Genetic characterization of the *Diorhabda* species complex released for biological control of *Tamarix*.

Dan Bean[1], David Kazmer[2] and David Thompson[3]

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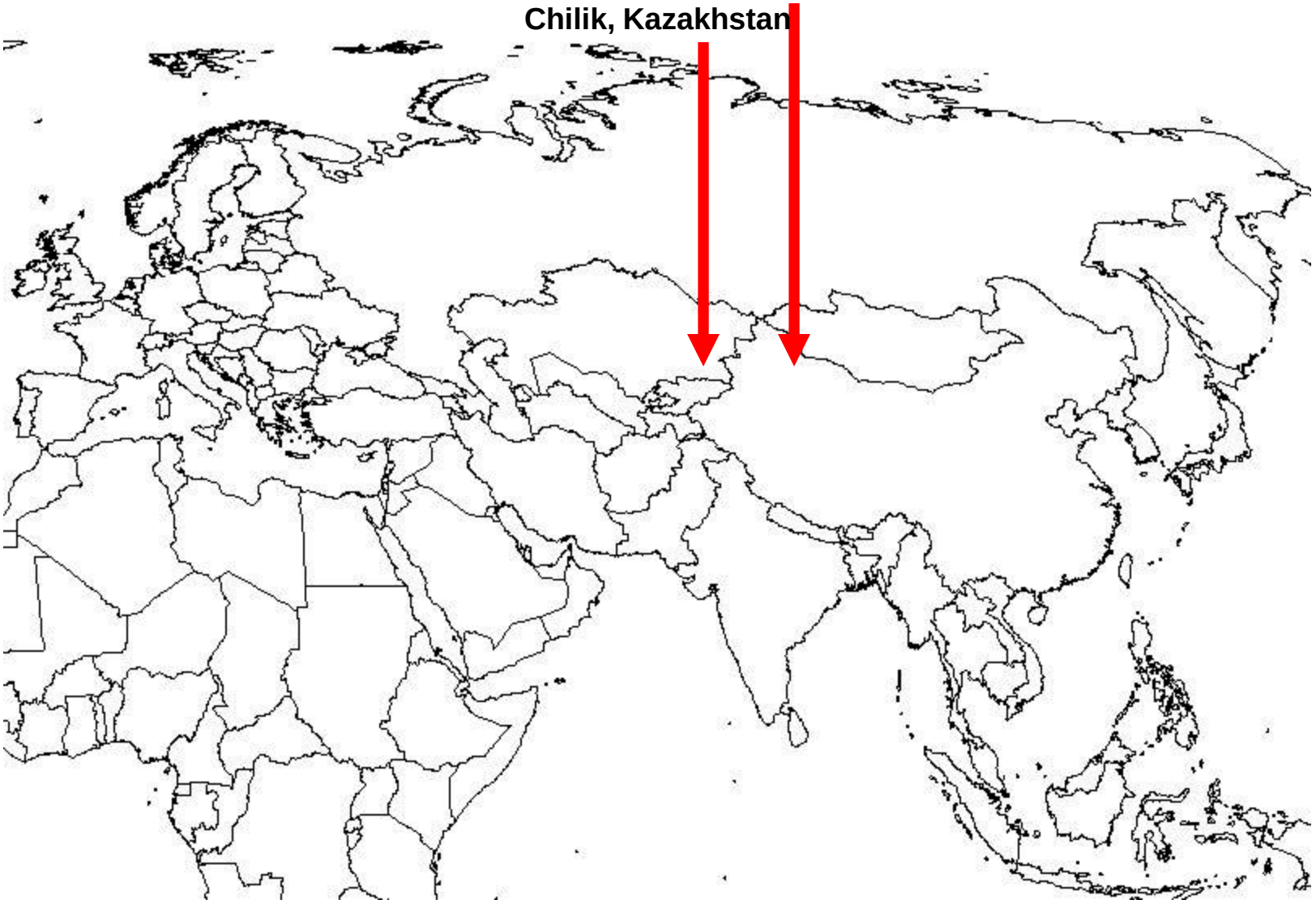
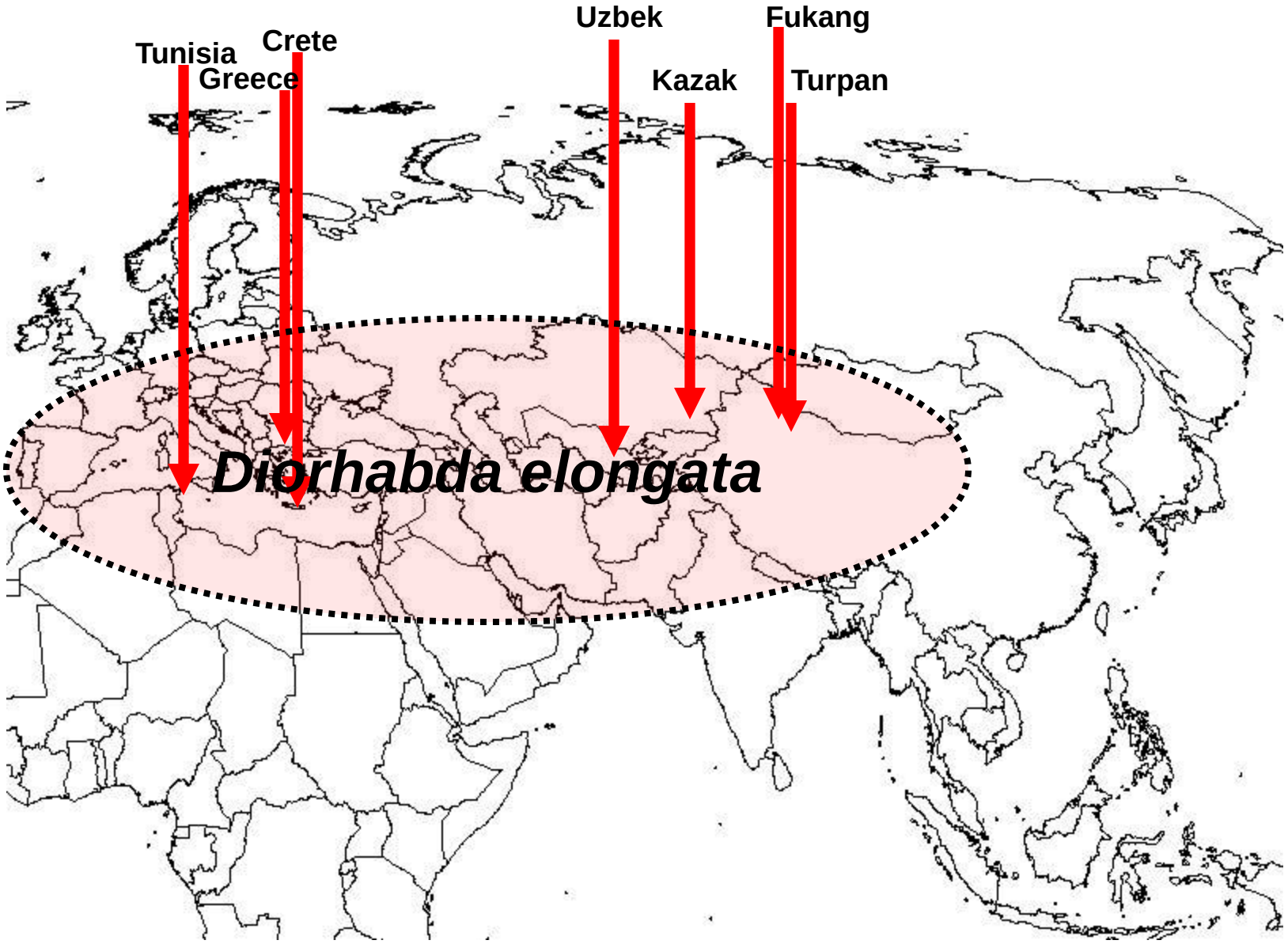
First Saltcedar Biological Control Agent Released in North America in May 2001

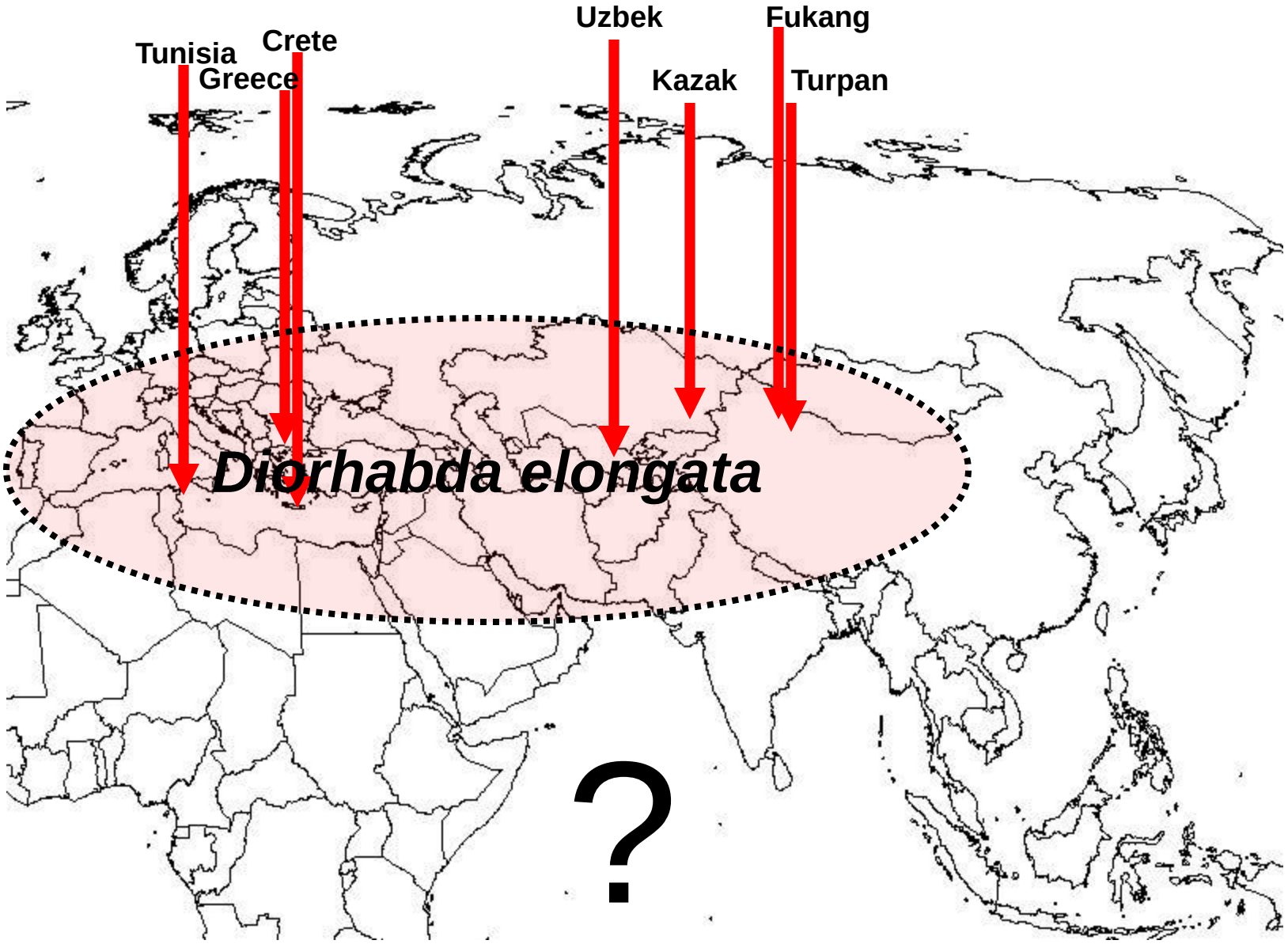
Saltcedar Leaf Beetle, *Diorhabda elongata deserticola* from China and Kazakhstan


