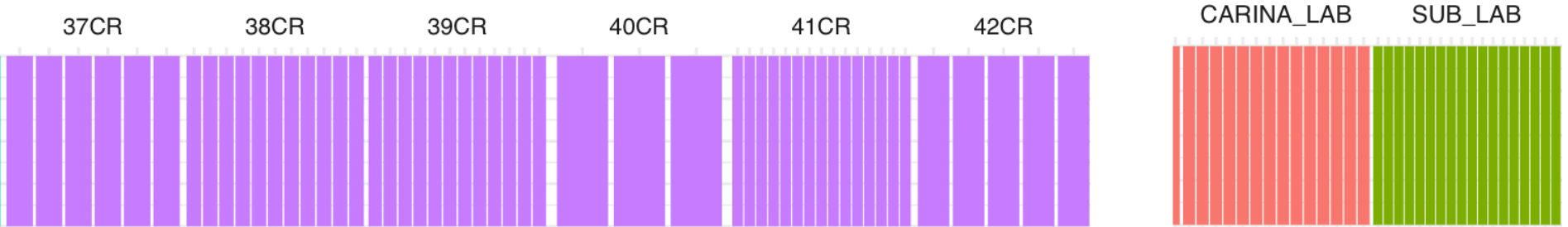
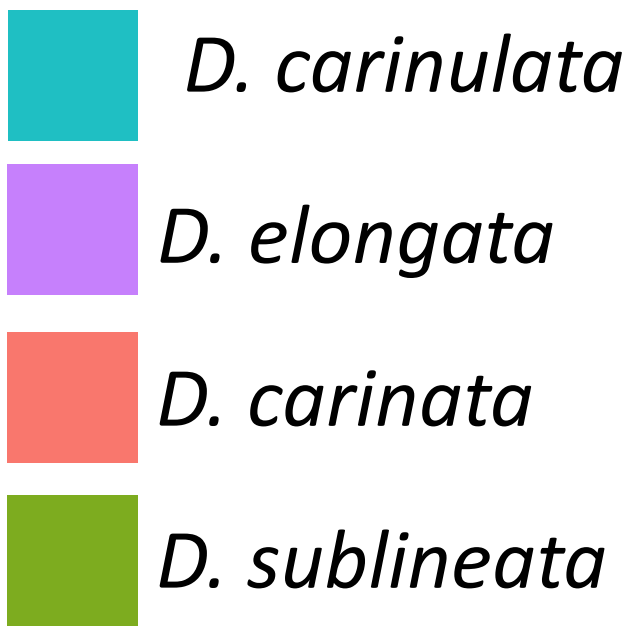
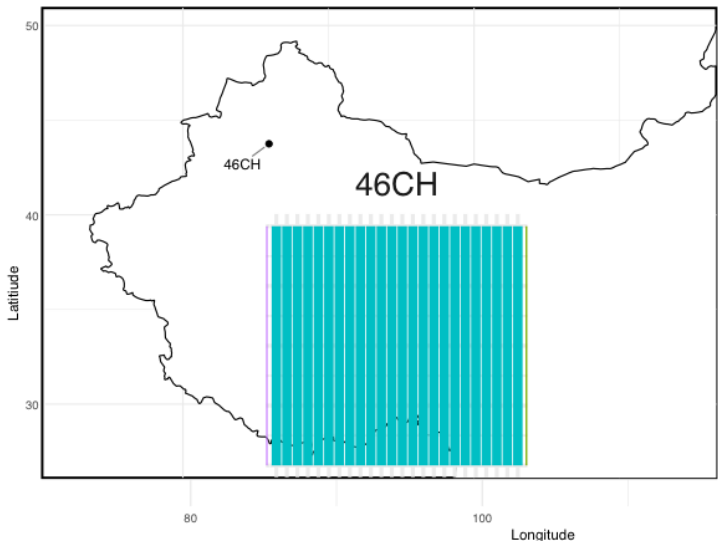


No hybridization was detected in the native range or lab.

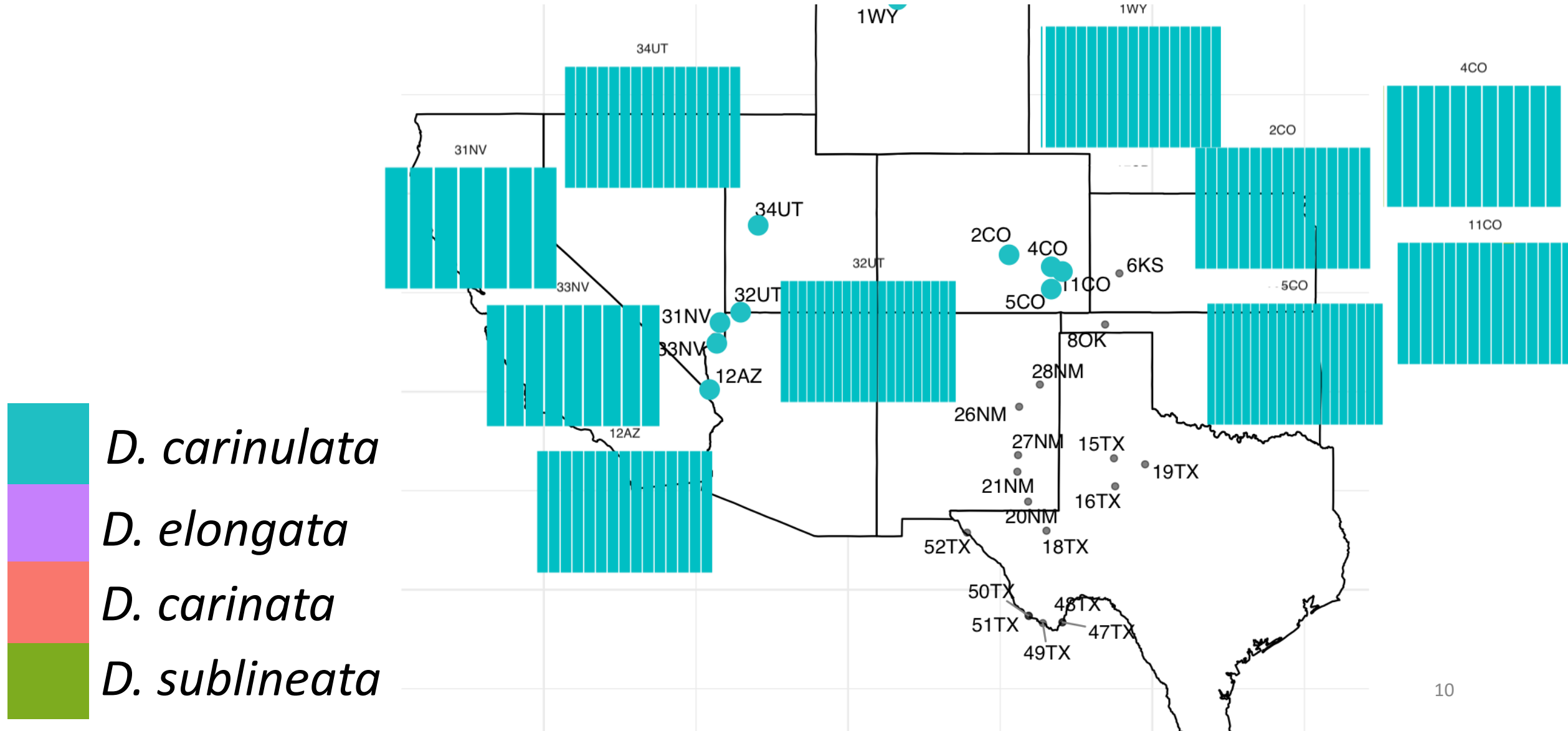
Mainland Greece & Crete



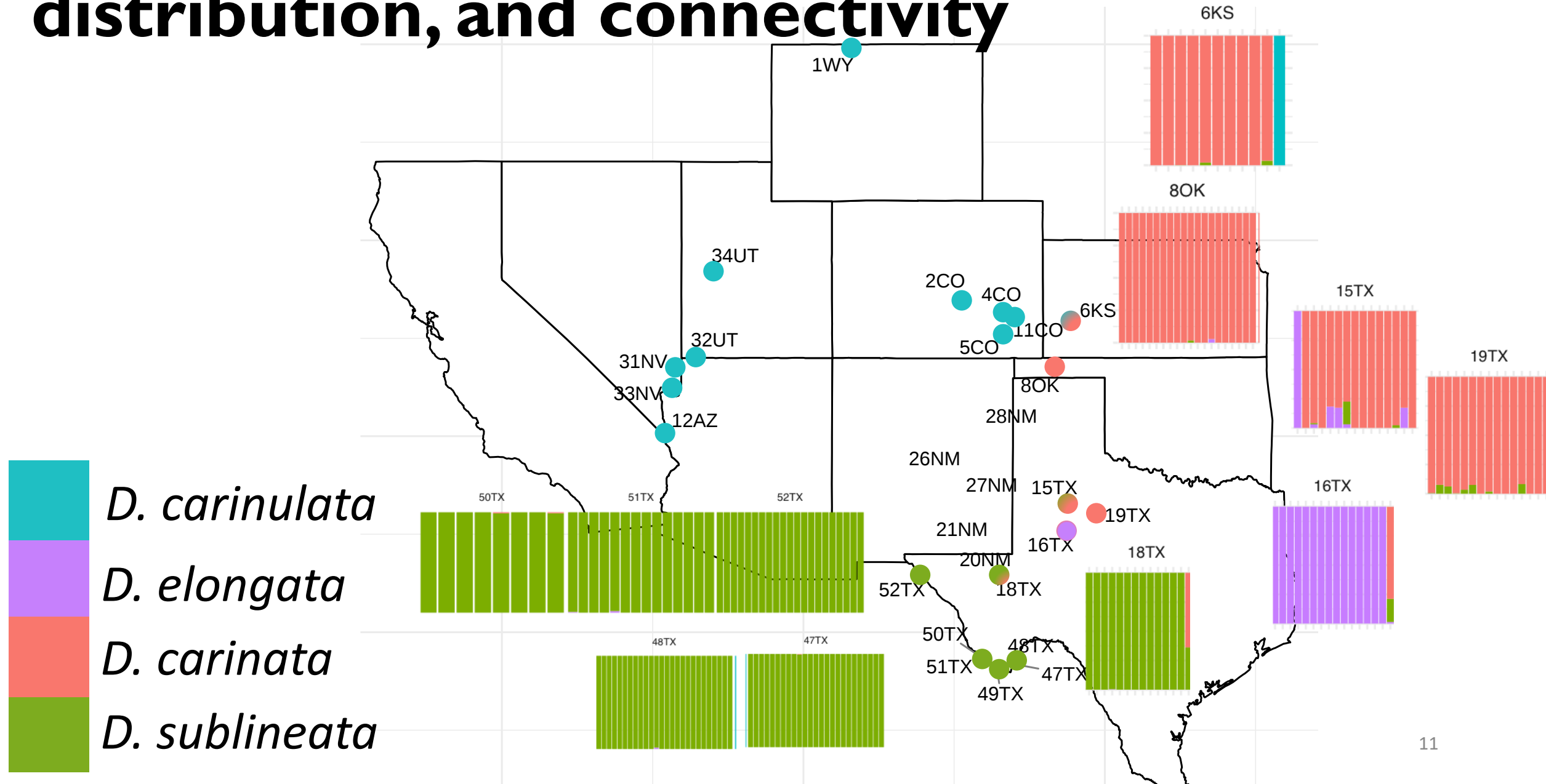
Xinjiang, China



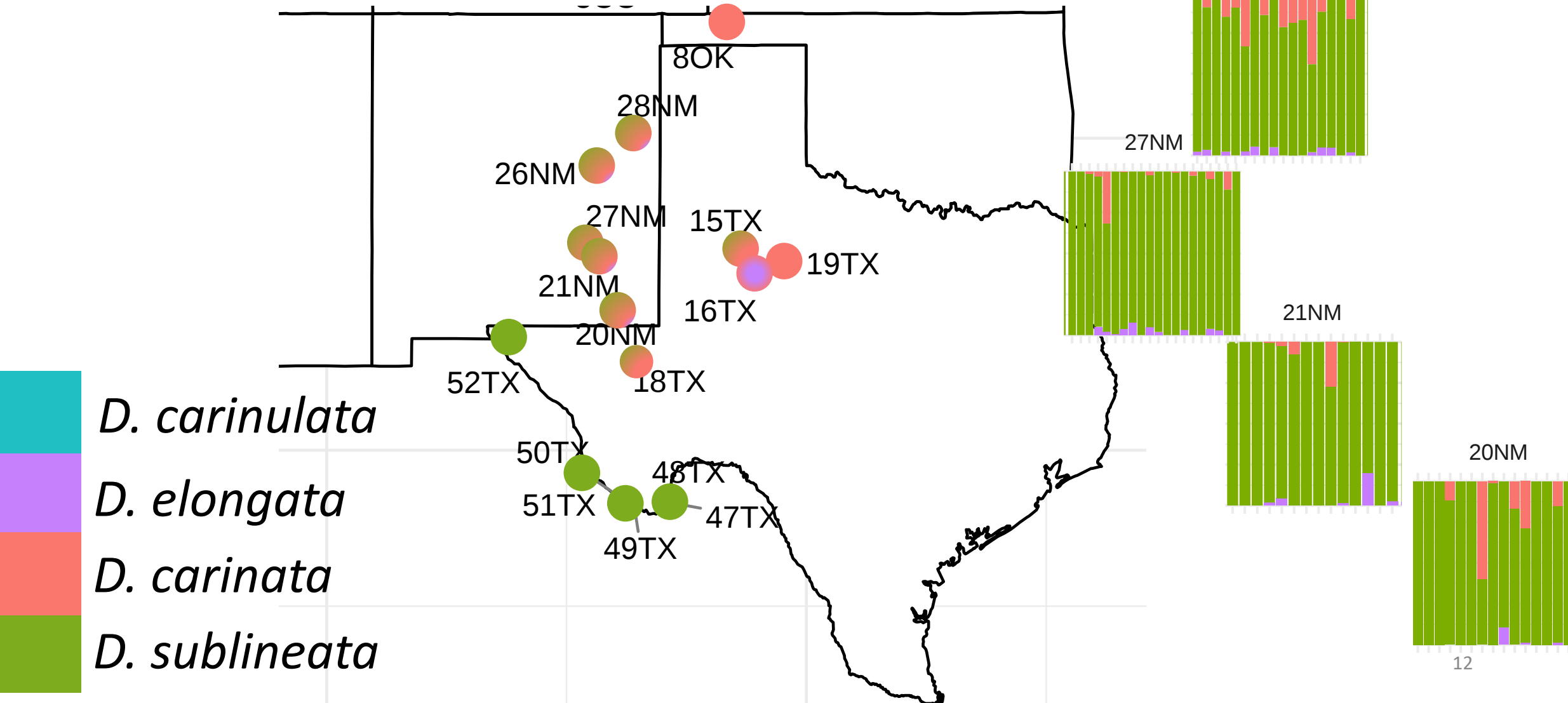
Ancestry assignment reflects introduction, re-distribution, and range expansion



Ancestry assignment reflects **introduction, re-distribution, and connectivity**



Ancestry assignment reveals widespread hybridization at contact zones



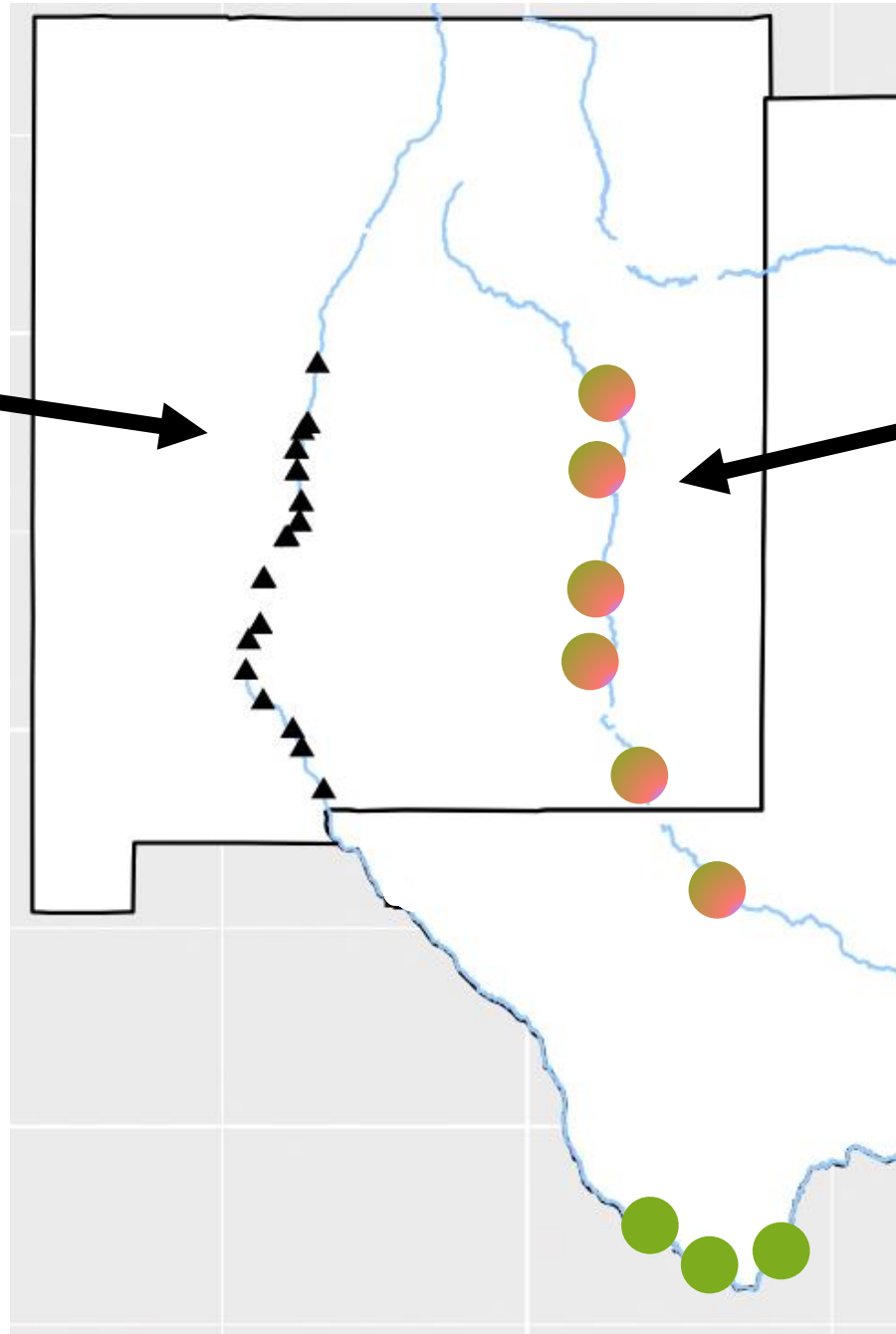


Rio Grande

Tracking
population
dynamics over
a full season



?



Pecos River

If present will
hybridize
Pure
populations
still existed on



Rio Grande

- Part of a focused effort to track range expansion and species interactions (Levi 2:00)
- 5 samples per site in May, July, and September
- Performed RADseq & sequenced mitochondrial marker COI

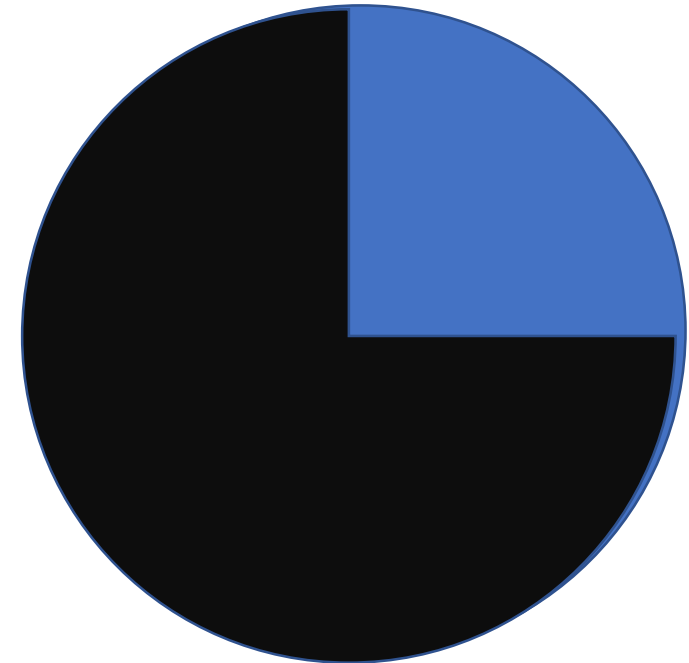
CO-I ancestry assignment

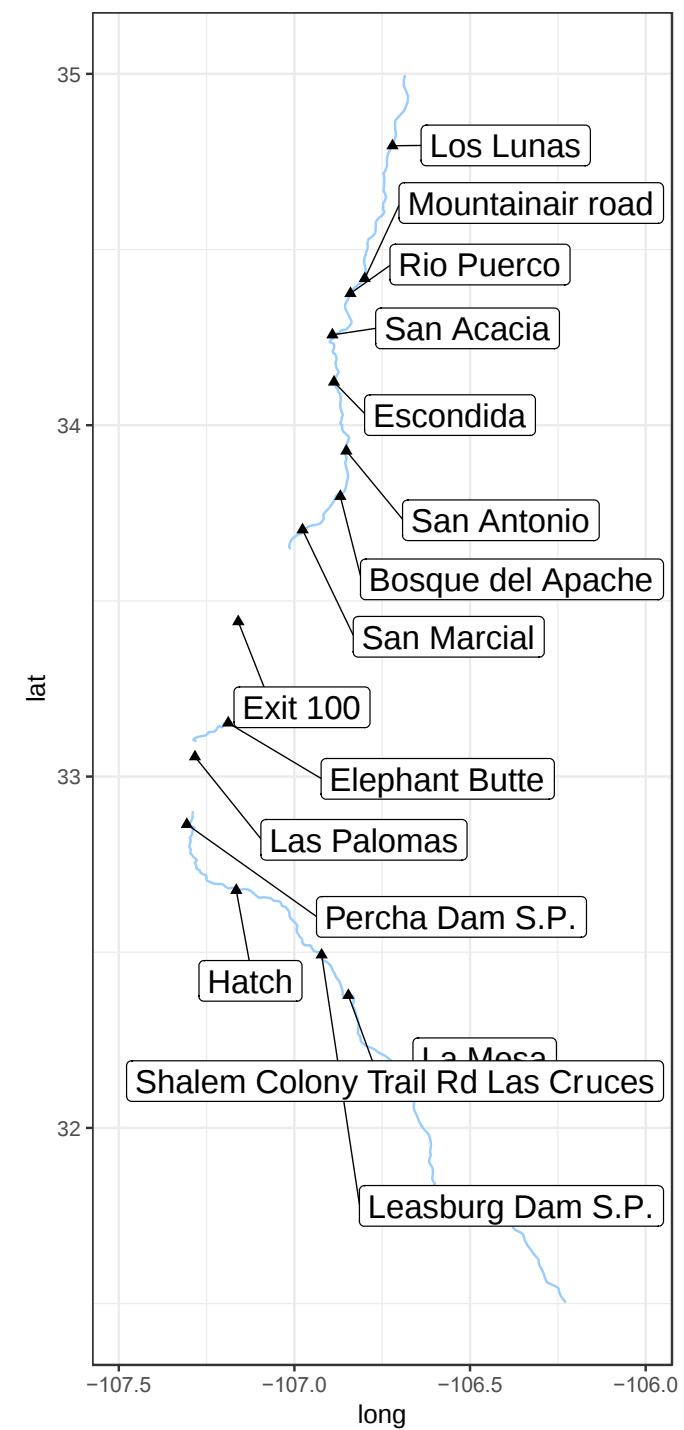
Mitochondrial marker
Cytochrome Oxidase subunit-I
1296 bp Sanger sequencing

Can handle more degraded
samples, single samples at a time

98% sequence homology with
morphologically IDed samples
(Bean et al 2013)

LOCALITY ID



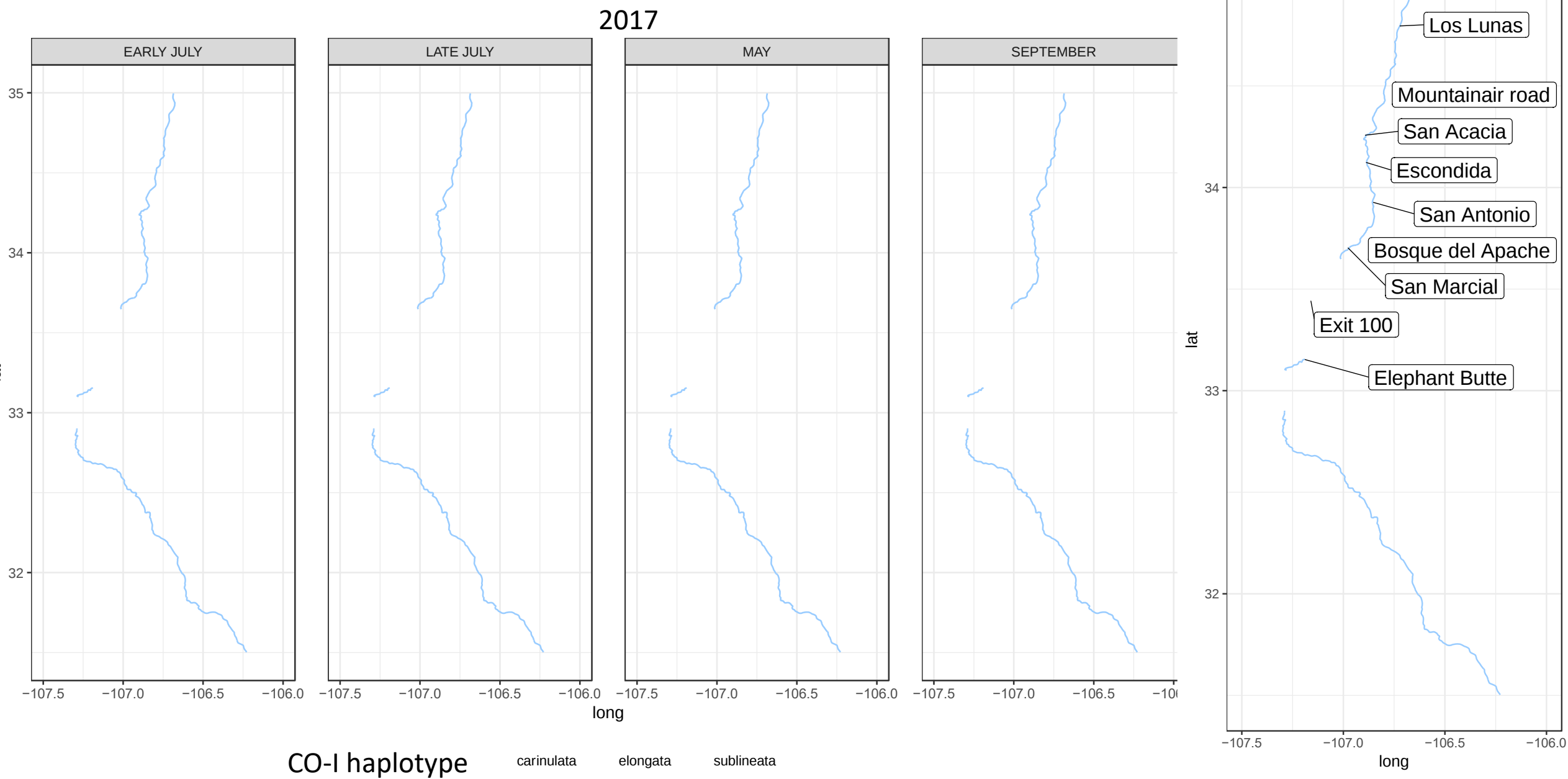


CO-I haplotype

carinulata

elongata

sublineata



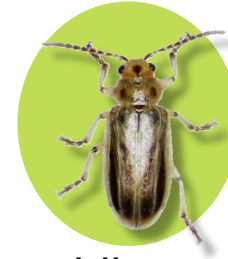
What's the best way to track *Diorhabda* spp?