2007 pre-beetle


2010 post-beetle

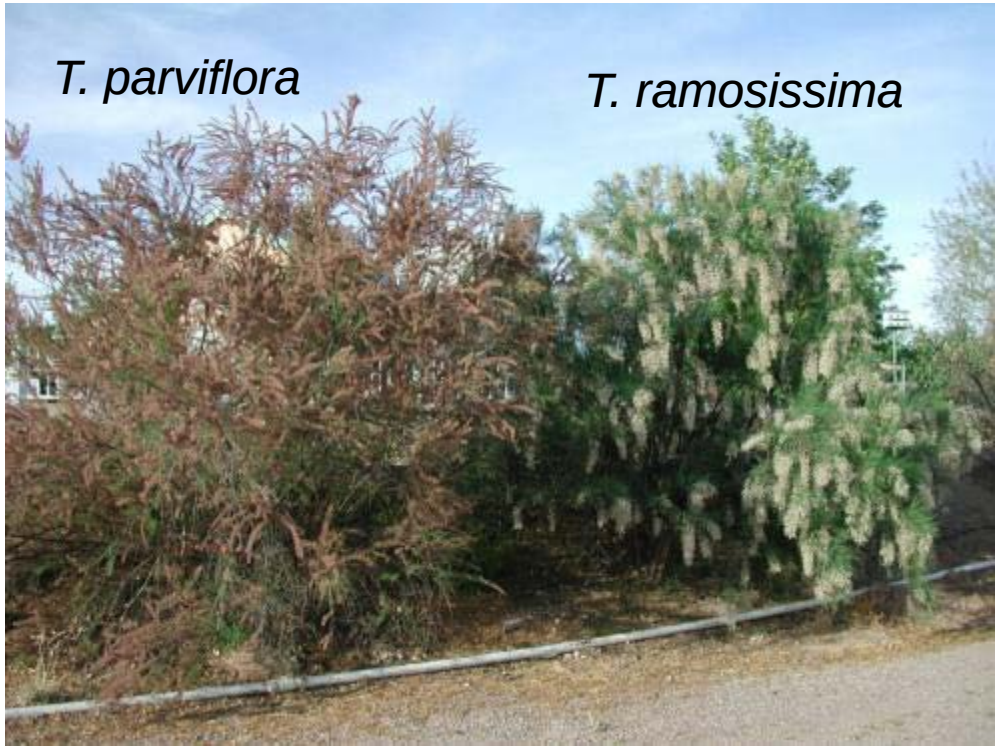

Fukang, China
Chilik, Kazakhstan


Tunisia
Crete
Greece
Uzbek
Kazak
Fukang
Turpan
Diorhabda elongata

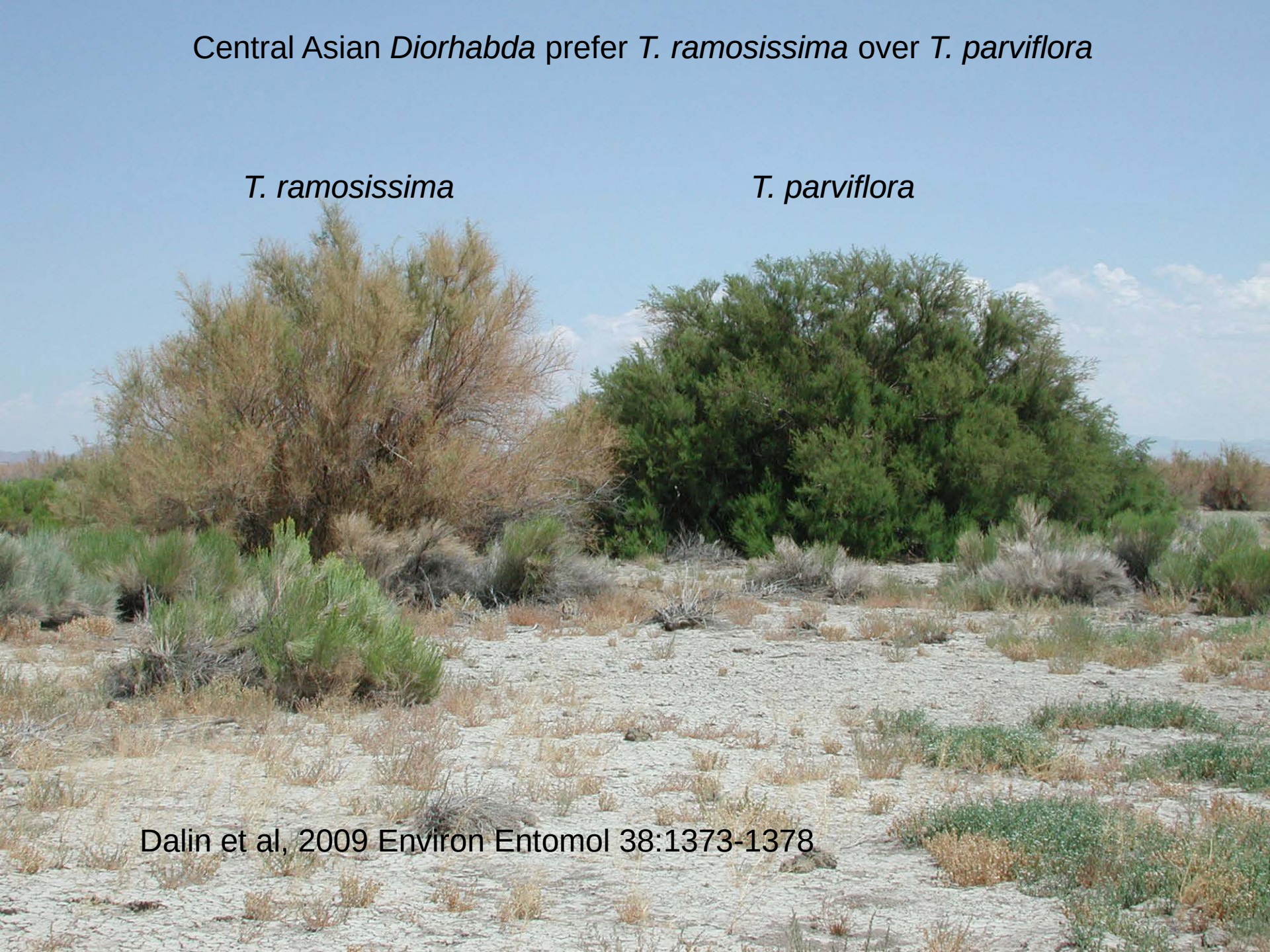

Tunisia
Crete
Greece
Uzbek
Kazak
Fukang
Turpan
Diorhabda elongata
?

Tamarix is a complex and wide ranging target genus

1. 5 invasive *Tamarix* species
2. Extensive hybridization in NA



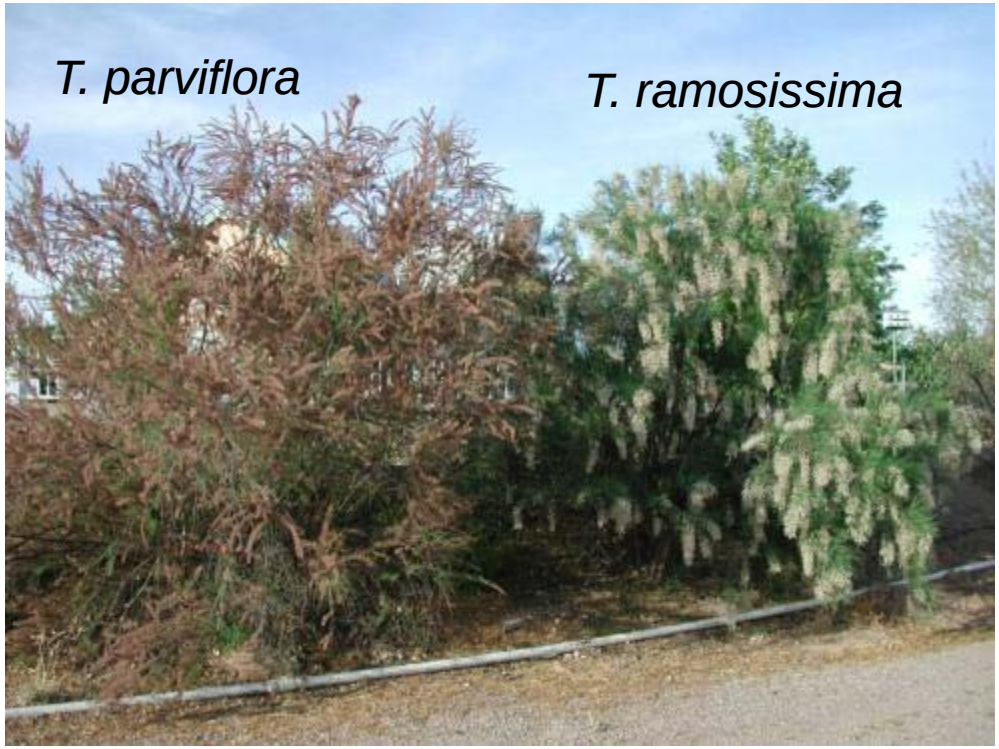
Central Asian *Diorhabda* prefer *T. ramosissima* over *T. parviflora*



Dalin et al, 2009 Environ Entomol 38:1373-1378

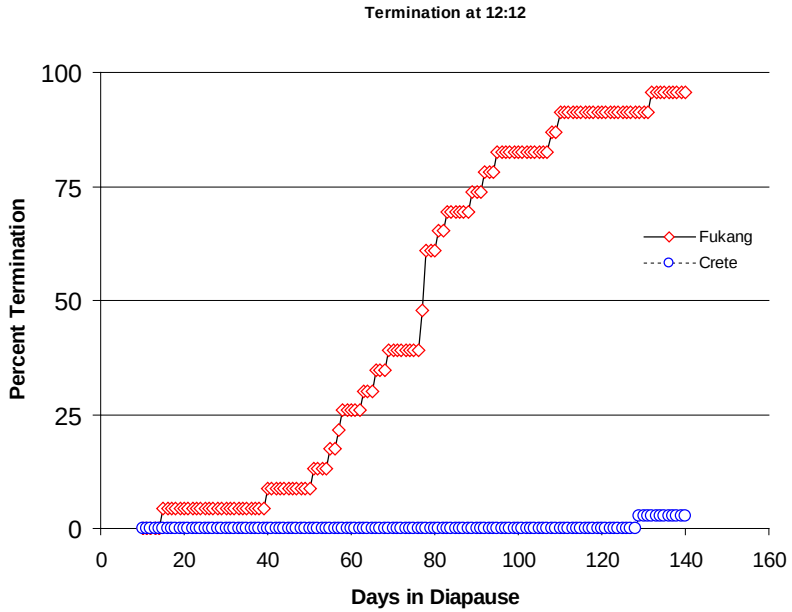
***Tamarix* is a complex and wide ranging target genus**

1. 5 invasive *Tamarix* species
2. Extensive hybridization in NA
3. ***Diorhabda* from one or two sites in central Asia would not be adapted to conditions over the entire range of *Tamarix* in North America**



Diorhabda from one or two sites in central Asia would not be adapted to conditions over the entire range of *Tamarix* in North America

Diorhabda from Crete remain in diapause longer under warm temperatures than *Diorhabda* from central Asia, probably an adaptation to higher winter temperatures in the Mediterranean



Diorhabda populations are adapted to the day length patterns, temperatures and possibly the predators found at their origin; this restricts range in North America.

Predators prevent establishment at some locations

Matching host plant (*Tamarix* species and there hybrids) and region with the appropriate *Diorhabda* ecotype is and will continue to be critical to the success of the *Tamarix* biocontrol program. We needed a way to distinguish *Diorhabda* ecotypes and recognize their potential for hybridization.

Molecular analysis

Analysis of morphology

Analysis of hybridization potential between ecotypes

Matching host plant (*Tamarix* species and there hybrids) and region with the appropriate *Diorhabda* ecotype is and will continue to be critical to the success of the *Tamarix* biocontrol program. We needed a way to distinguish *Diorhabda* ecotypes and recognize their potential for hybridization.

Kazmer

Molecular analysis

Tracy and Robbins

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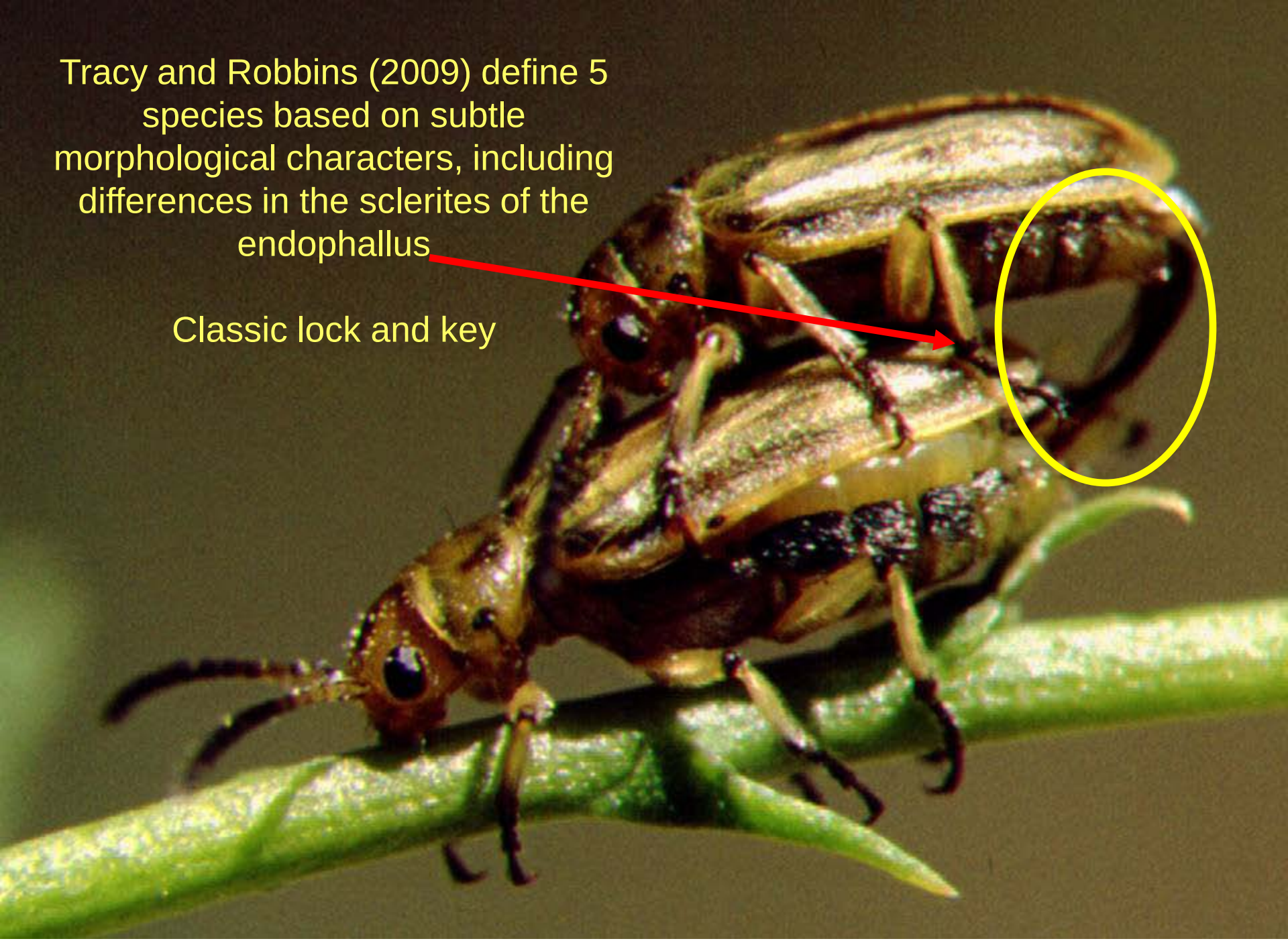
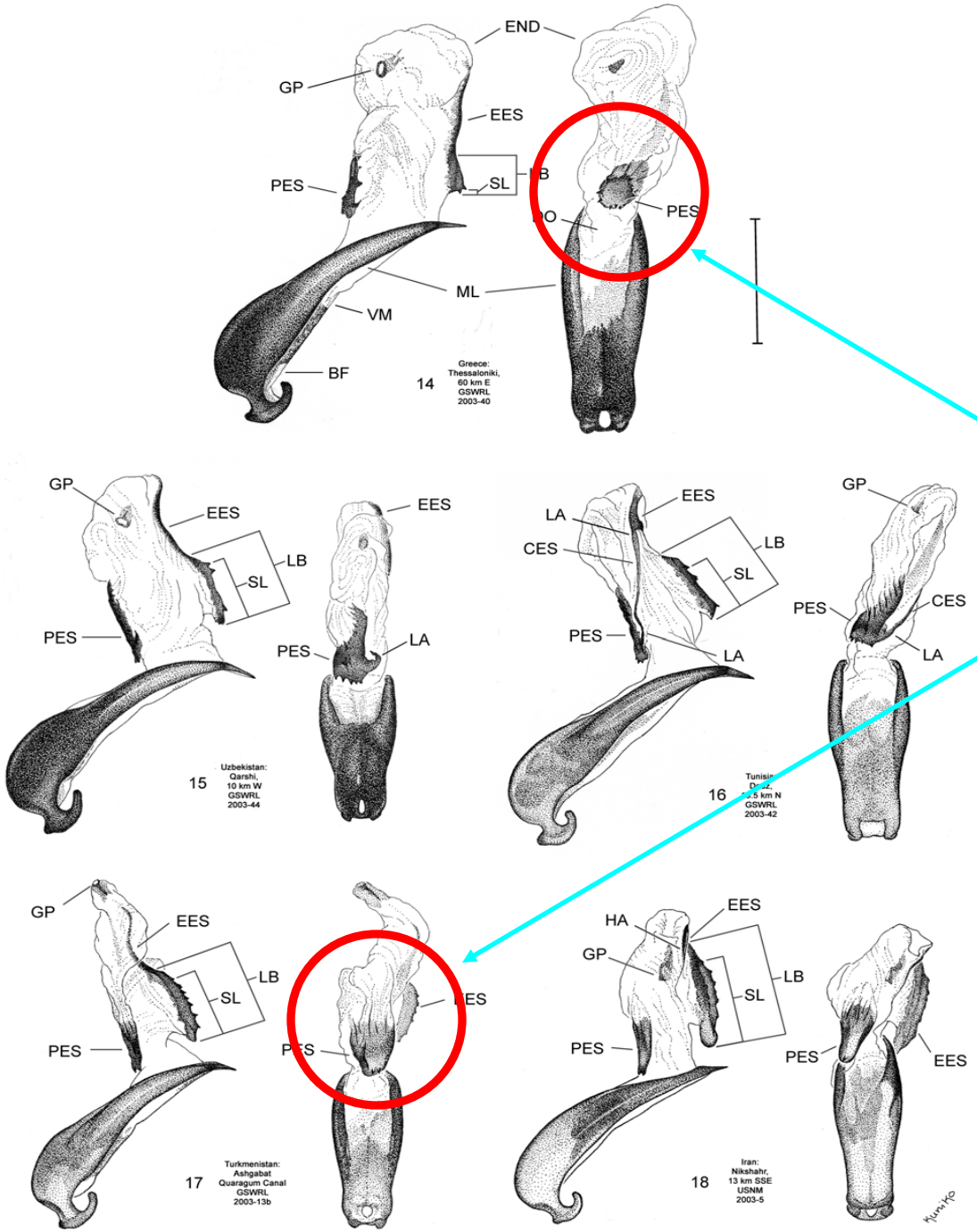
Thompson and Bean