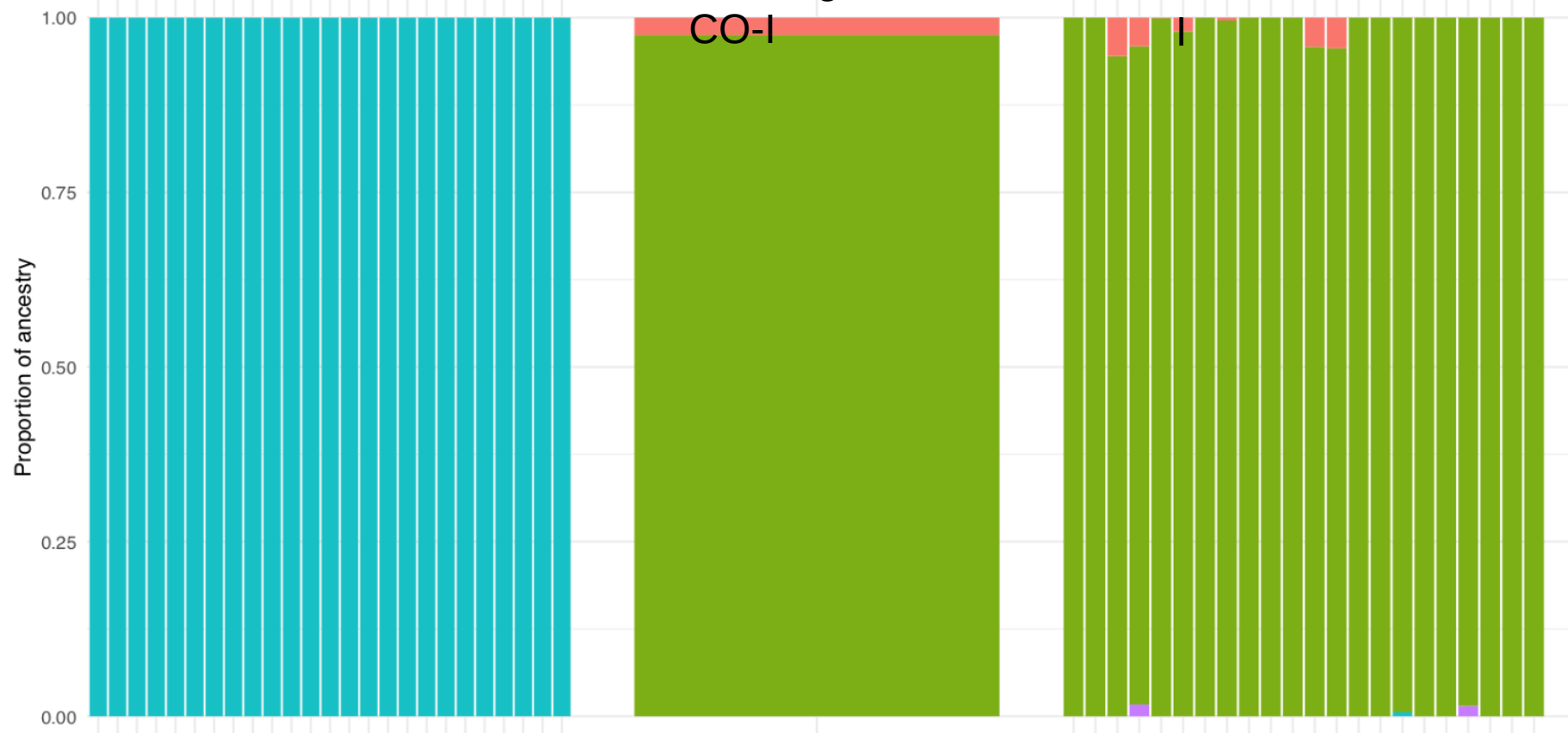
D. Carinulata CO-I



D. elongata
CO-I



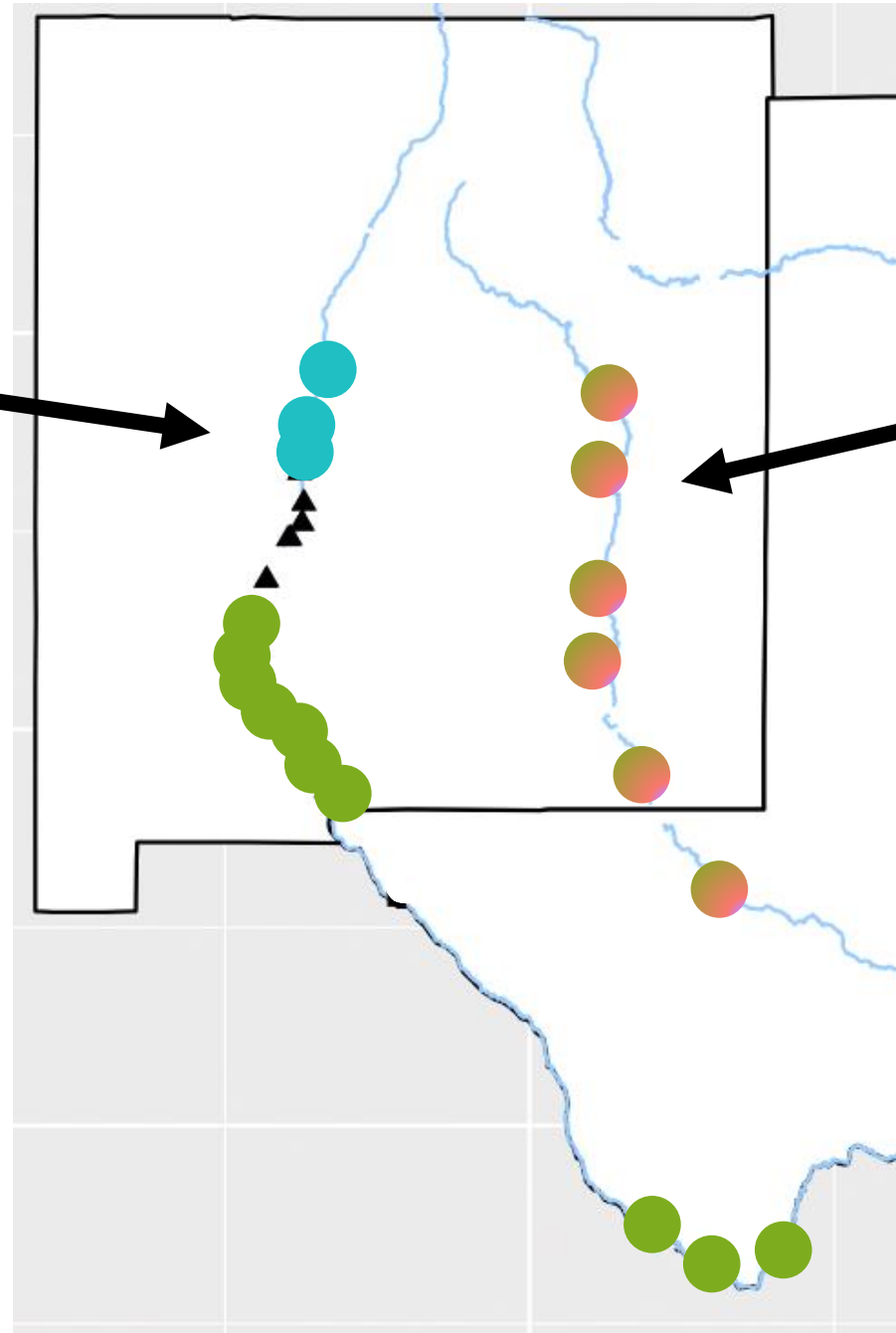
D. sublineata CO-I



Depends on your question...

Rio Grande

- Population composition changed rapidly over a season,
- But competition could slow range expansion
- CO-I remains useful for rapid ID



Pecos River

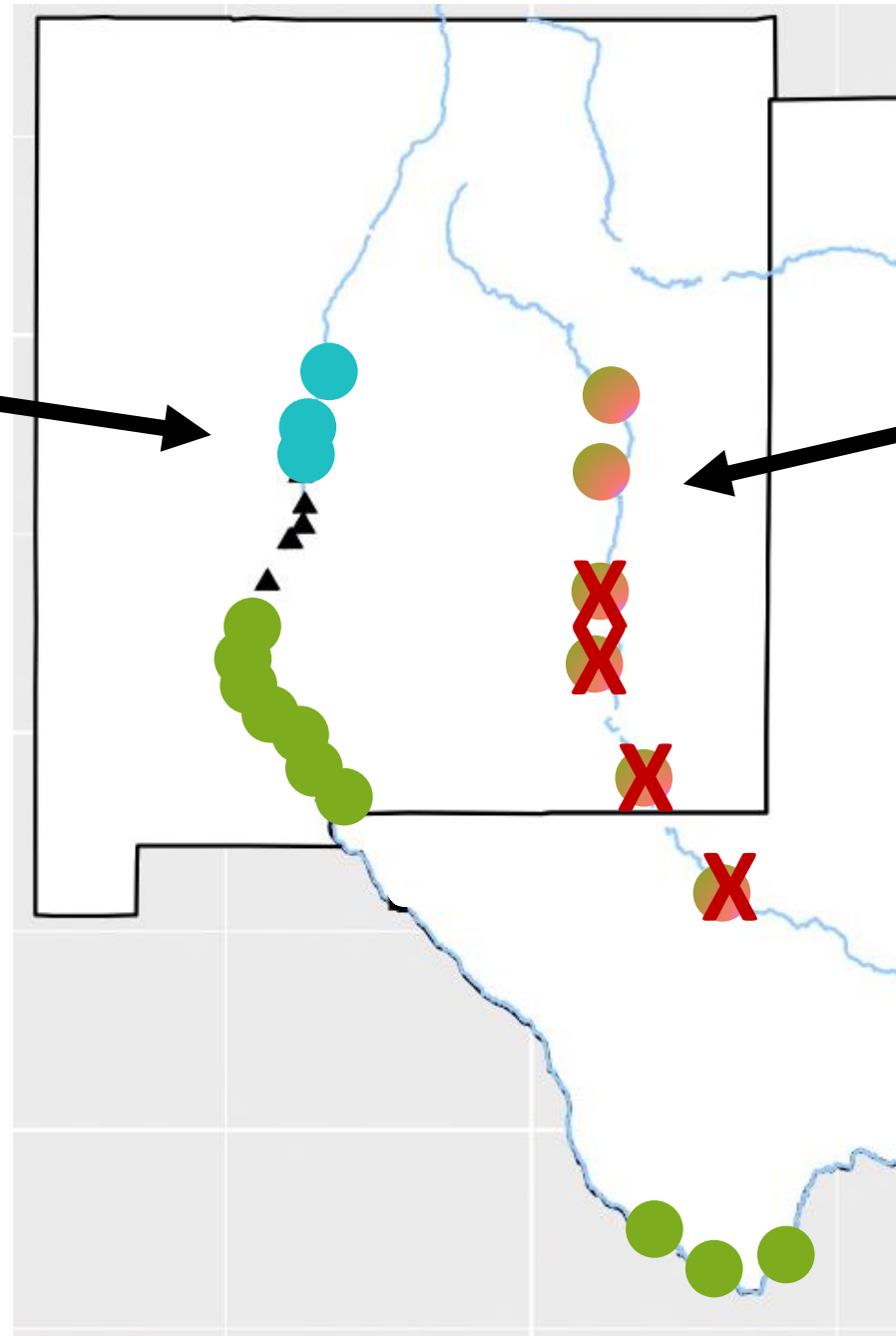
- If present, will hybridize
- Lack of hybrids elsewhere: strong role for connectivity? possible role for maladaptation?

Rio Grande

- Population composition changed rapidly over a season,
- But competition could slow range expansion
- CO-I remains useful for rapid ID

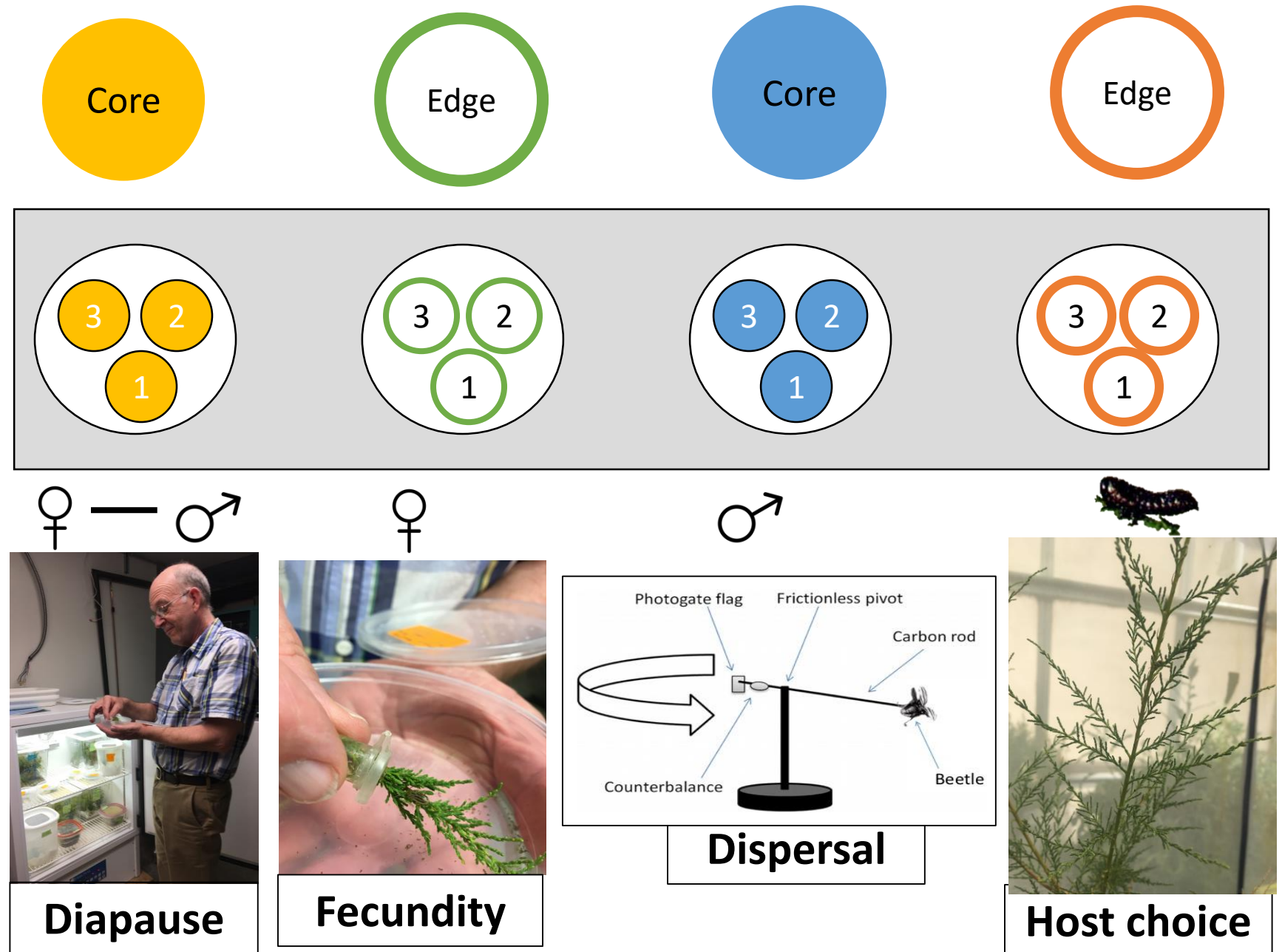
Pecos River

- If present, will hybridize
- Lack of hybrids elsewhere: strong role for connectivity? possible role for maladaptation?



Many hybrid populations seem to have disappeared

Phenotyping



Acknowledgments

USDA Agricultural Research Service

Natalie West

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Patrick Moran

Marie Claud Bon

René Sforza

Palisade Insectary (CO Dep. Ag.)

Karen Rosen

Nina Loudon

Sonya Daly

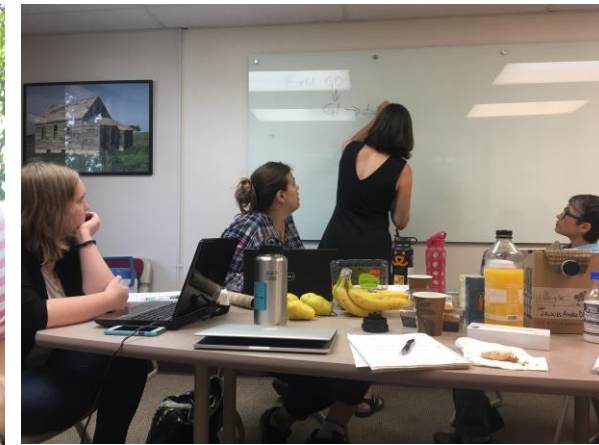
Beyond

Hohenlohe Lab

Tom Dudley

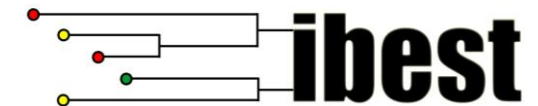
James Tracy

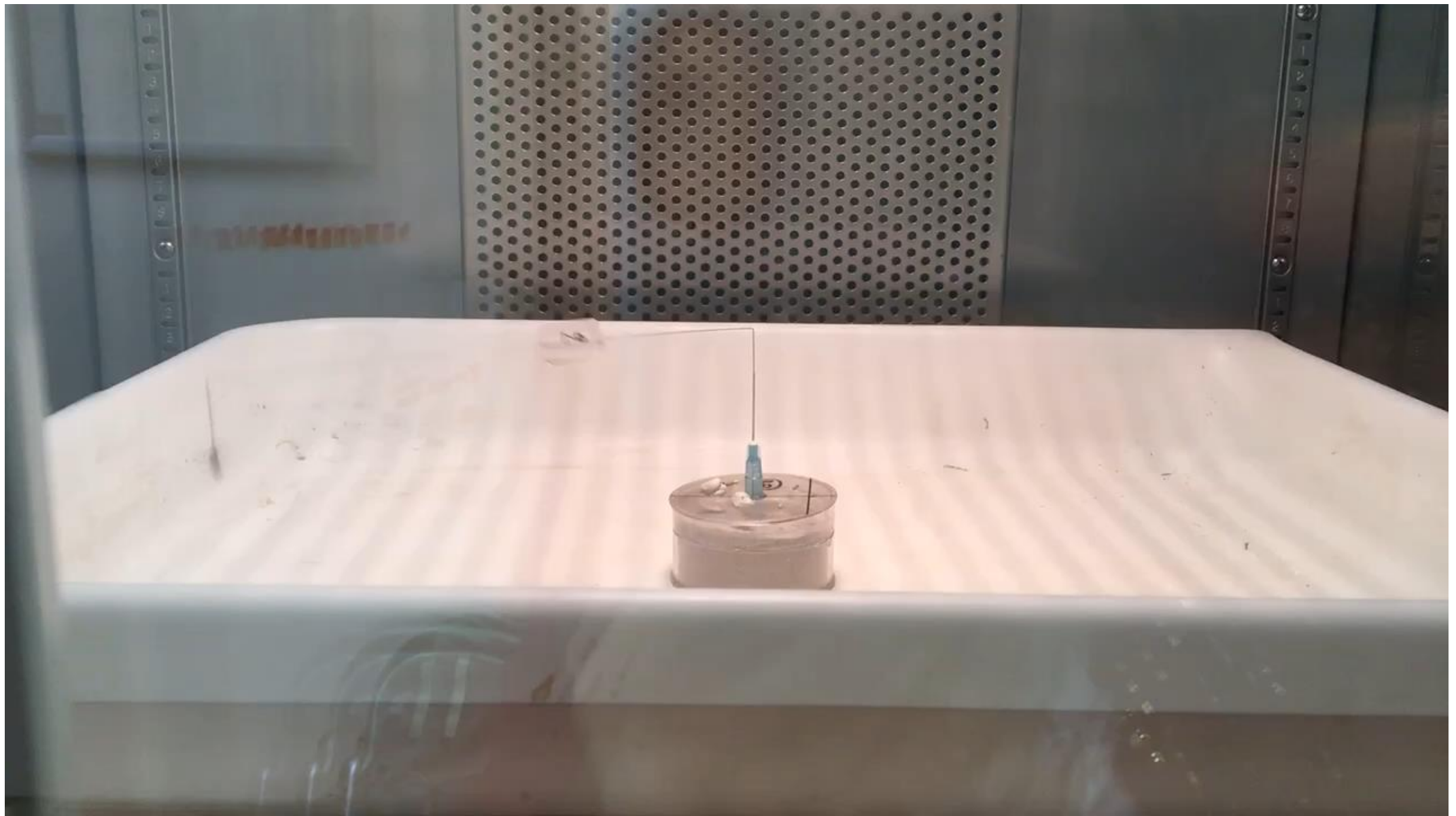
Massimo Cristofaro



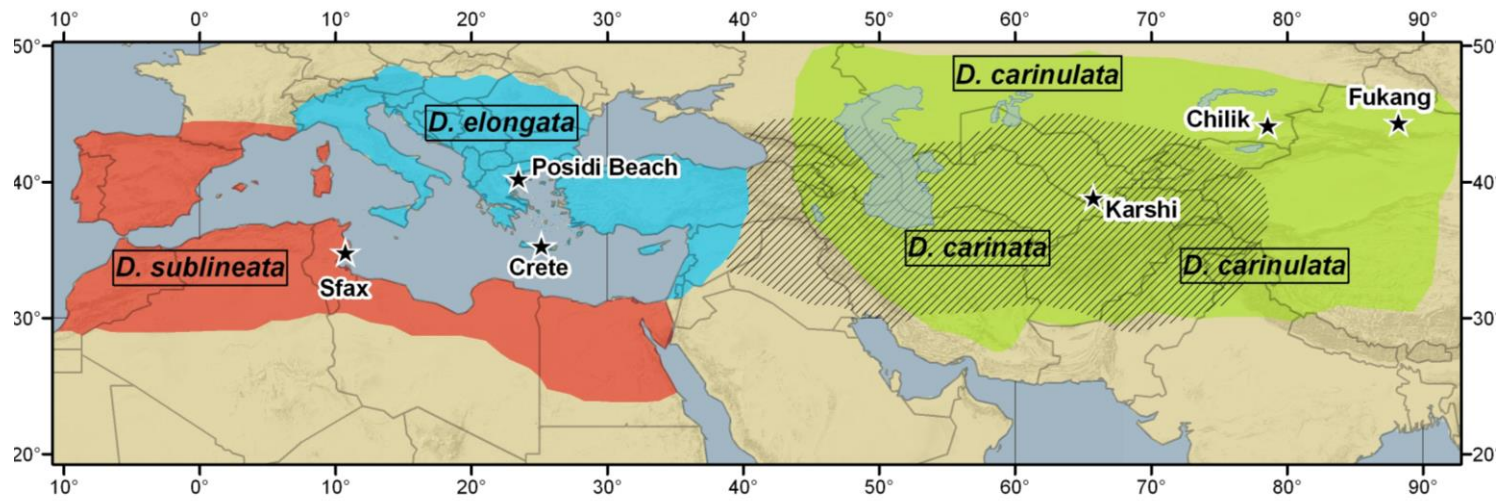
United States Department of Agriculture
National Institute of Food and Agriculture

USDA G-13528

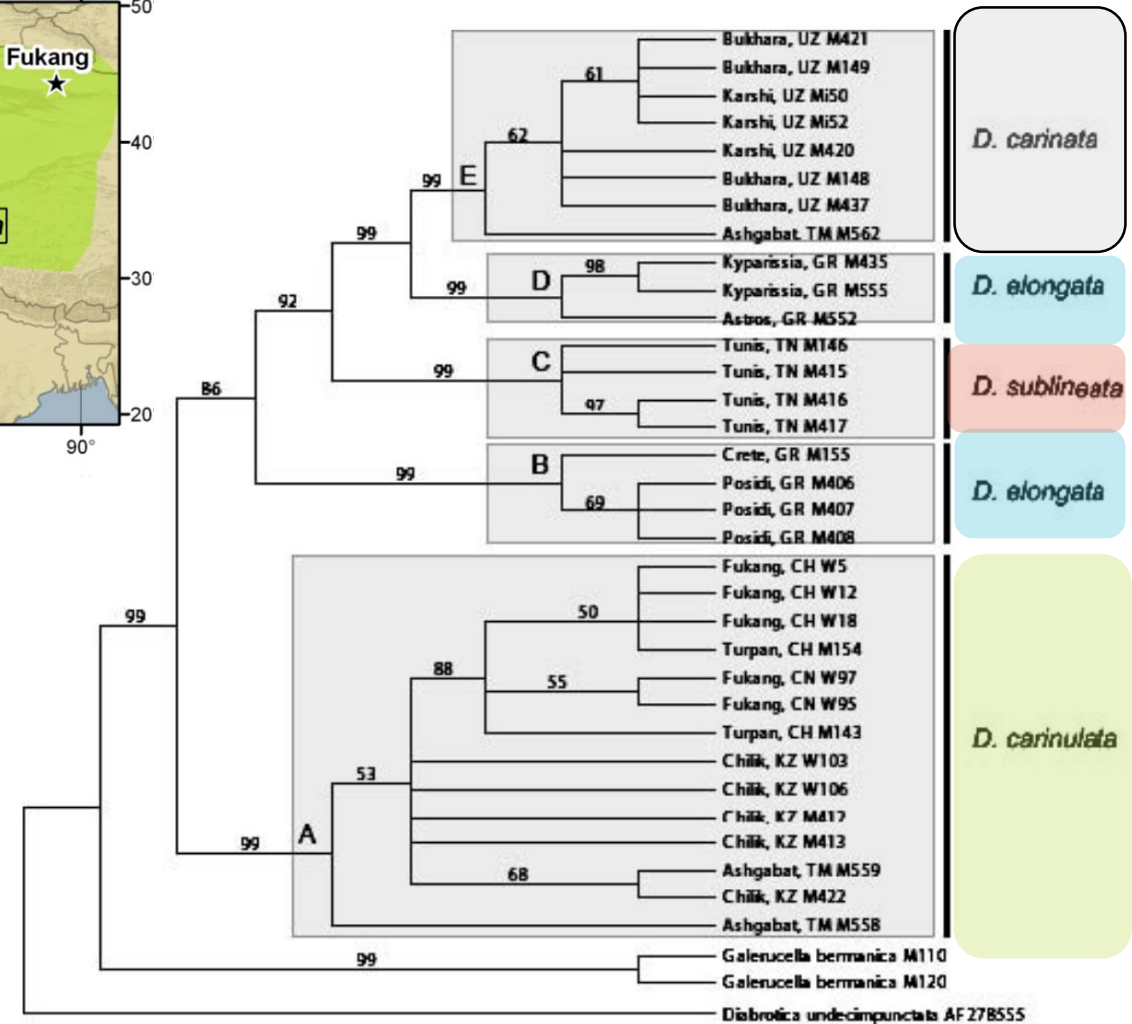




Thank you!