Tracy and Robbins (2009) define 5 species based on subtle morphological characters, including differences in the sclerites of the endophallus
Classic lock and key

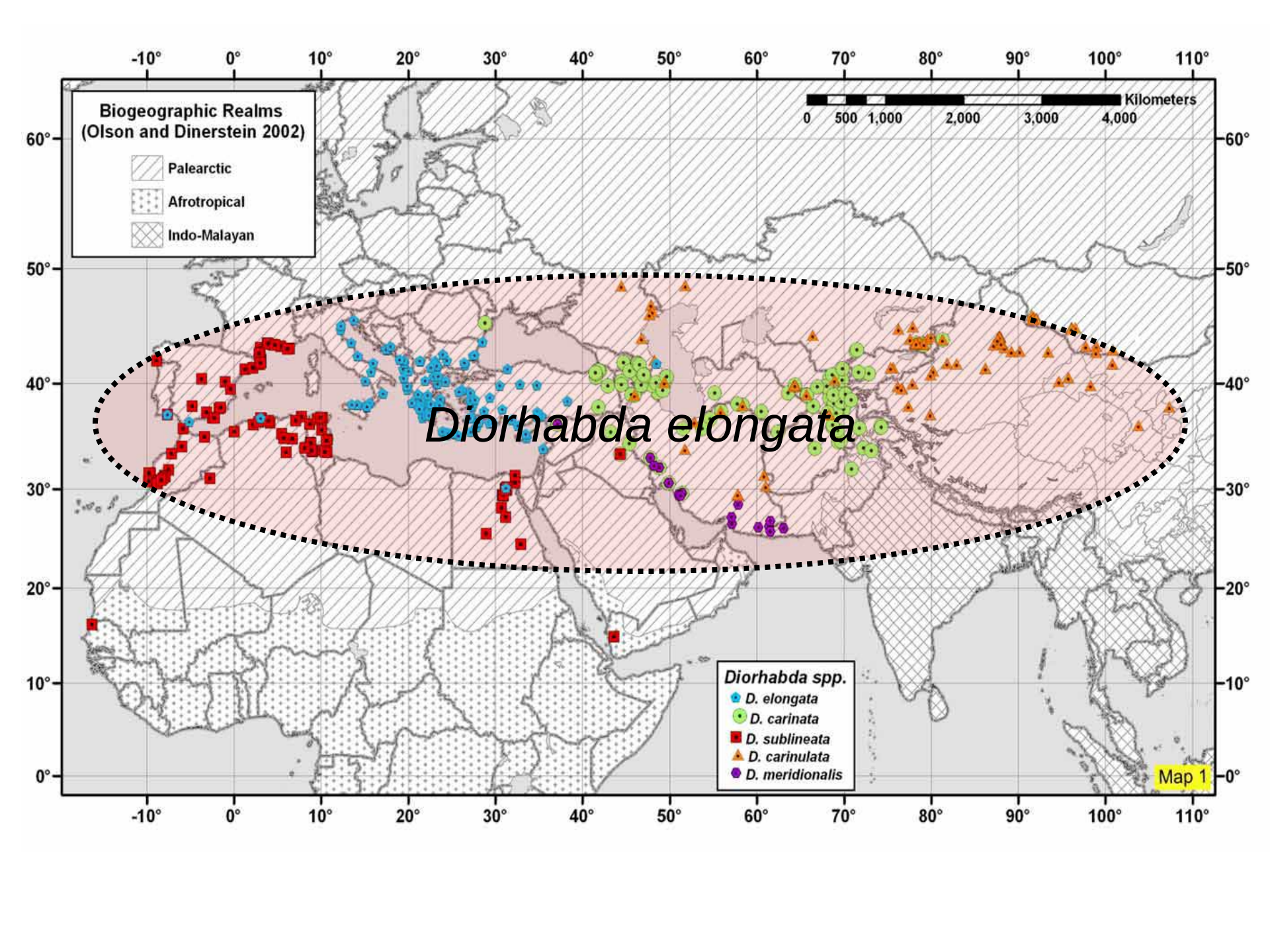


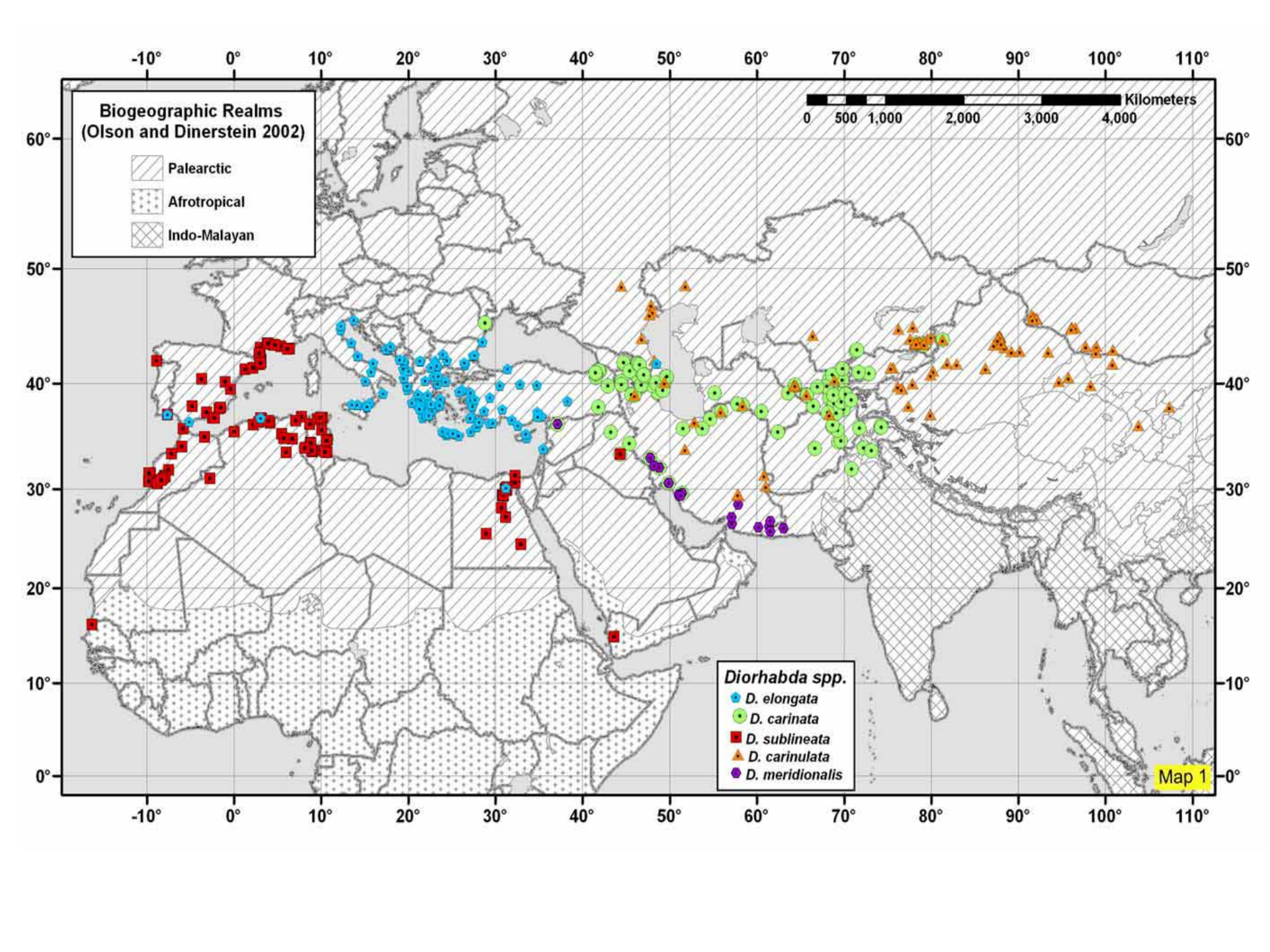
Structural differences in sclerites of the endophallus are used to divide *Diorhabda* into 5 species

D. elongata

D. carinulata

From: Tracy and Robbins (2009) Zootaxa 2101


Biogeographic Realms
(Olson and Dinerstein 2002)
Palearctic
Afrotropical
Indo-Malayan
Kilometers
0 500 1,000 2,000 3,000 4,000
Diorhabda elongata
Diorhabda spp.
D. elongata
D. carinata
D. sublineata
D. carinulata
D. meridionalis
Map 1
-10° 0° 10° 20° 30° 40° 50° 60° 70° 80° 90° 100° 110°
60° 50° 40° 30° 20° 10° 0°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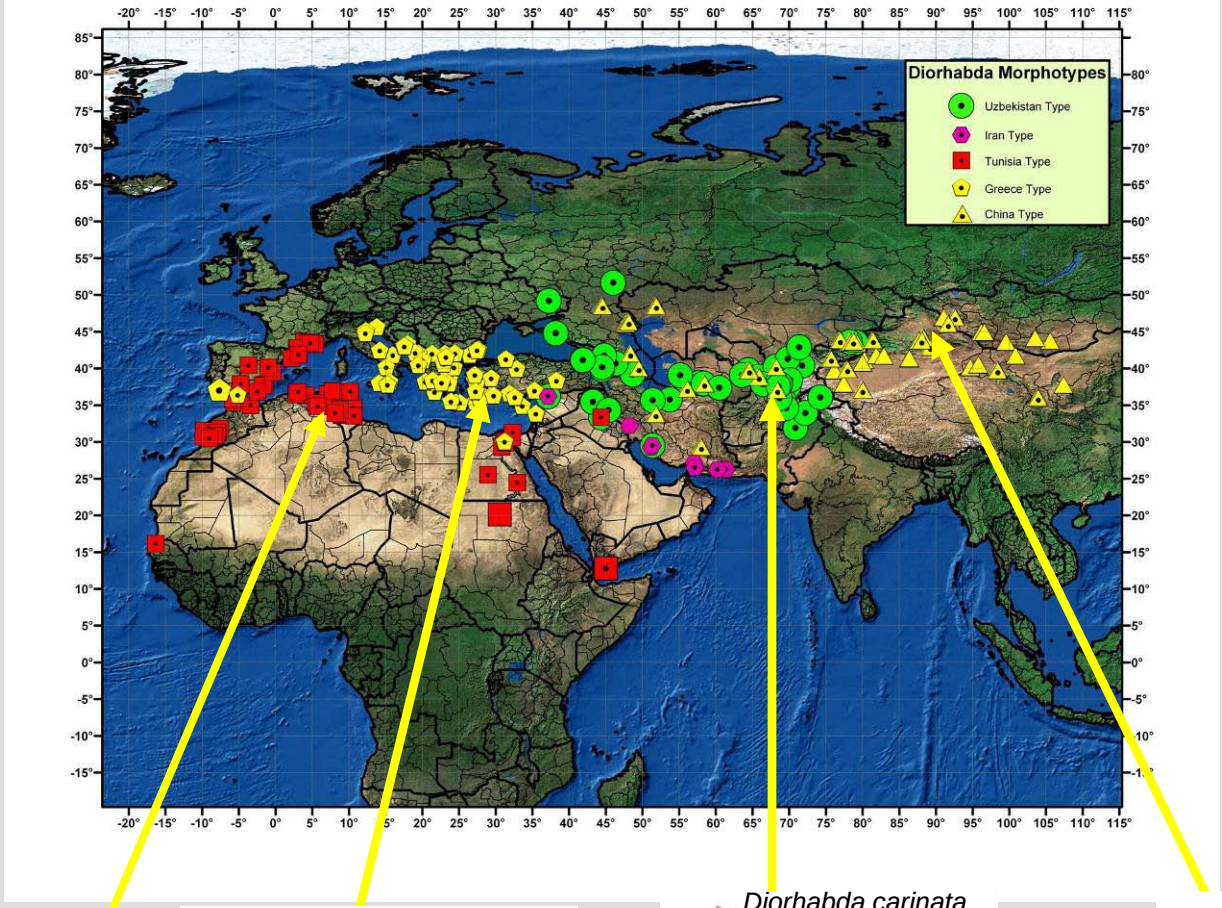