Bean, Kazmer, Garndner, Thompson, Reynolds, Keller, Gaskin (2013).



The Tamarisk Leaf Beetle, *Diorhabda* s



D. sublineata



D. elongata



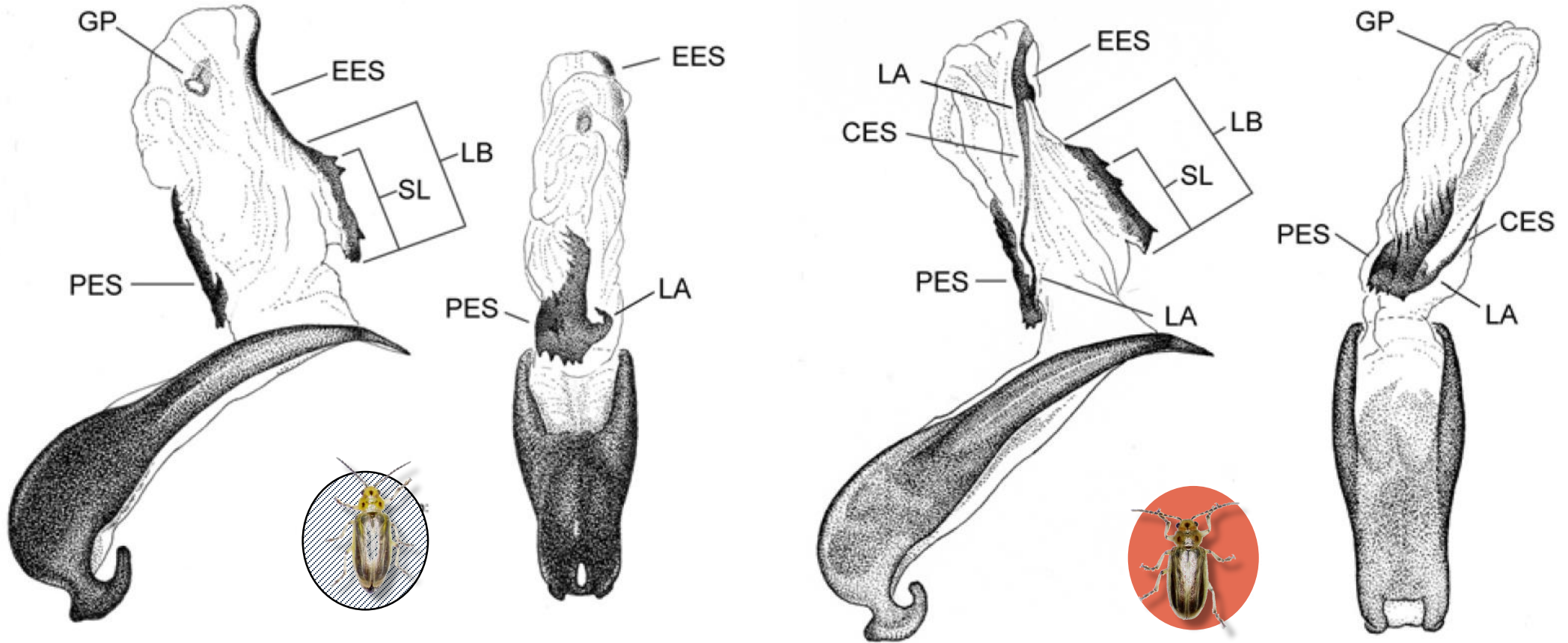
D. carinata



D. carinata

Maximum parsimony 50% majority consensus mtCOI

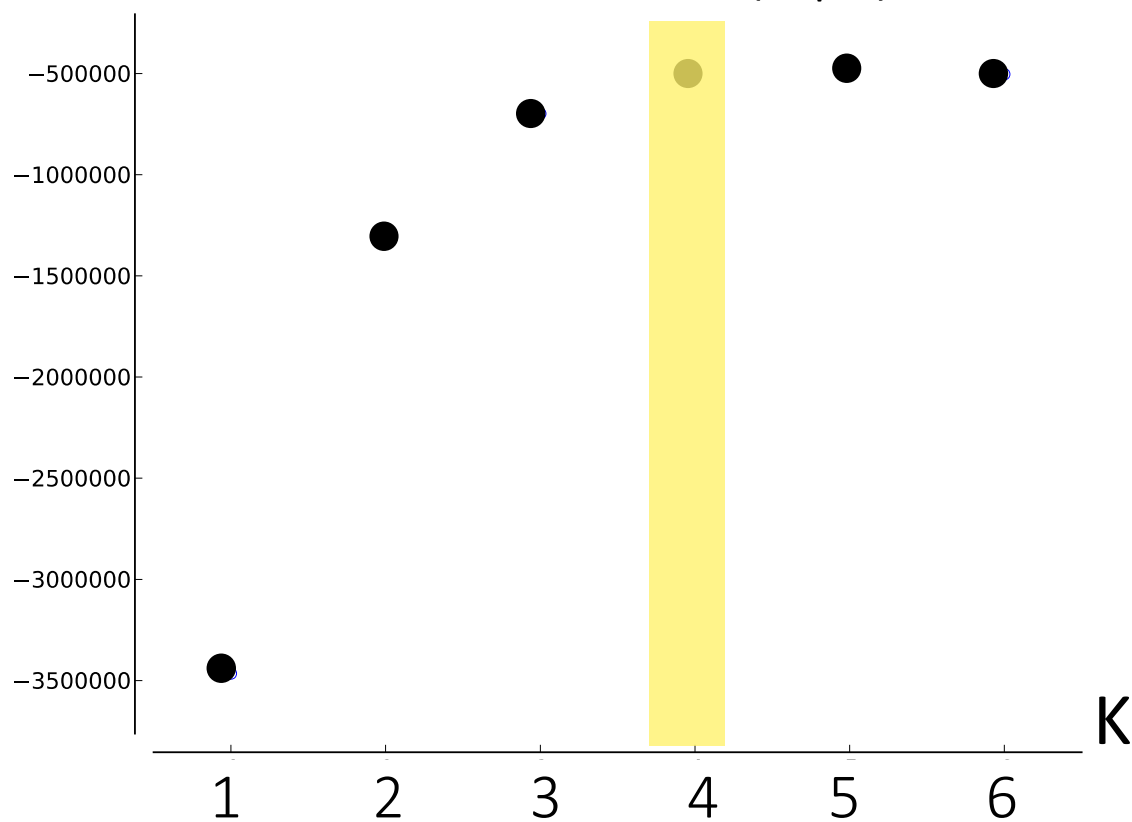
Internal morphology defines species delimitation and identification



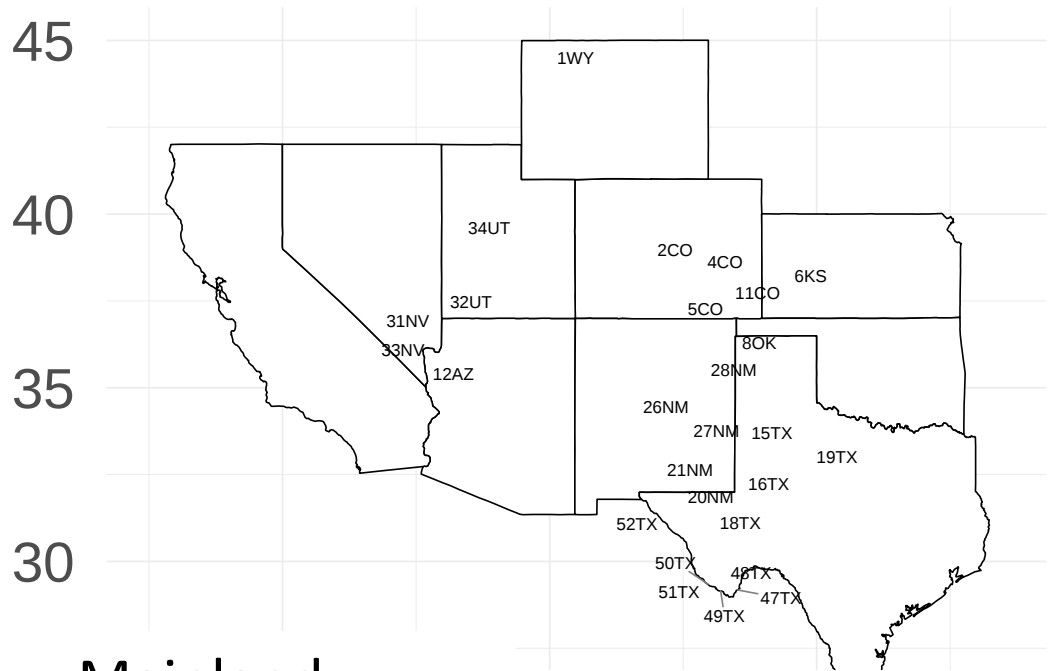
Tracy & Robbins. 2009. Zootaxa.

K=4 reflects species identity for known samples

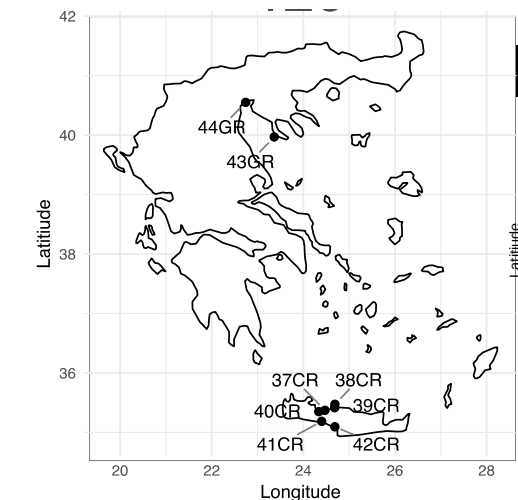
Likelihood(X|K)