Diorhabda sublineata

Tunisia

Diorhabda elongata

Crete

Diorhabda carinata

Uzbekistan

Diorhabda carinulata

Chilik, Fukang, Turpan

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Kazmer

Molecular analysis

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Analysis of morphology

Analysis of hybridization potential between ecotypes

Thompson and Bean

Objectives

- Determine the molecular genetic relationships among the *Tamarix*-feeding members of the *Diorhabda elongata* species complex
- Examine concordance of molecular genetic traits with morphological, behavioral and ecological traits
- Develop molecular genetic assays for determining hybridization between the important genetic lineages

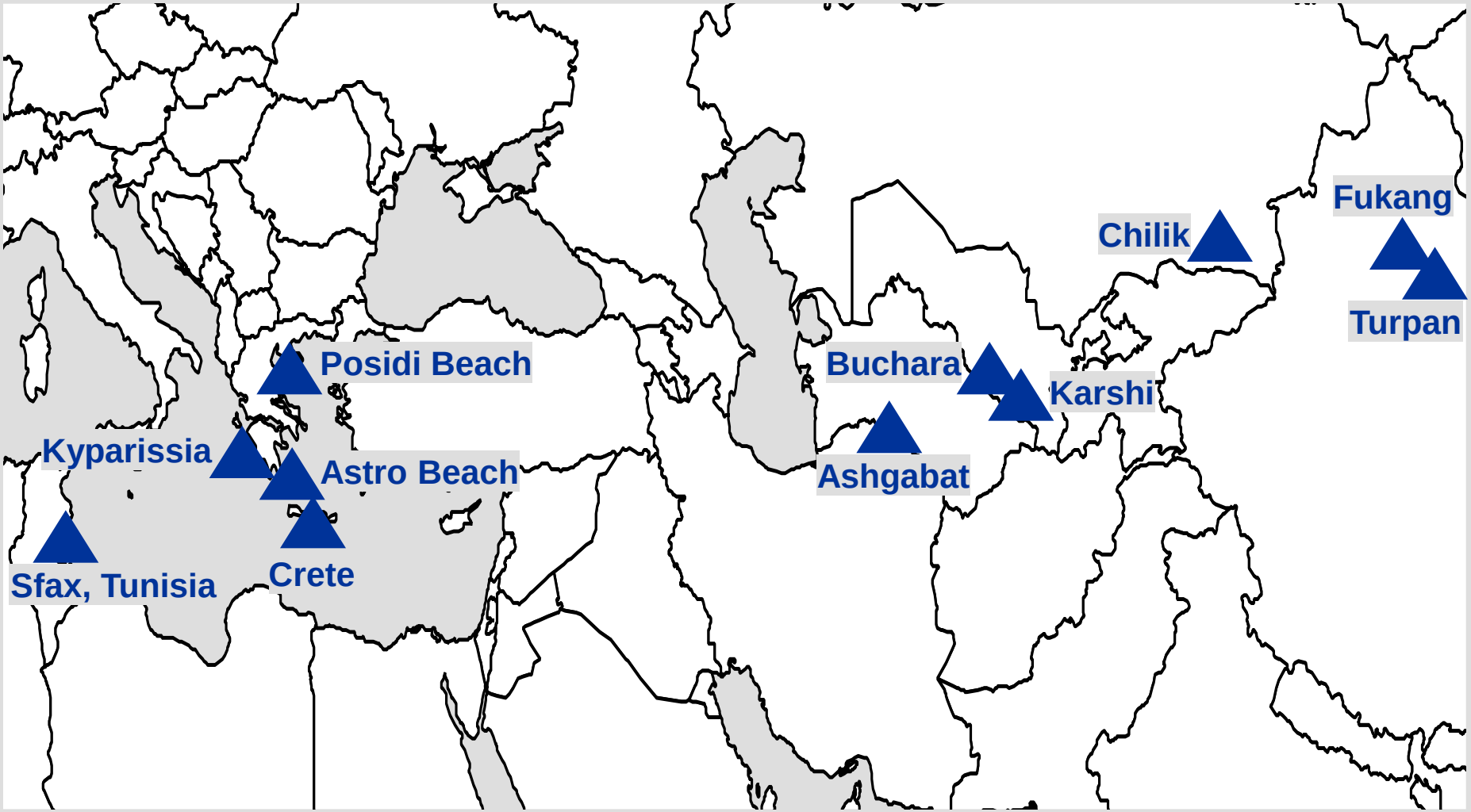
Outgroups and Analysis

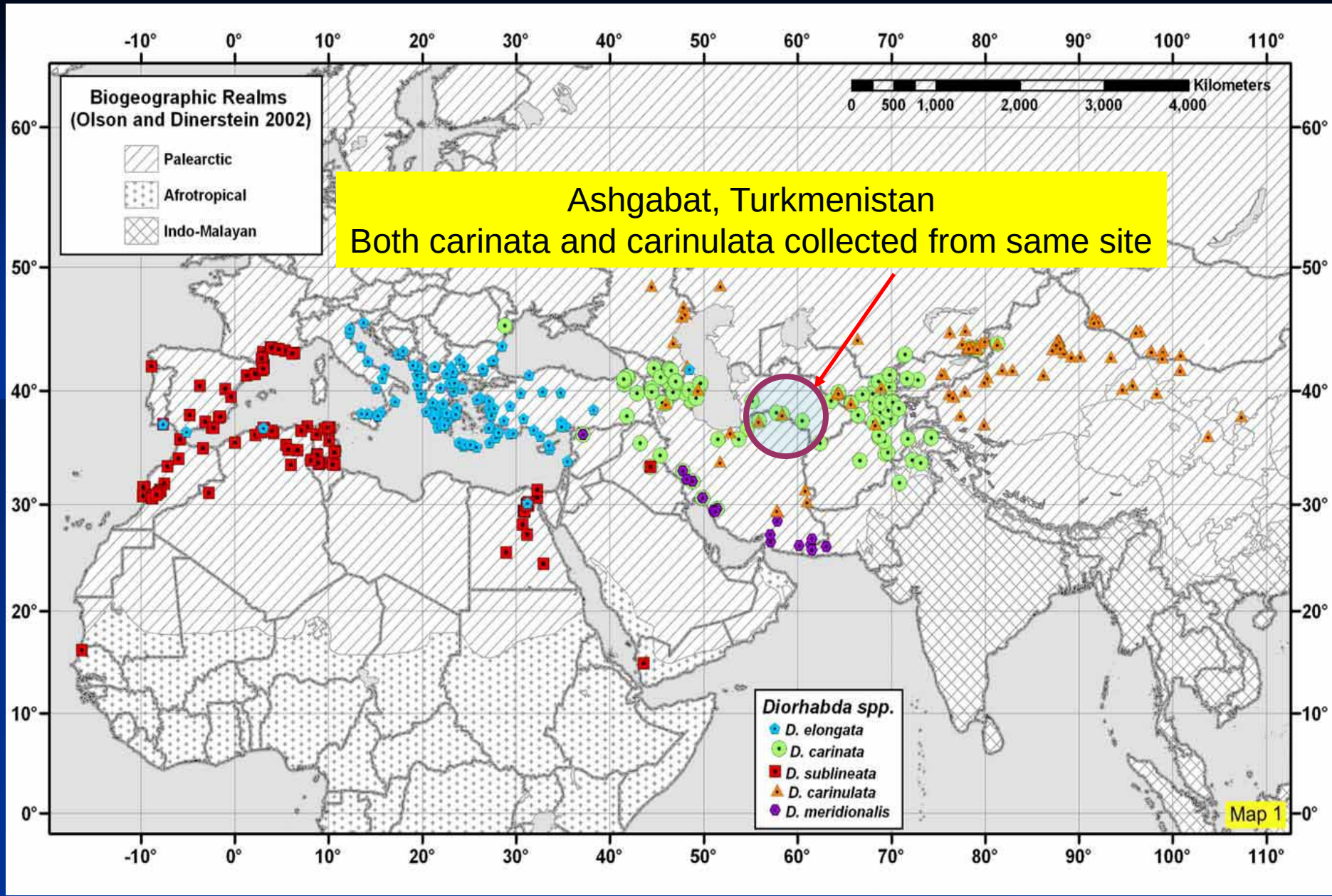
- Ingroup:
  - *Diorhabda* spp:
    - Galerucinae: Galerucini
- Outgroups:
  - *Galerucella birmanica* from China
    - Galerucinae: Galerucini
  - *Diabrotica* sp.
    - Galerucinae: Luperini
- Phylogenetic inference:
  - Distance, parsimony and likelihood methods

DNA Regions/Markers

- Mitochondrial DNA
  - Sequenced 1270 nt of cytochrome oxidase I (COI)
  - 316 variable sites
  - 240 parsimony-informative sites
  - 3rd codon position sites most variable
  - Transition/transversion ratio near 4:1
  - 36 of 49 sequences unique
- Amplified Fragment Length Polymorphisms (AFLPs)
  - Identified 115 AFLPs using 4 selective primer pairs
  - 100% repeatability

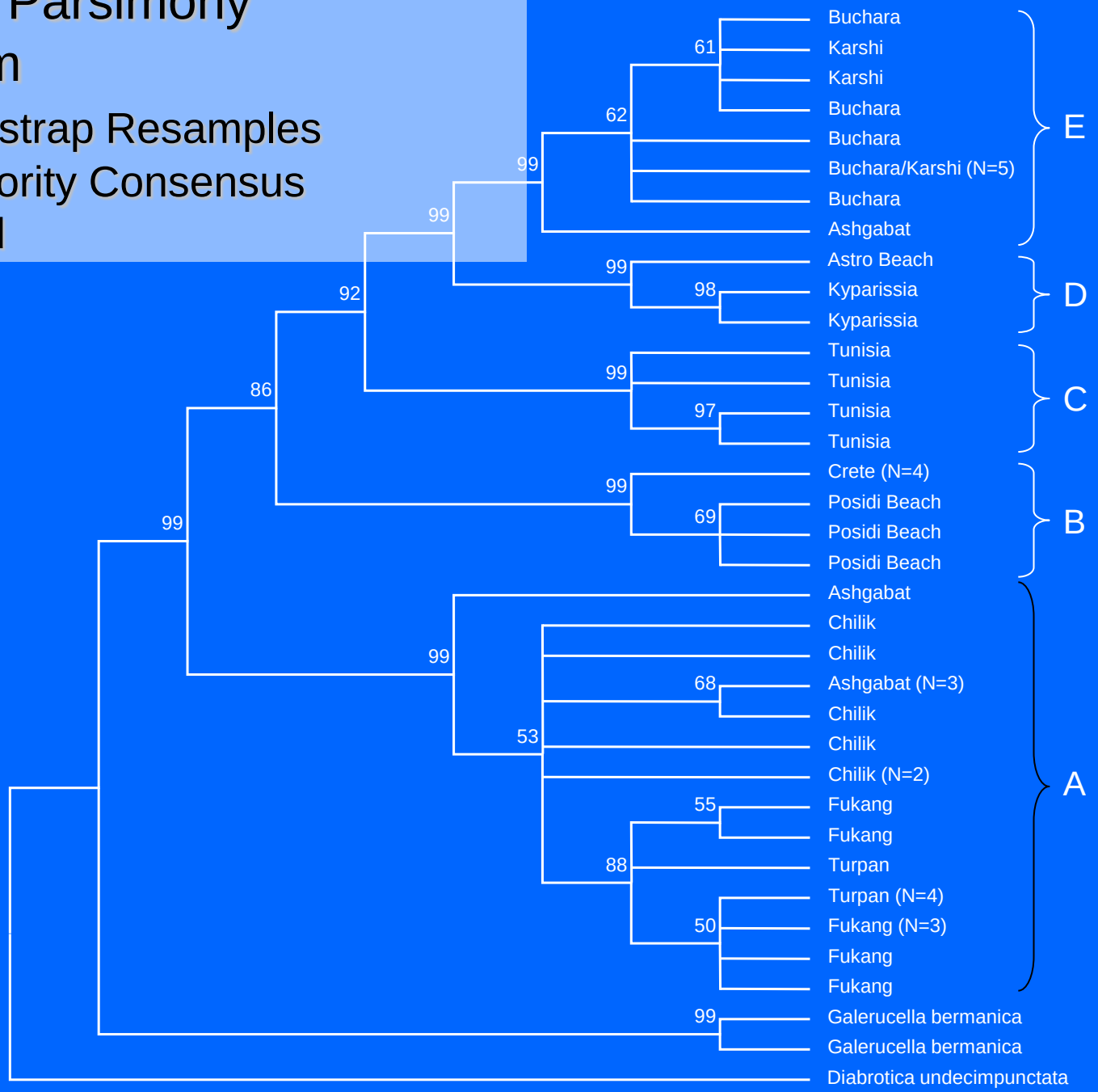
Cultures/Populations




Biogeographic Realms
(Olson and Dinerstein 2002)
Palearctic
Afrotropical
Indo-Malayan
Kilometers
0 500 1,000 2,000 3,000 4,000
Ashgabat, Turkmenistan
Both carinata and carinulata collected from same site
Diorhabda spp.
D. elongata
D. carinata
D. sublineata
D. carinulata
D. meridionalis
Map 1
-10° 0° 10° 20° 30° 40° 50° 60° 70° 80° 90° 100° 110°
60° 50° 40° 30° 20° 10° 0°

Maximum Parsimony Cladogram

500 Bootstrap Resamples
50% Majority Consensus
Unrooted



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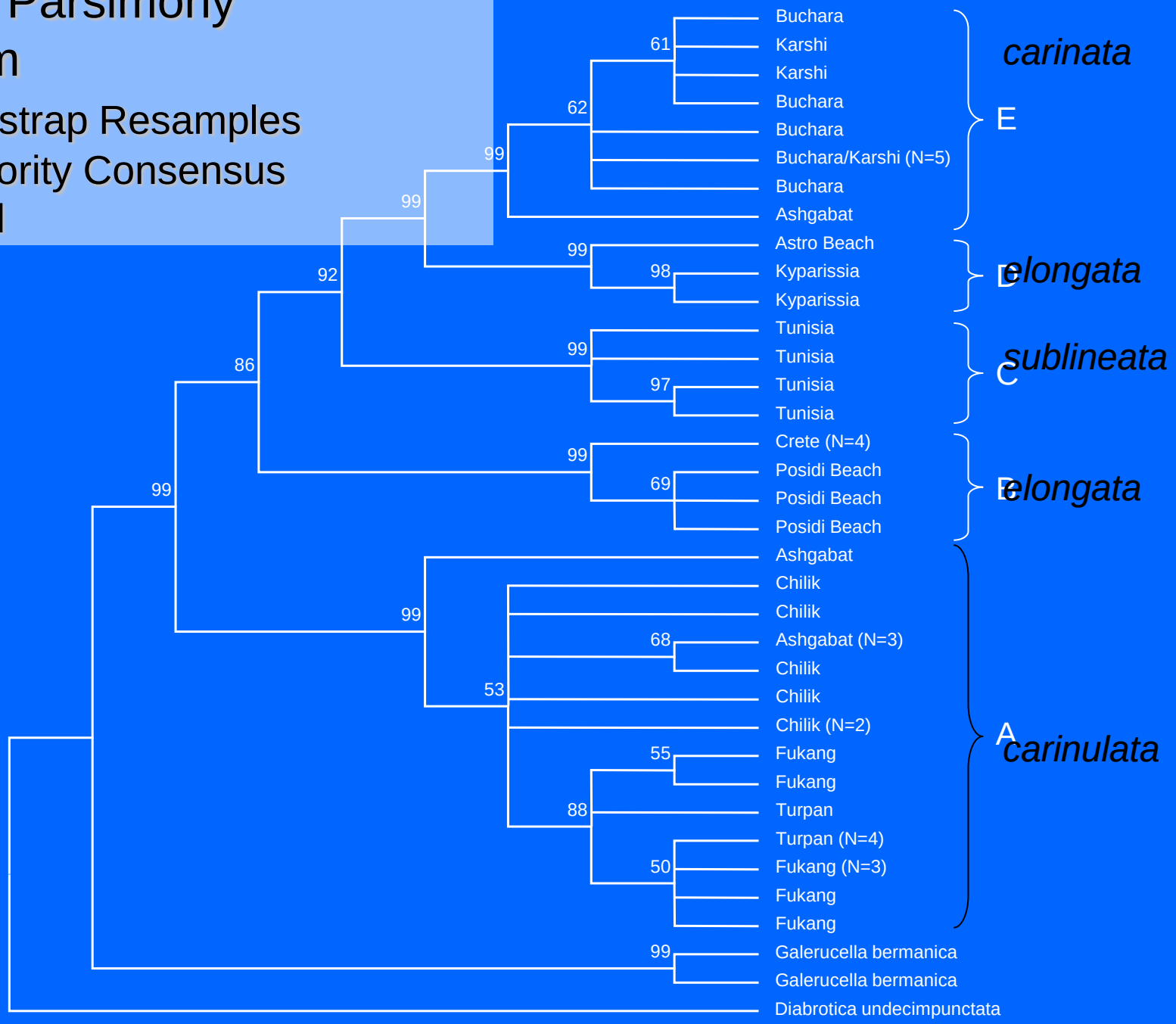
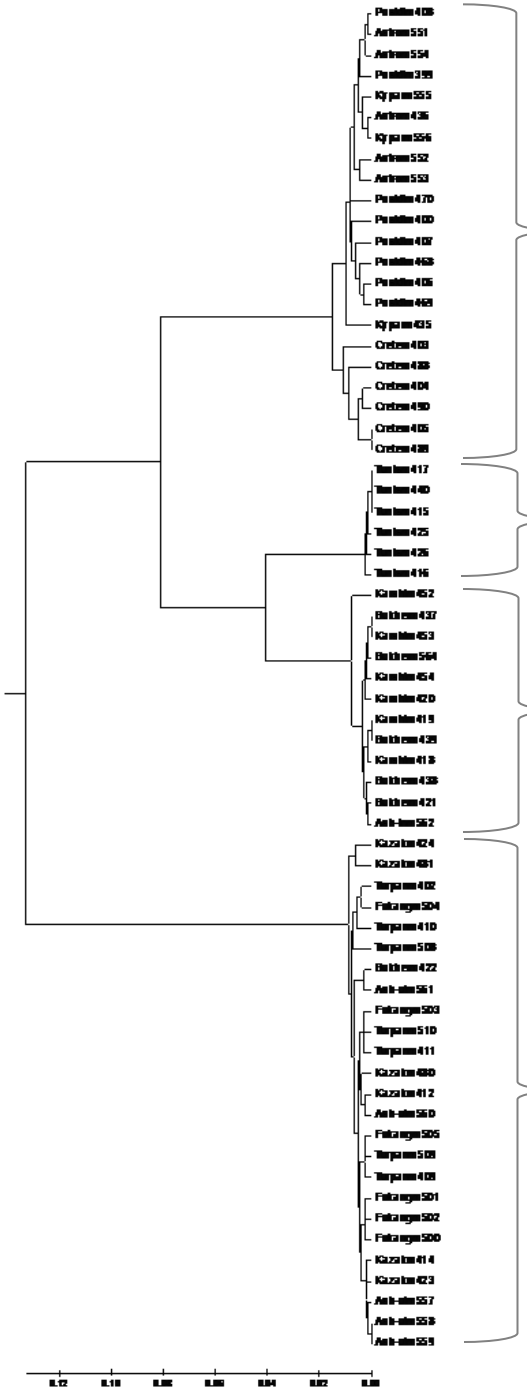


AFLP-Based UPGMA Dendogram



Astro Beach, Posidi Beach, Kyparissia, Crete → *Diorhabda elongata*

Tunisia → *Diorhabda sublineata*

Karshi, Buchara, Ashgabat → *Diorhabda carinata*

Kazakhstan, Turpan, Fukang, Ashgabat → *Diorhabda carinulata*

Conclusions

- 4 or 5 robust, deep DNA lineages are present in the *Tamarix*-feeding *Diorhabda* examined to date
  - The additional mt-DNA lineage may be due to lineage sorting
- The lineages are largely, but not completely, consistent with:
  - Proposed species delimitations based on morphology
  - Reproductive compatibility relationships within and among lineages

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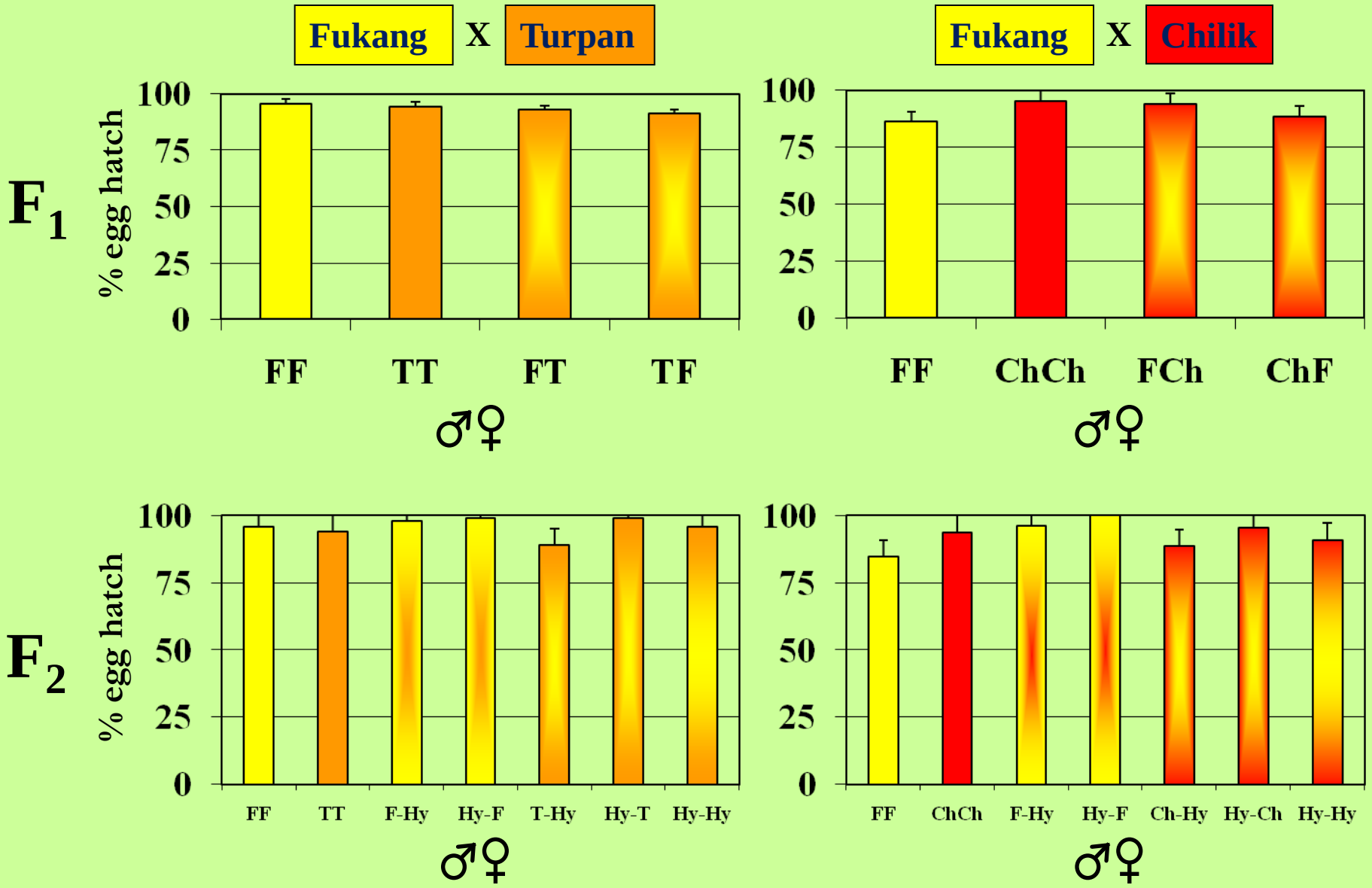
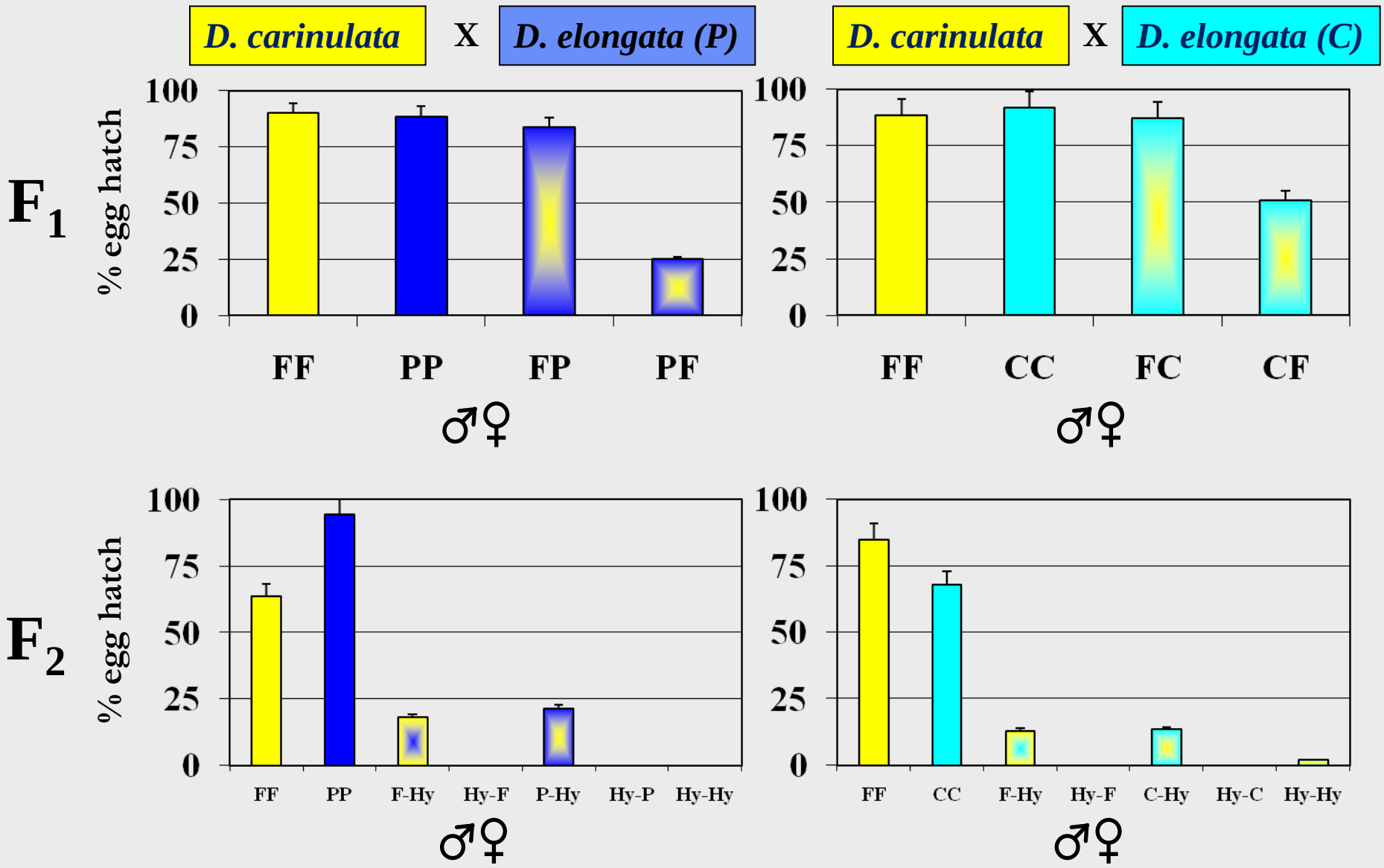
Hybridization potential of Saltcedar leaf beetle, *Diorhabda elongata*, ecotypes

- Measured:
  - Egg viability.
  - Time and percentage of larvae into pupation.
  - Time and percentage of pupae becoming adults.
- Almost all tests performed in small cup cages.
- F_1 and F_2 Generations with backcrosses for most.