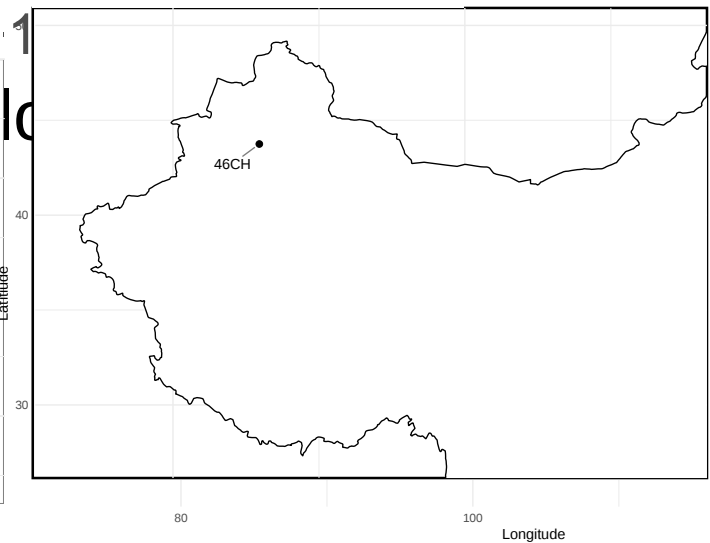
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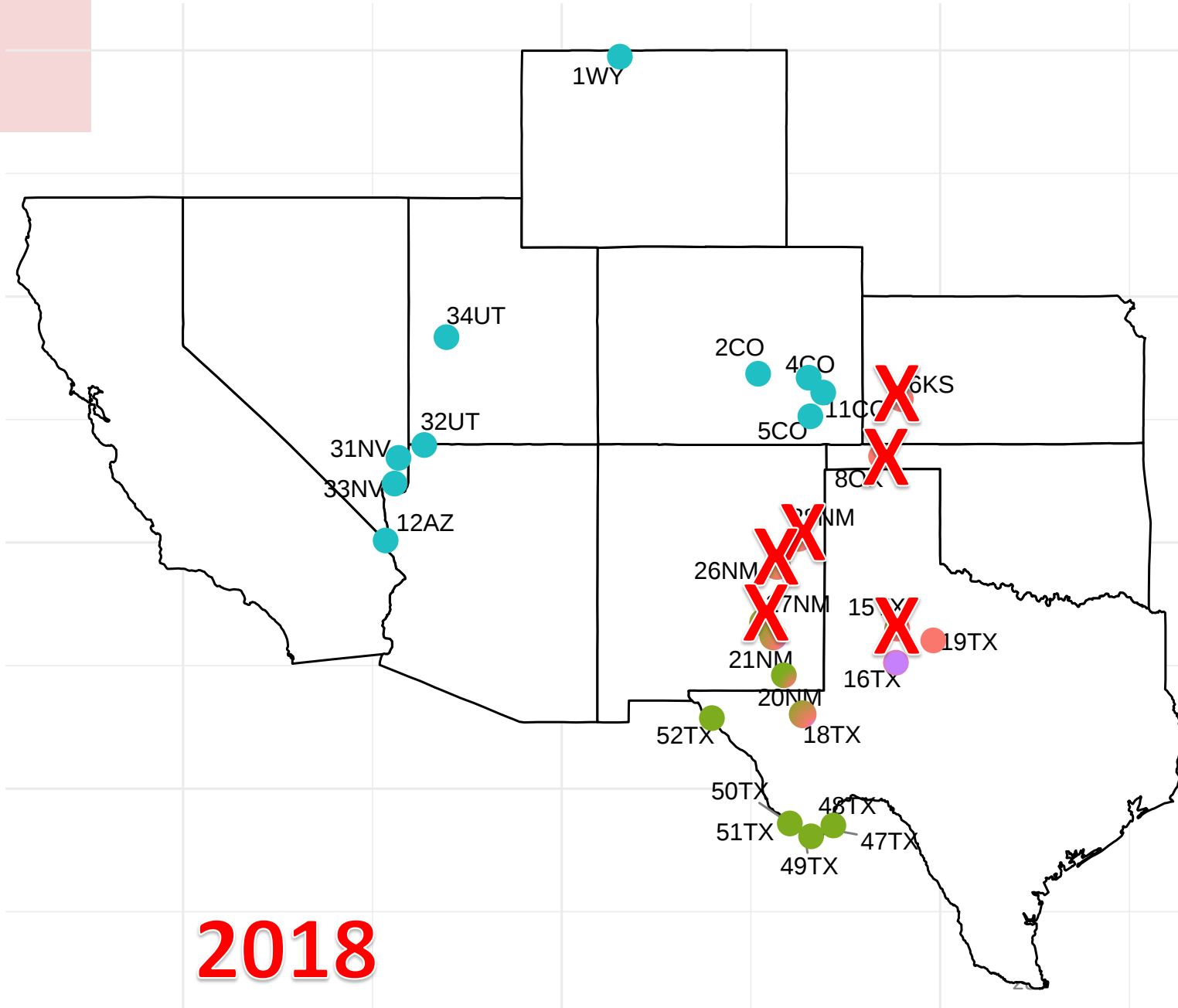
Collected field samples from lab cultures and 36 sites within introduced and native range



Prospects

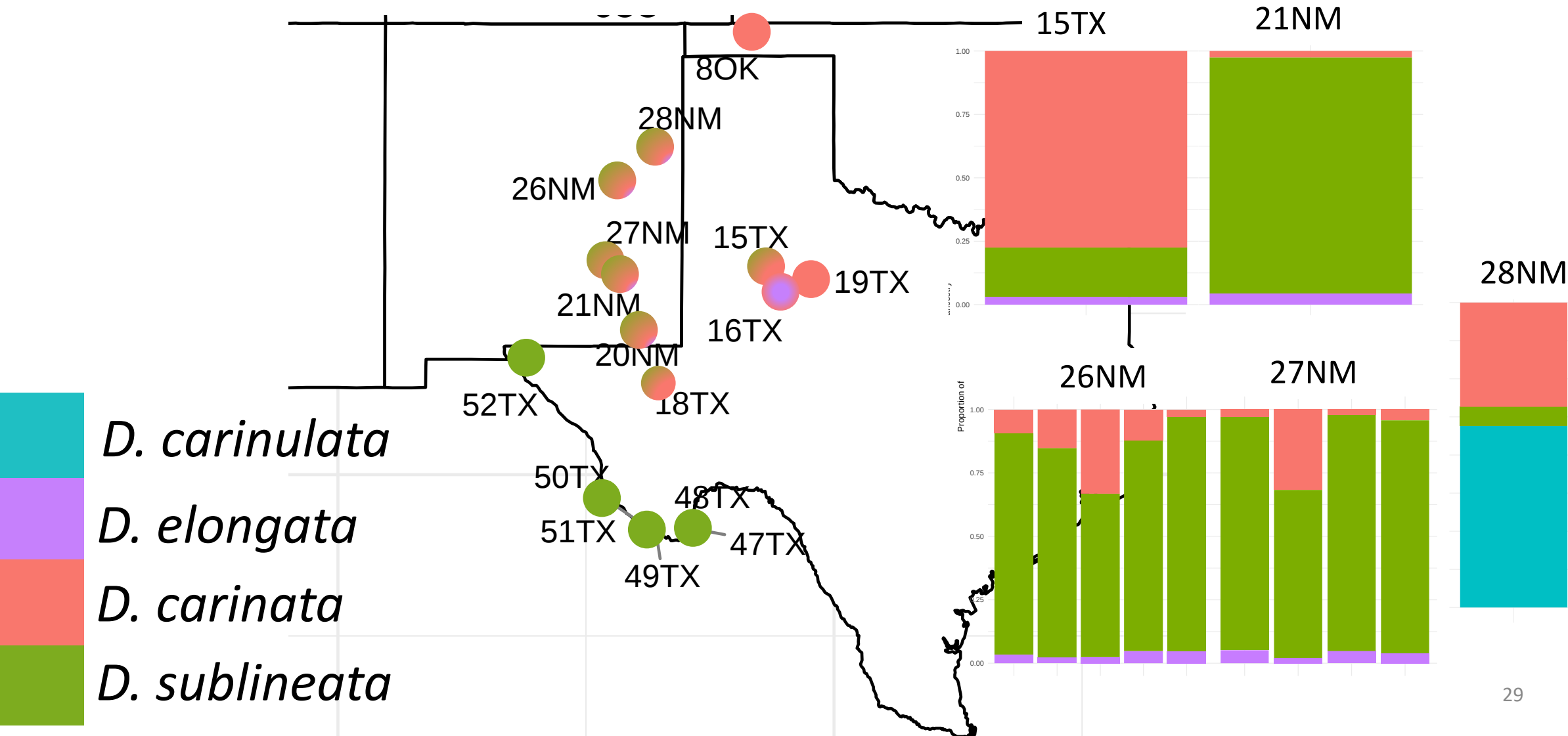
Hybrids and "pure"
populations were
abundant in 2014.

Neither hybridization
status nor genomic
diversity alone seem to
predict establishment
and spread.



2018

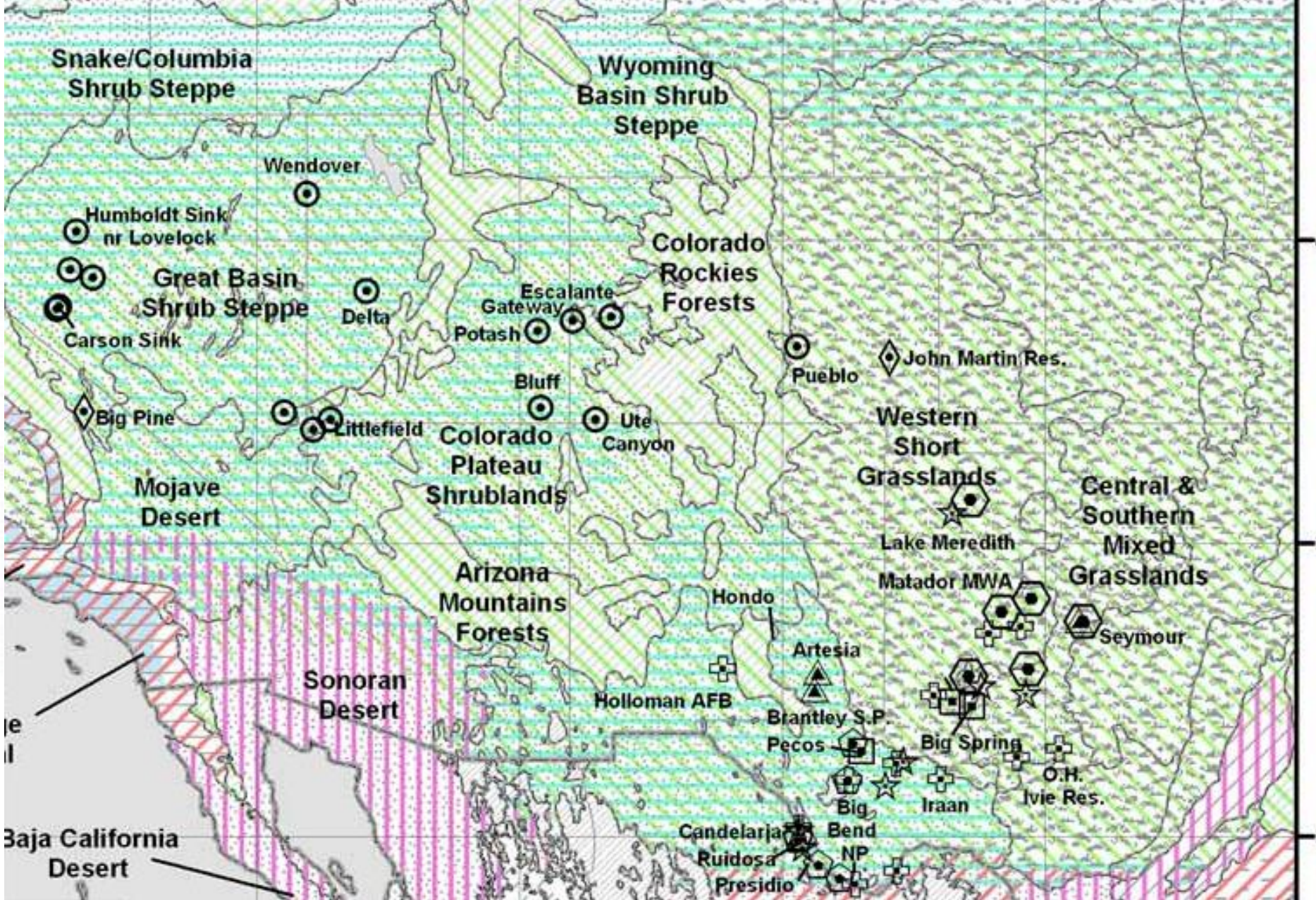
Ancestry assignment reveals tri-specific hybrids



Subject Name	Scientific Name
flea beetle	<i>Altica carduorum</i>
brown dot leafy spurge flea beetle	<i>Aphthona cyparissiae</i>
black leafy spurge flea beetle	<i>Aphthona czwalinae</i>
copper leafy spurge flea beetle	<i>Aphthona flava</i>
brown-legged spurge flea beetle	<i>Aphthona lacertosa</i>
black dot spurge flea beetle	<i>Aphthona nigriscutis</i>
flea beetles	<i>Aphthona</i> spp.
broom seed beetle	<i>Bruchidius villosus</i>
thistle tortoise beetle	<i>Cassida rubiginosa</i>
lesser St. John's wort beetle	<i>Chrysolina hyperici</i>
greater St. John's wort beetle	<i>Chrysolina quadrigemina</i>
leaf beetle	<i>Chrysolina</i> spp.
Mediterranean tamarisk beetle	<i>Diorhabda elongata</i>
black-margined loosestrife beetle	<i>Galerucella californiensis</i>
golden loosestrife beetle	<i>Galerucella pusilla</i>
leaf beetles	<i>Galerucella</i> spp.
ragwort flea beetle	<i>Longitarsus jacobaea</i>

A reference genome allows us to anchor short reads, provides gene-specific context, and serves as a resource to other species

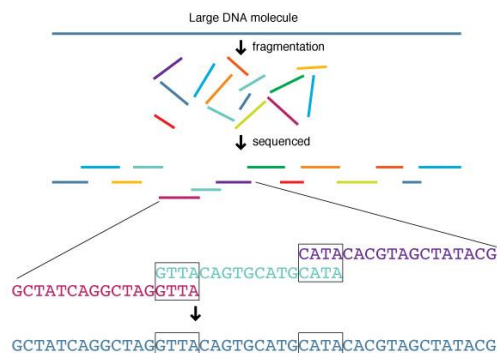
Predicted ranges (Tracy & Robbins 2009)



Cost-effective assembly of (one of) the first weed biocontrol (insect) agent reference genome (coming soon!)