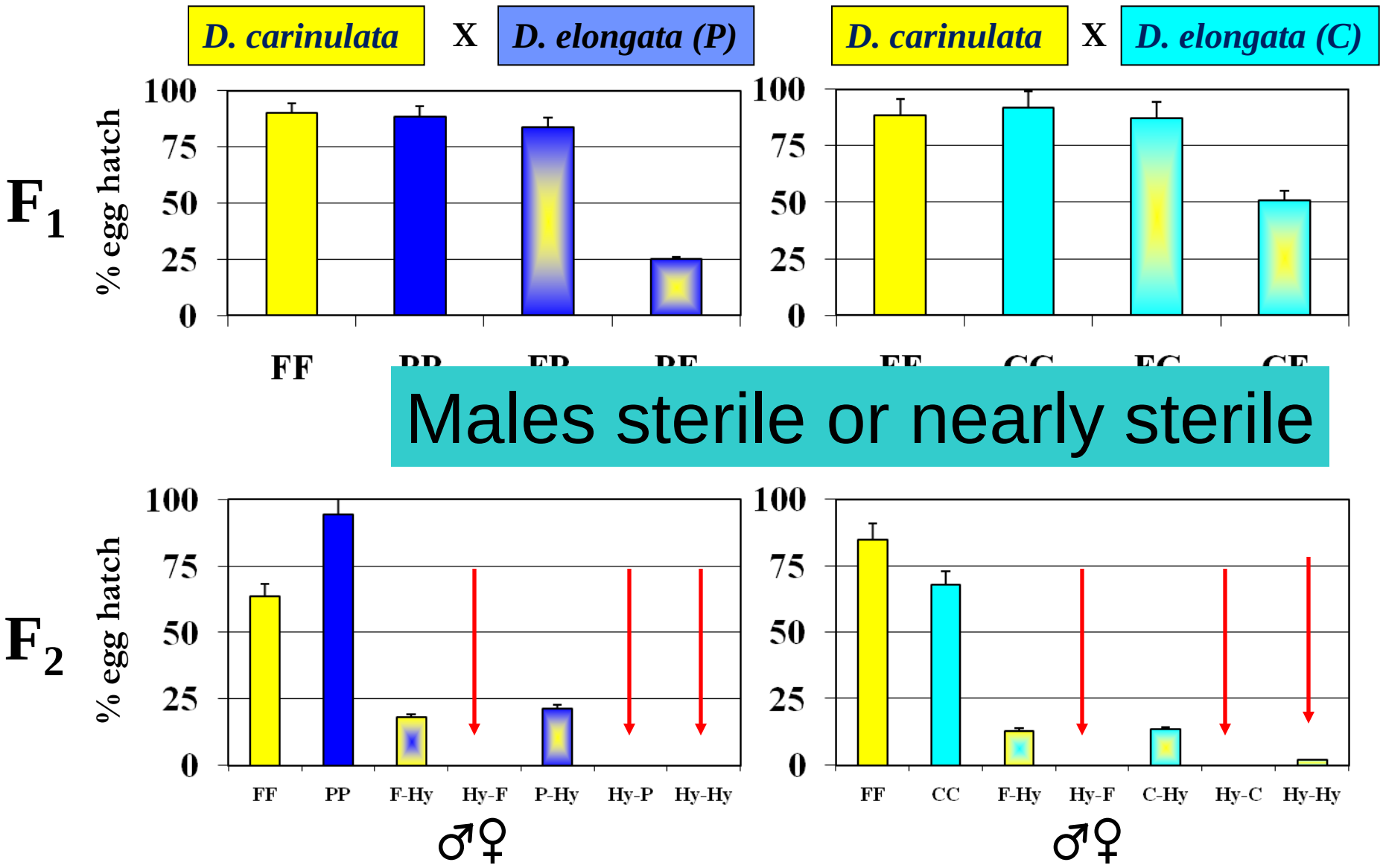
Crosses within *D. carinulata* are viable



Crosses between *D. carinulata* and *D. elongata* show low egg viability in the F_2



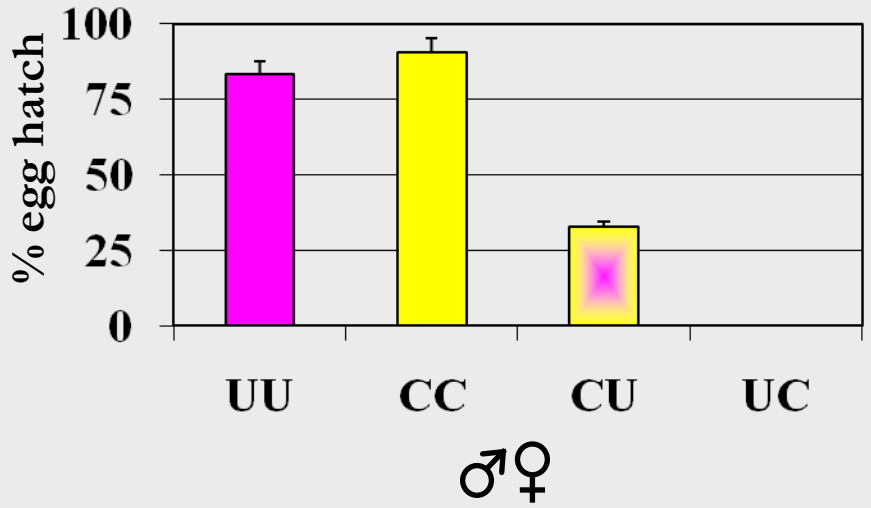
Crosses between *D. carinulata* and *D. elongata* show low egg viability in the F_2



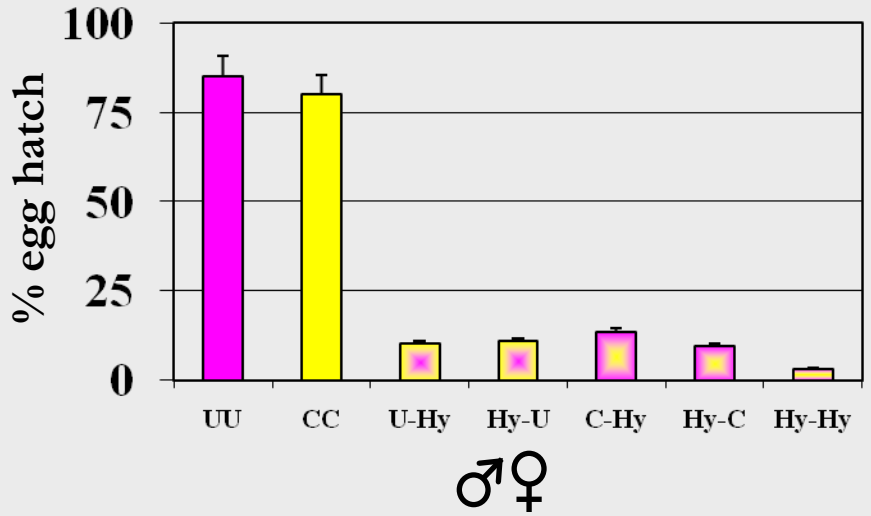
Crosses between *D. carinulata* and *D. carinata* show low egg viability in the F2
D. carinulata and *D. sublineata* crosses need to be done

D. carinulata X ***D. carinata***

F_1



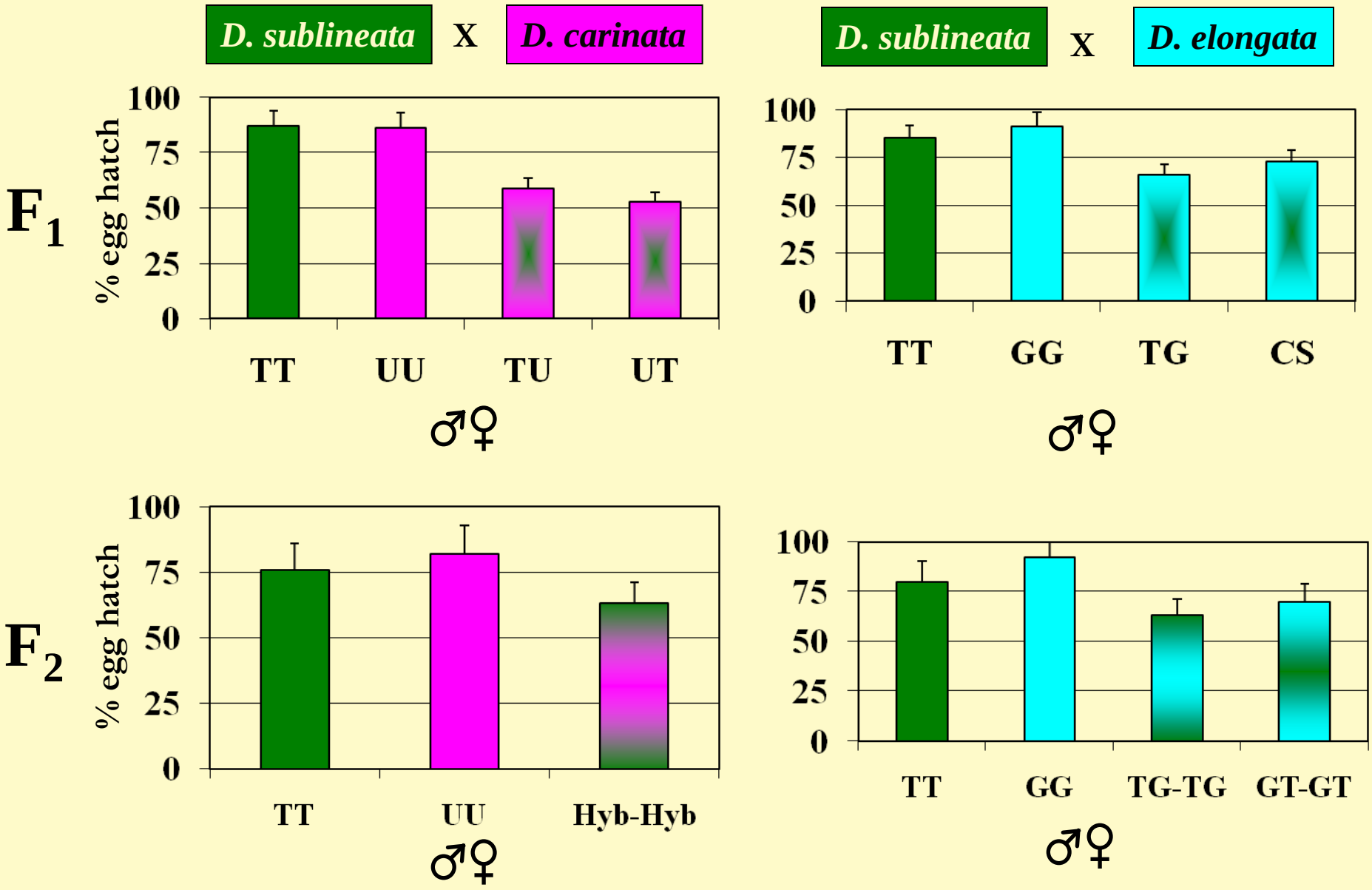
F_2



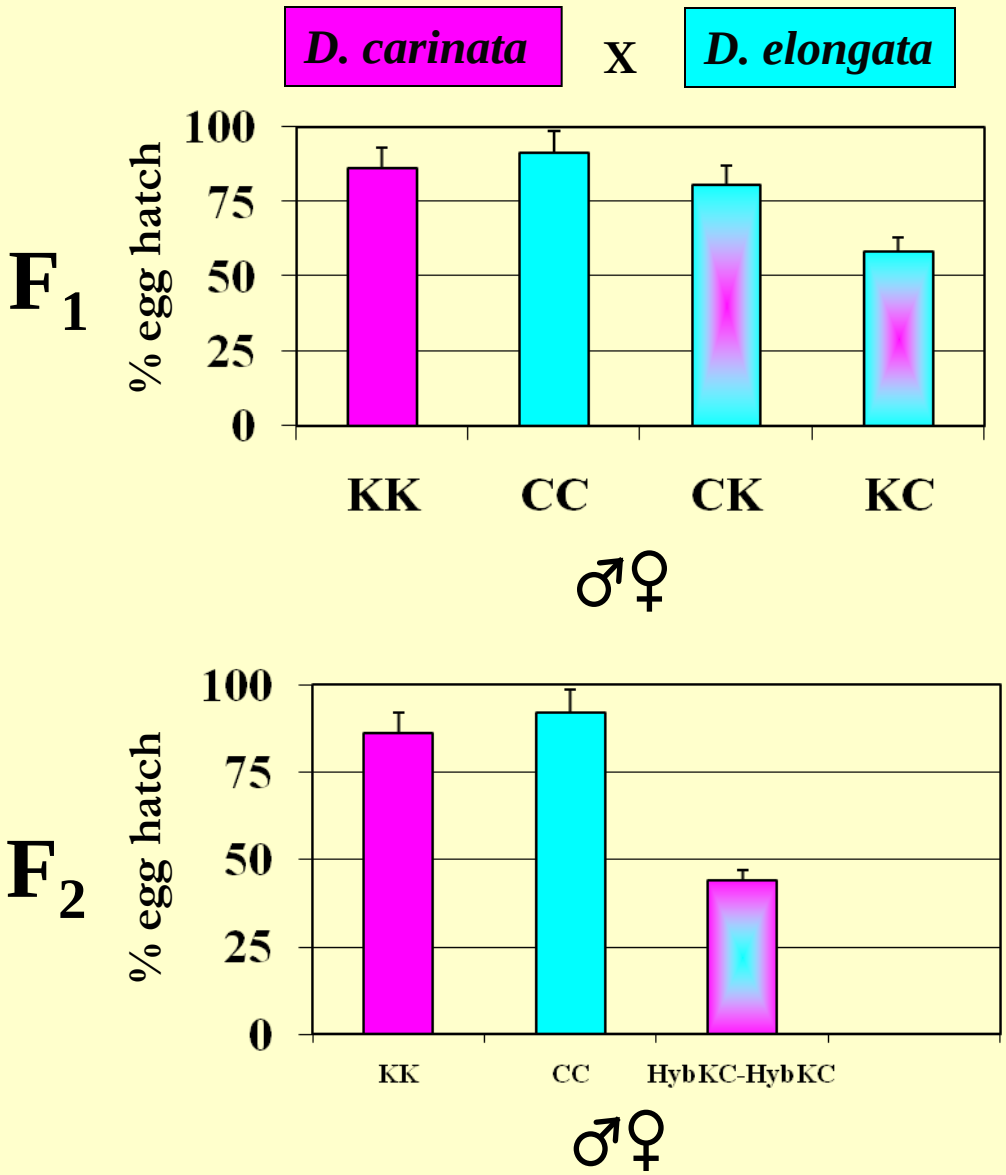
D. carinulata X ***D. sublineata***

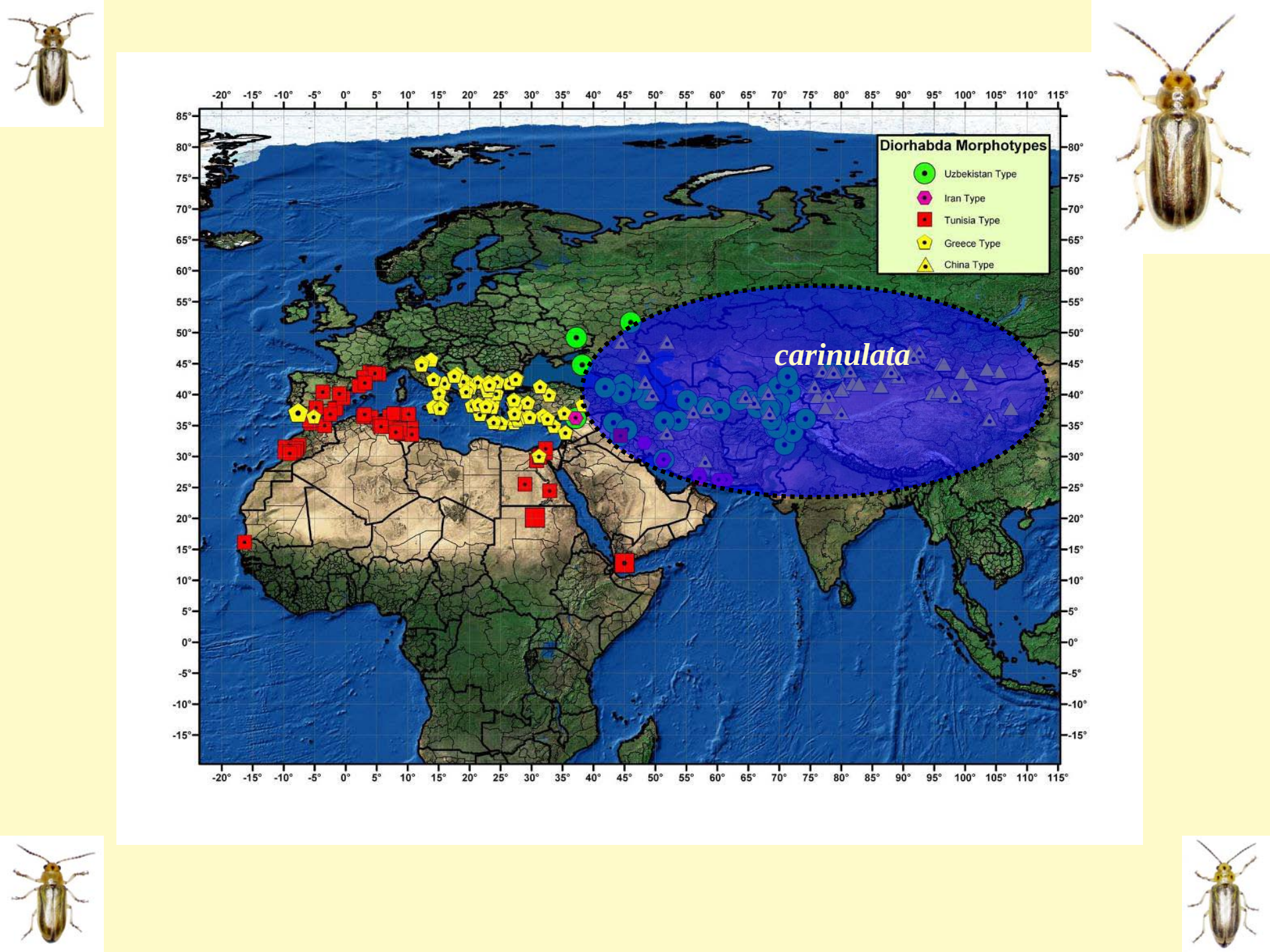
?

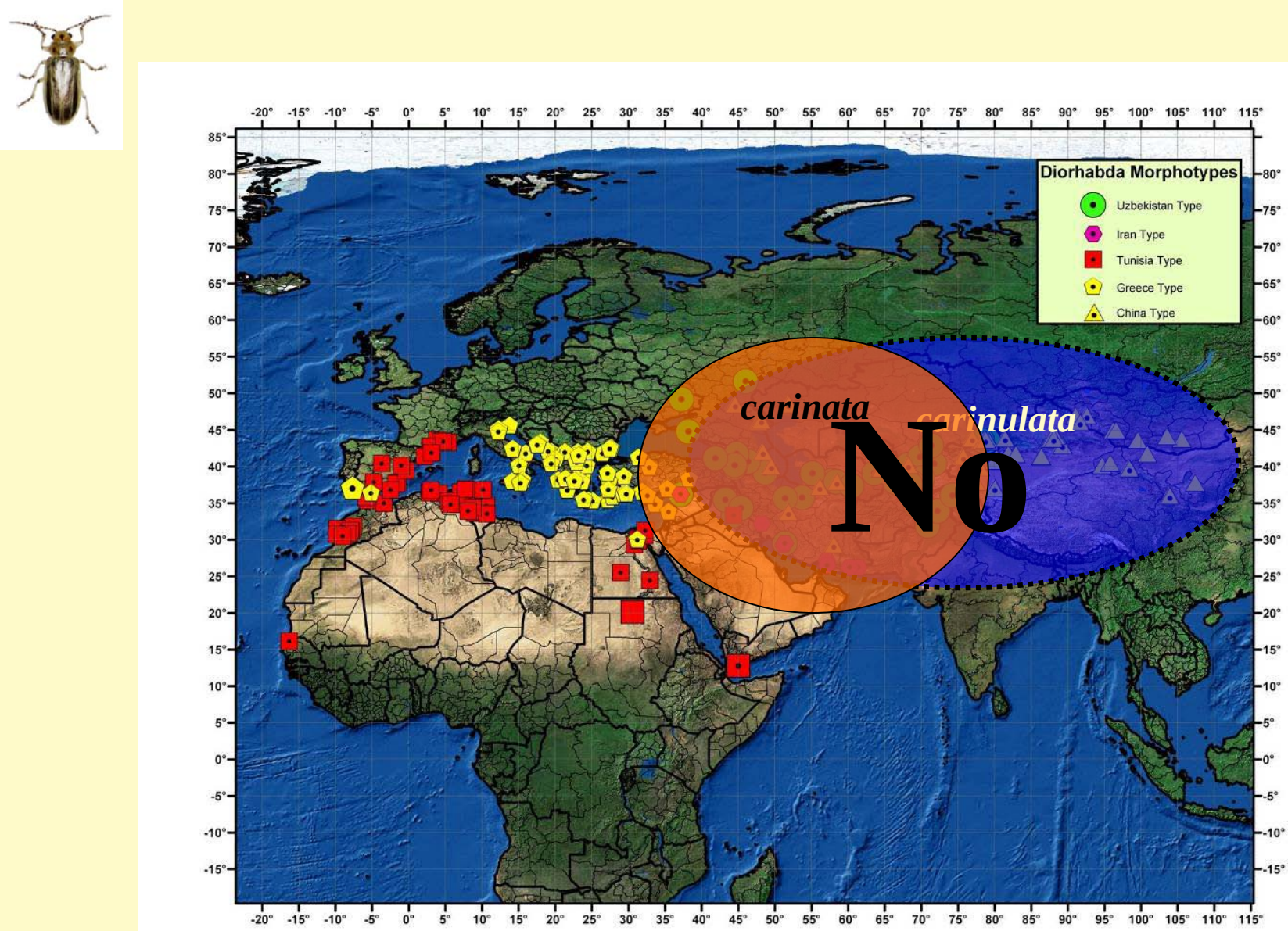
Crosses between *D. sublineata* and *D. carinata* or *D. elongata* yield high egg viability