Input data	③	① Shotgun	② 10X
ng DNA		2400	200
Cost		\$2,700	\$3,300
# contigs		21,901	79
Largest contig (Mbp)		.575	1.87
Total length (Mbp)		368.69	20.90
N50 (Kbp)		5.57	1.05
# N's per 100 kbp		40.11	2707.62

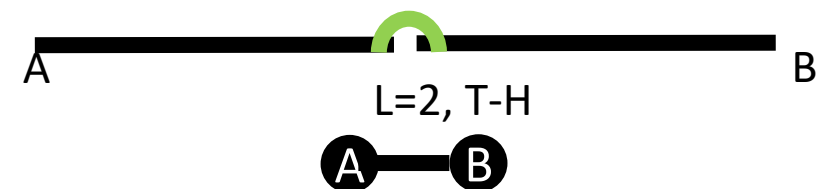
①

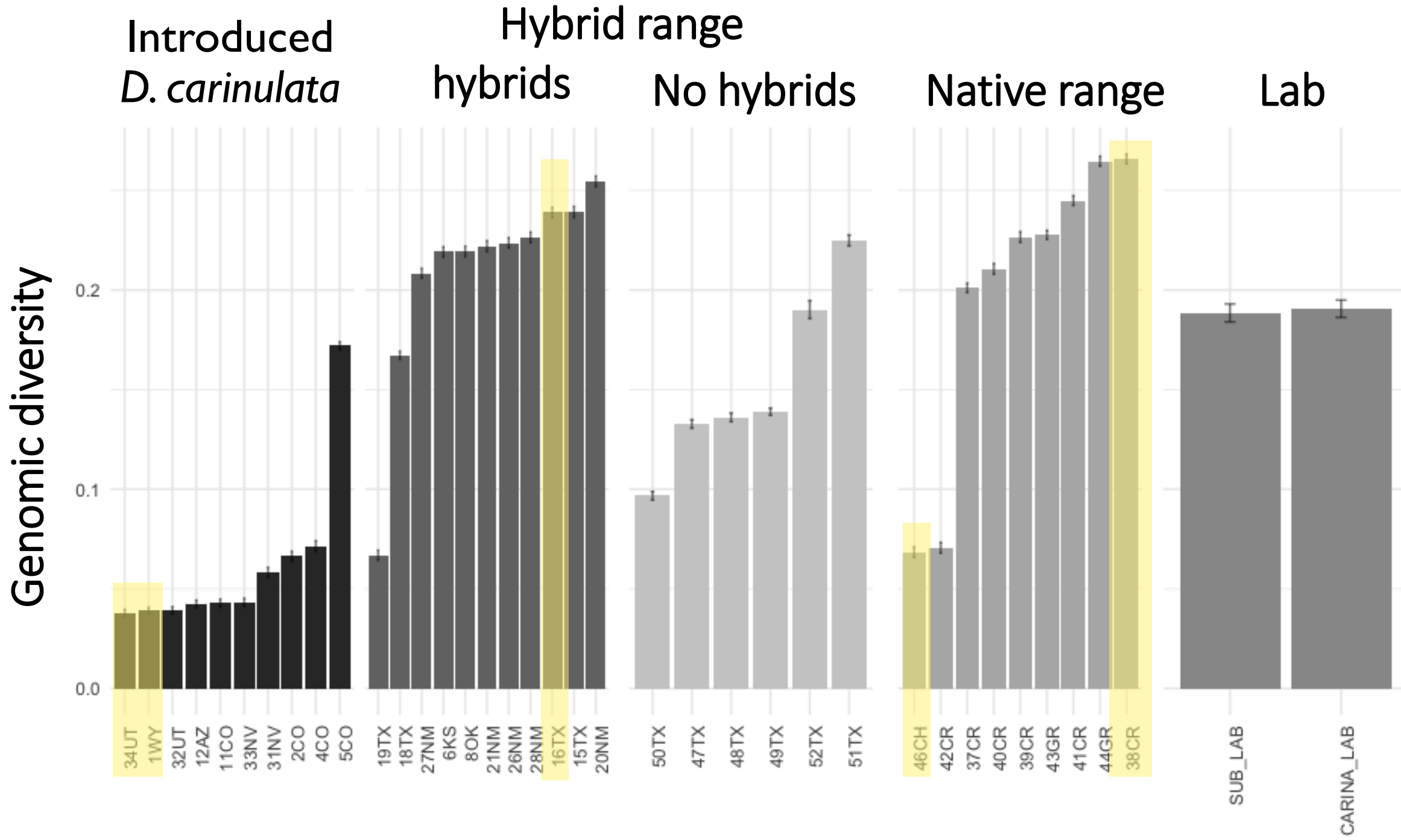


②



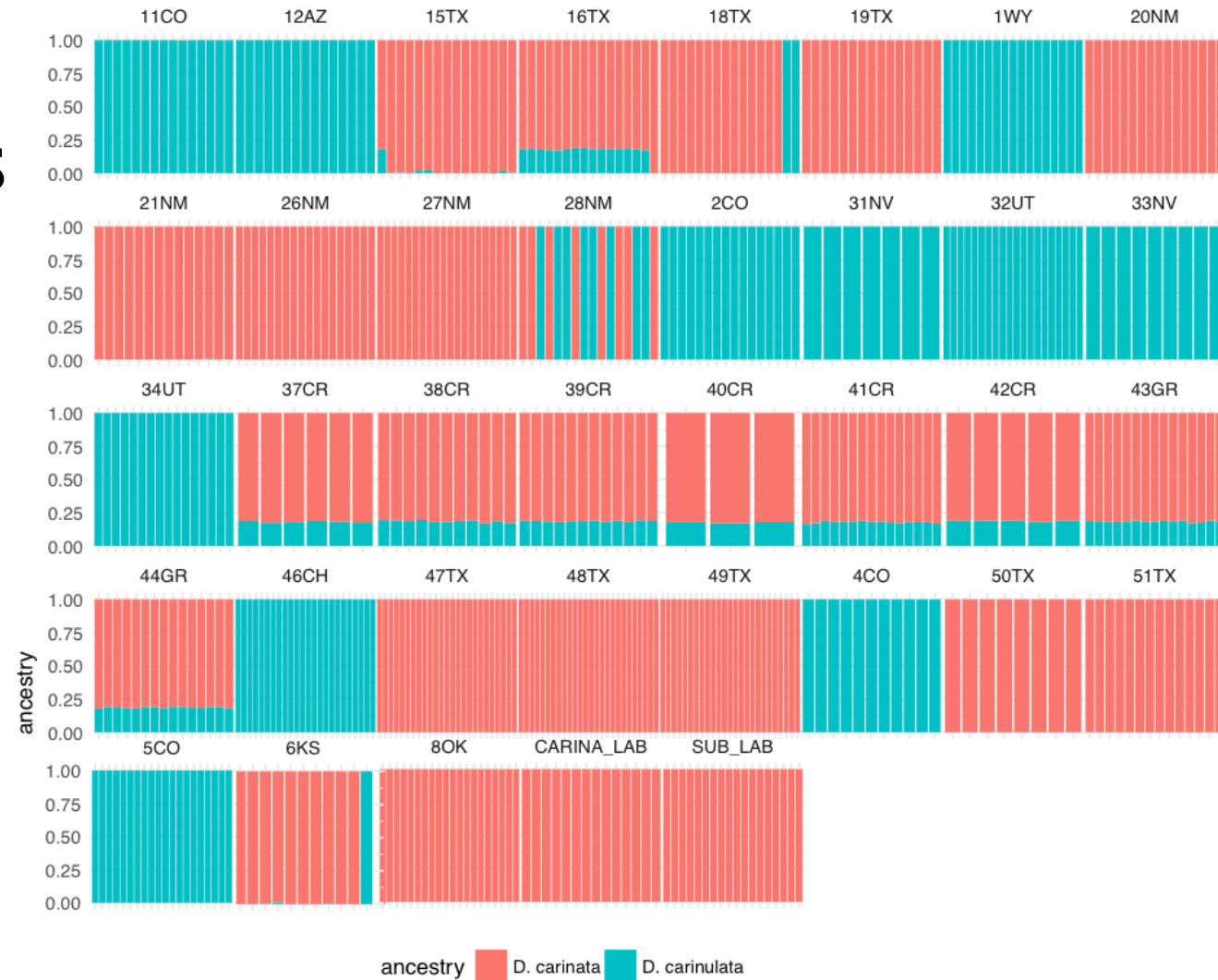
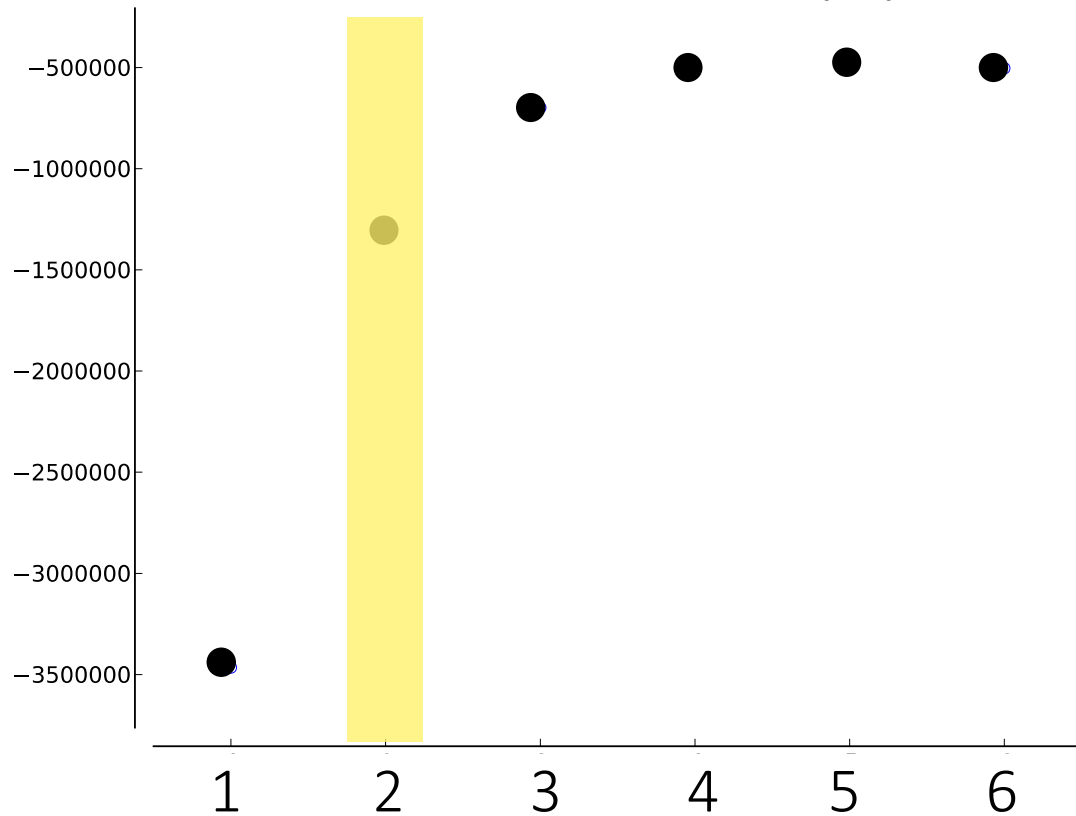
③





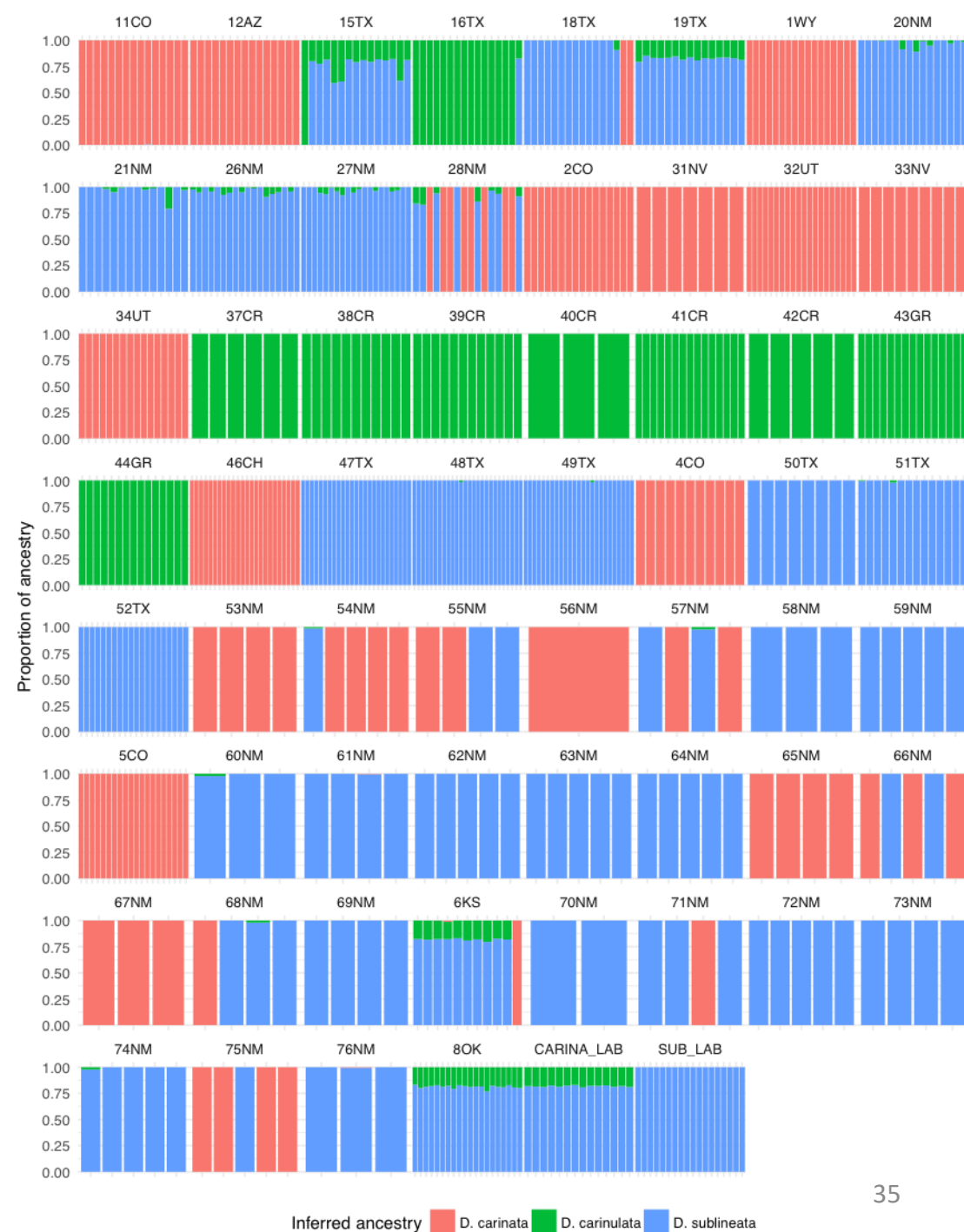
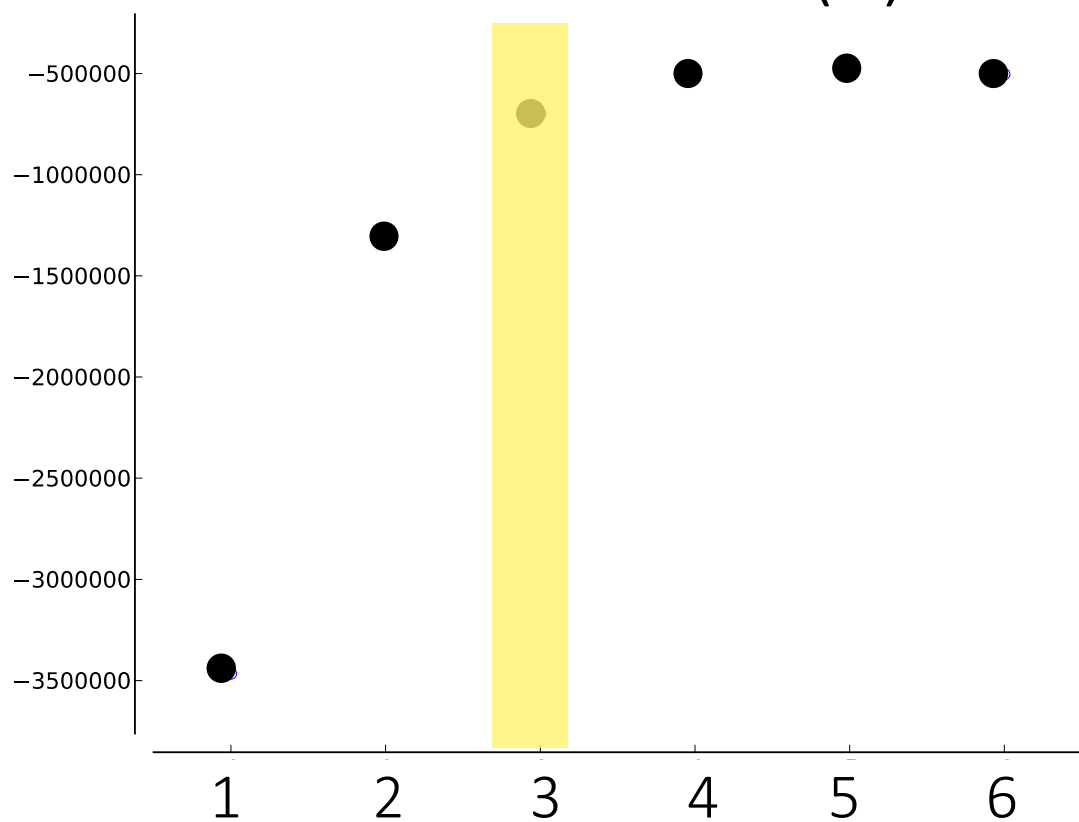
Hierarchical population structure reflects inter-specific relationships

Likelihood(K)



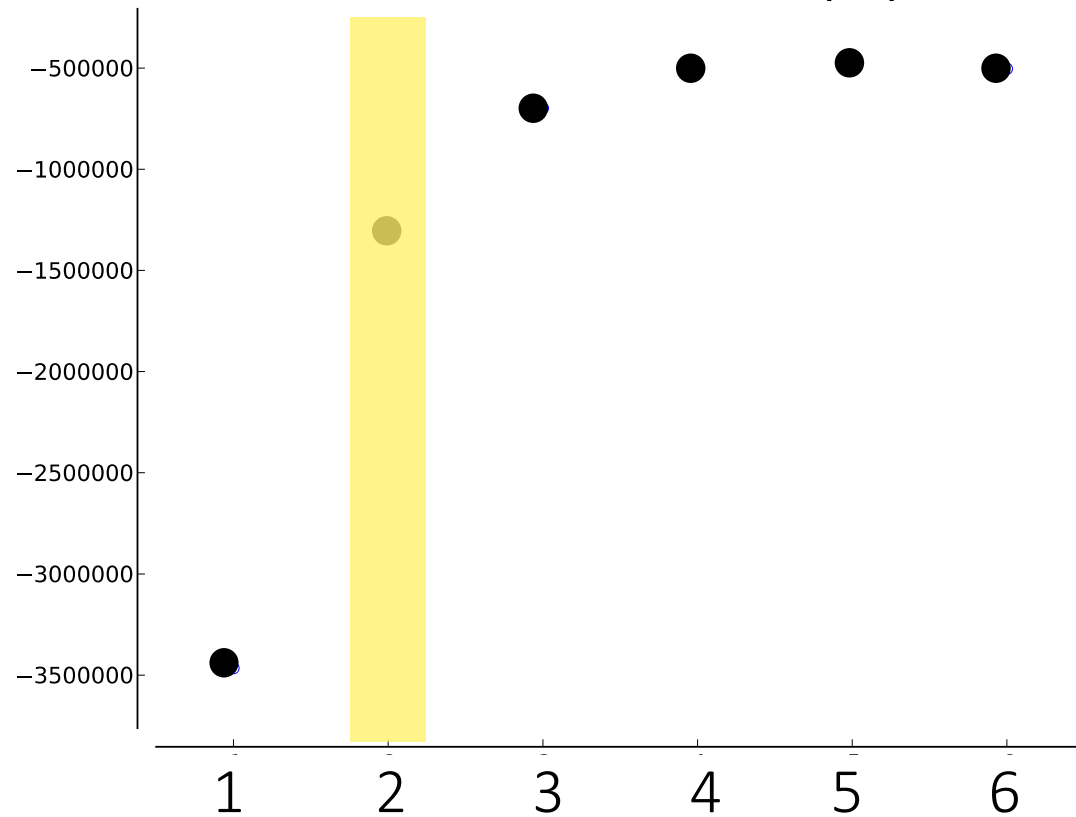
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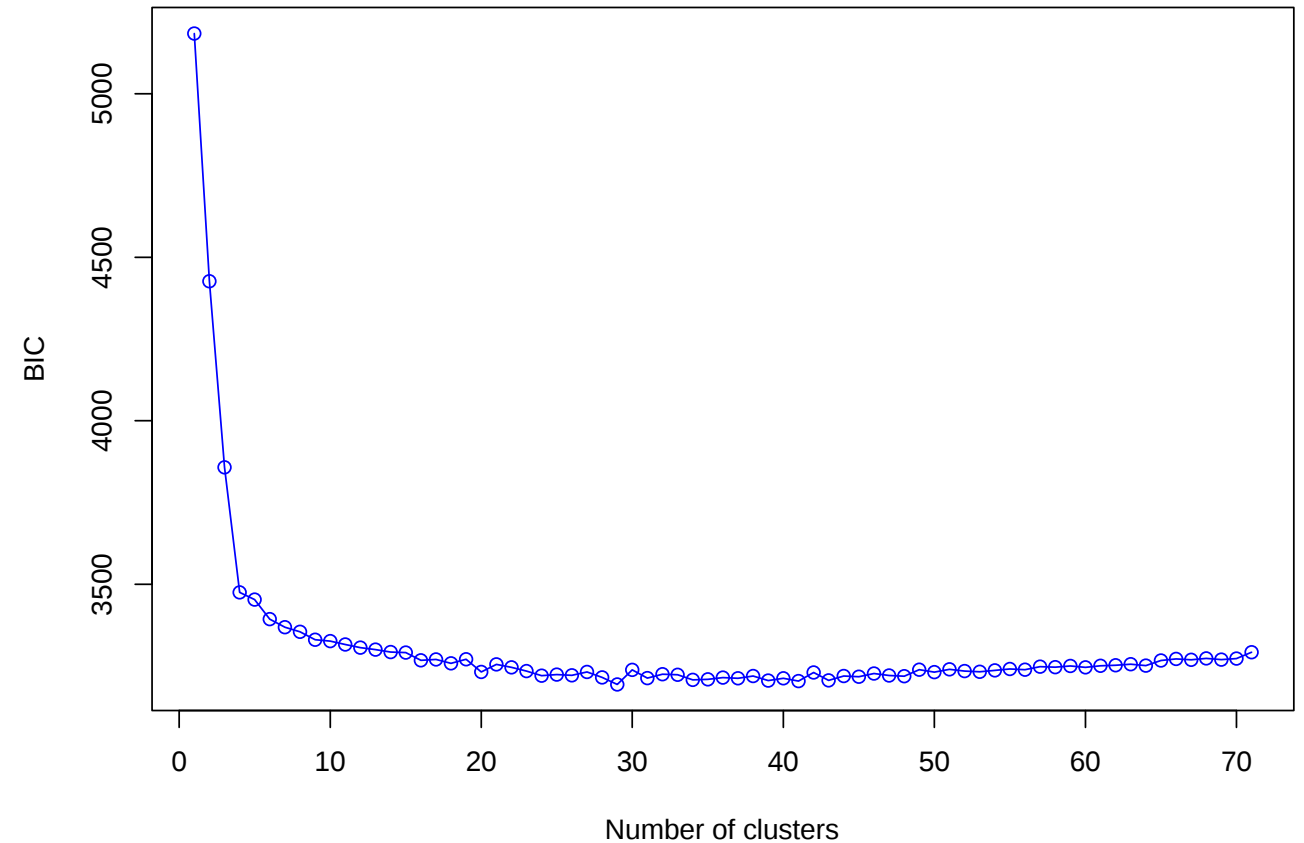


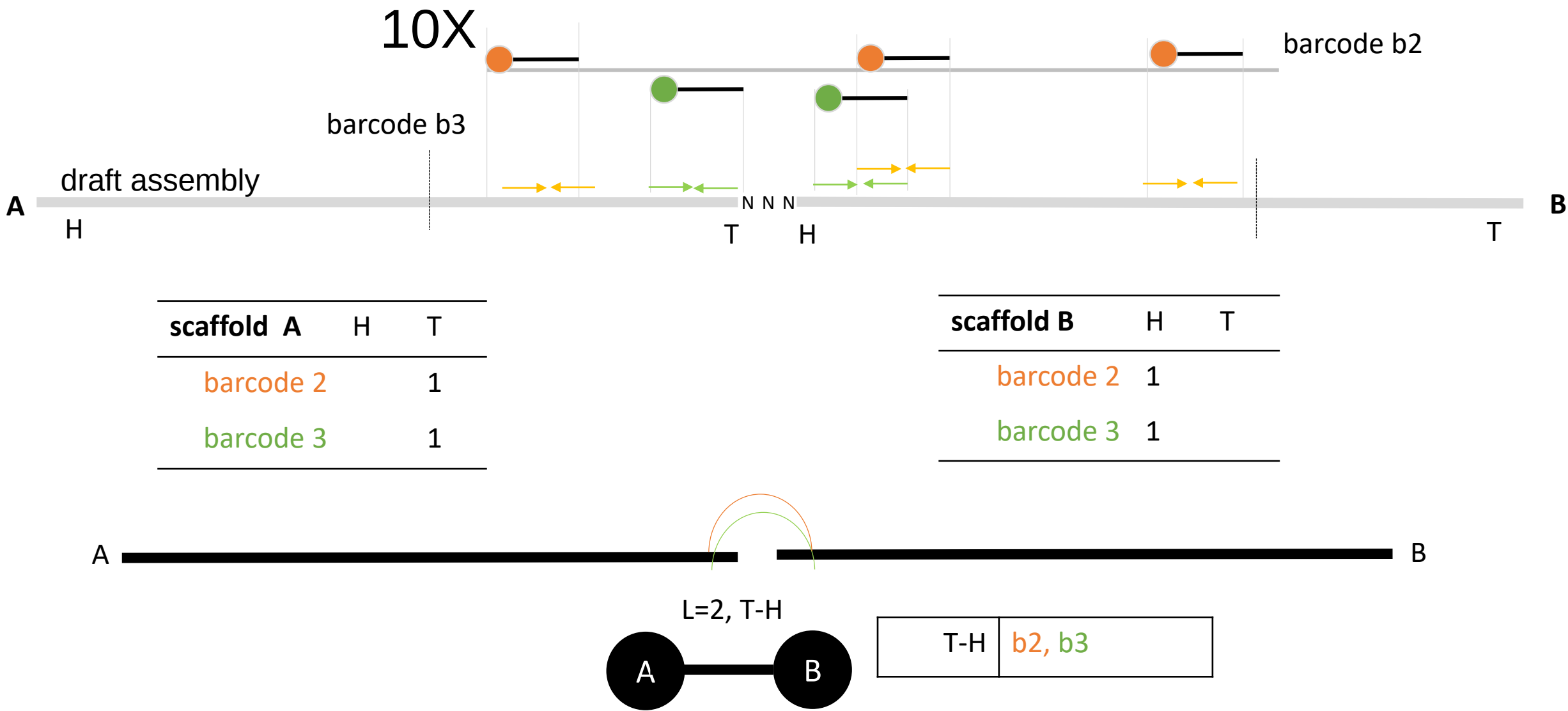
Choosing K

Likelihood(K)



Value of BIC
versus number of clusters





RADseq capitalizes on existing restriction enzyme technology, PCR, and high-throughput sequencing