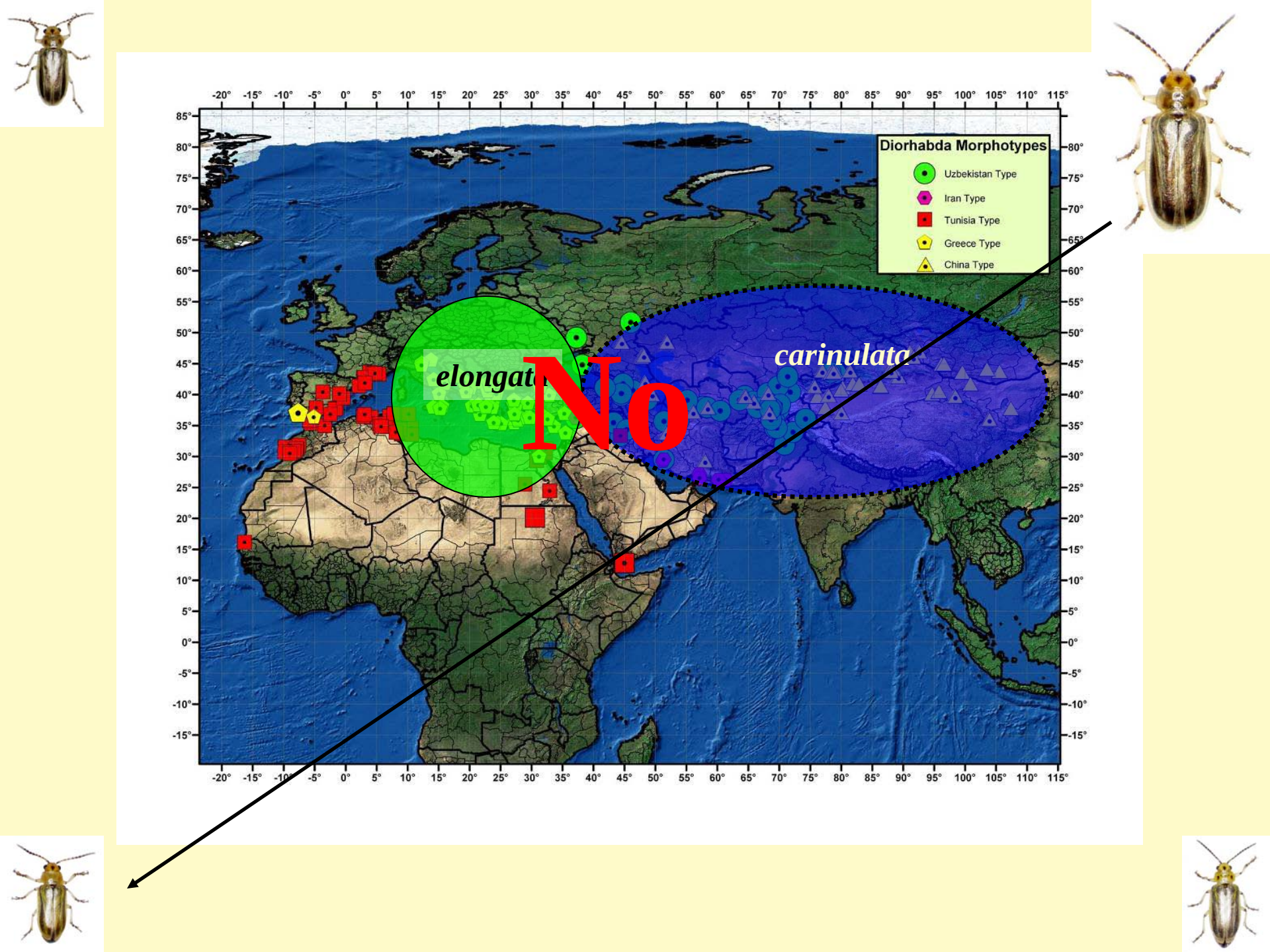


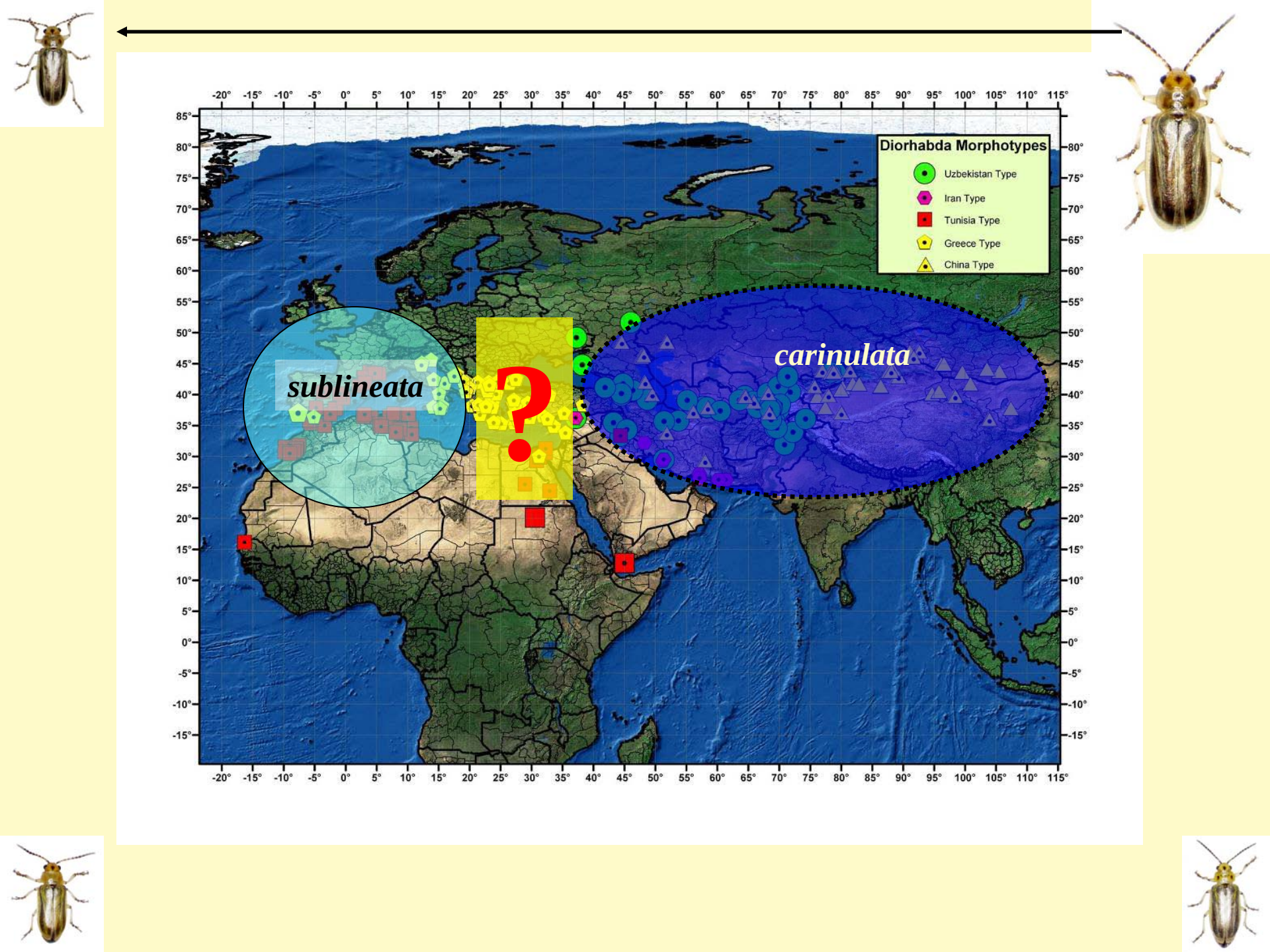
D. carinata and *D. elongata* hybrids are viable



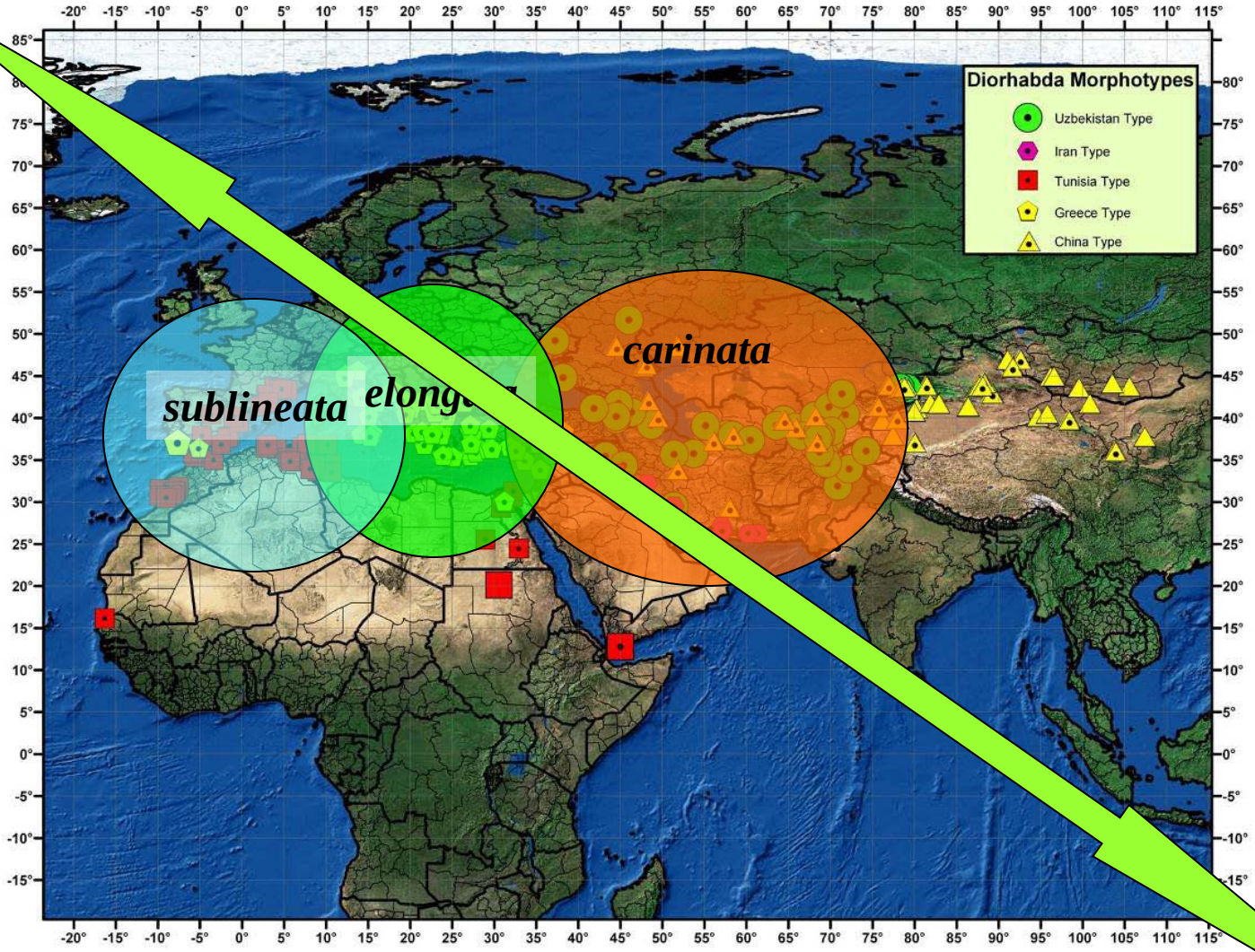

Diorhabda Morphotypes
Uzbekistan Type
Iran Type
Tunisia Type
Greece Type
China Type
carinulata


Diorhabda Morphotypes
Uzbekistan Type
Iran Type
Tunisia Type
Greece Type
China Type
carinata
carinulata
No

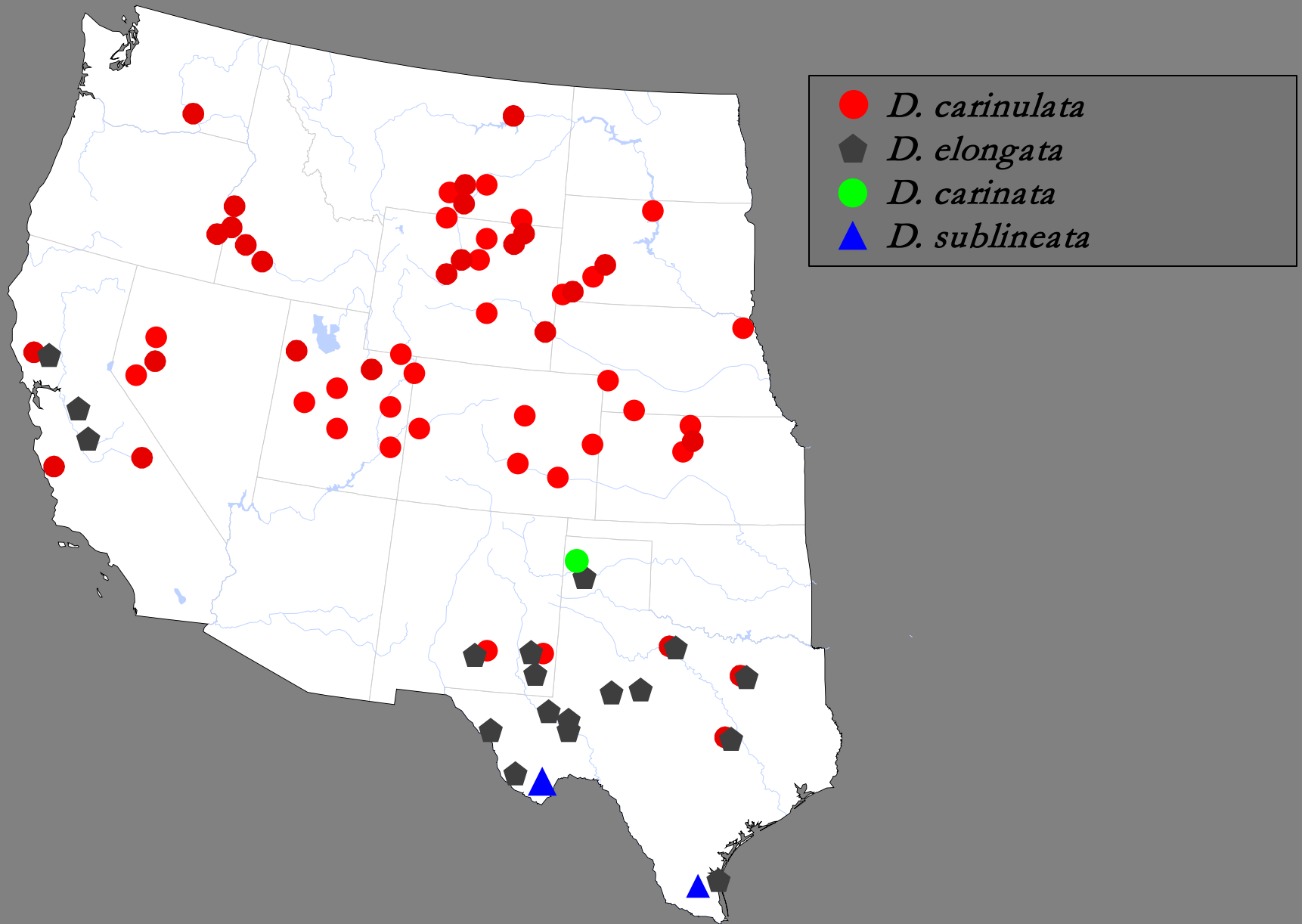

Diorhabda Morphotypes
Uzbekistan Type
Iran Type
Tunisia Type
Greece Type
China Type
elongata
carinulata
No


Diorhabda Morphotypes
Uzbekistan Type
Iran Type
Tunisia Type
Greece Type
China Type
sublineata
?
carinulata

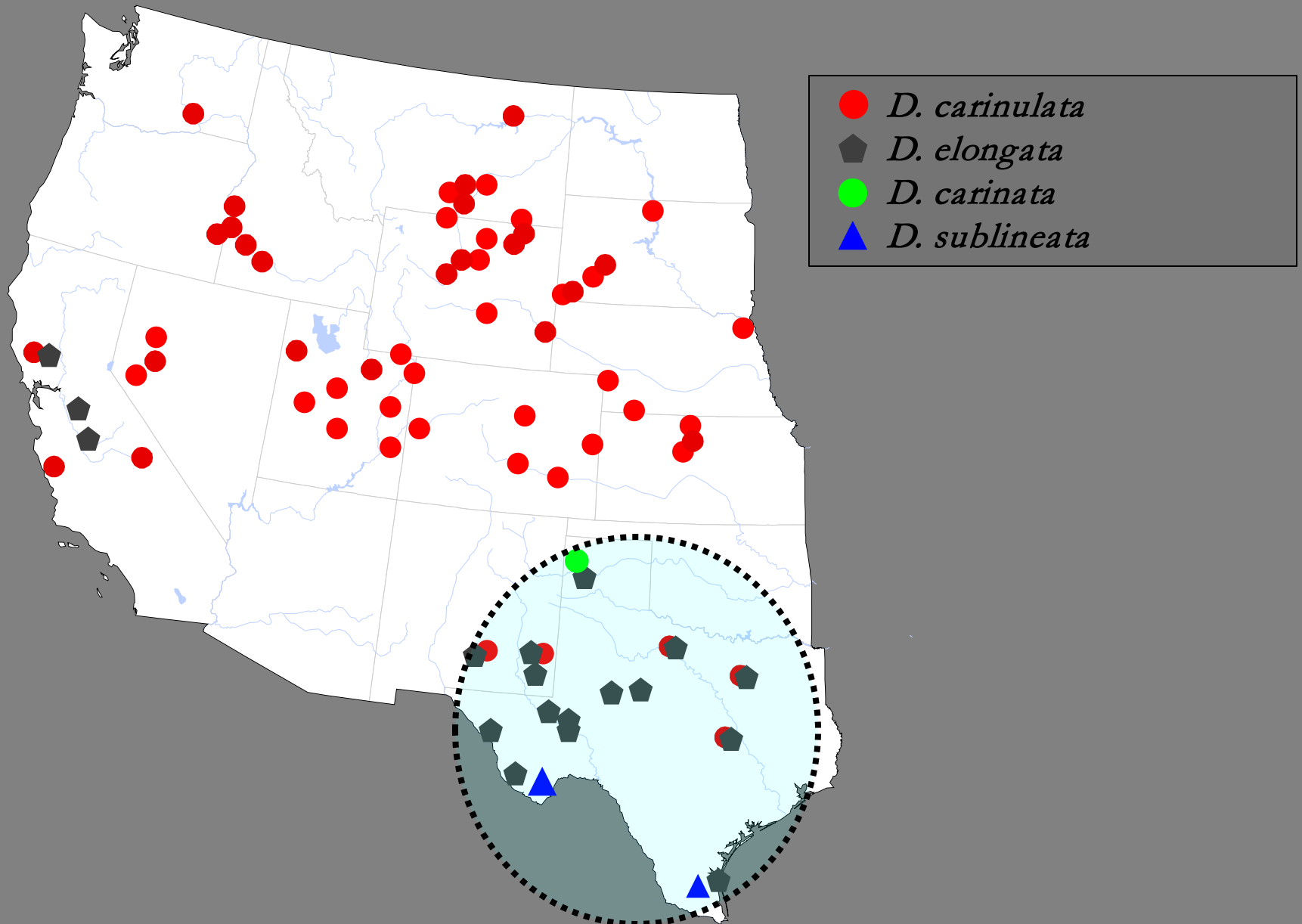
Hybrids show 50% egg viability, hybrid lines are stable



Diorhabda Releases (in part)



Diorhabda Releases (in part)



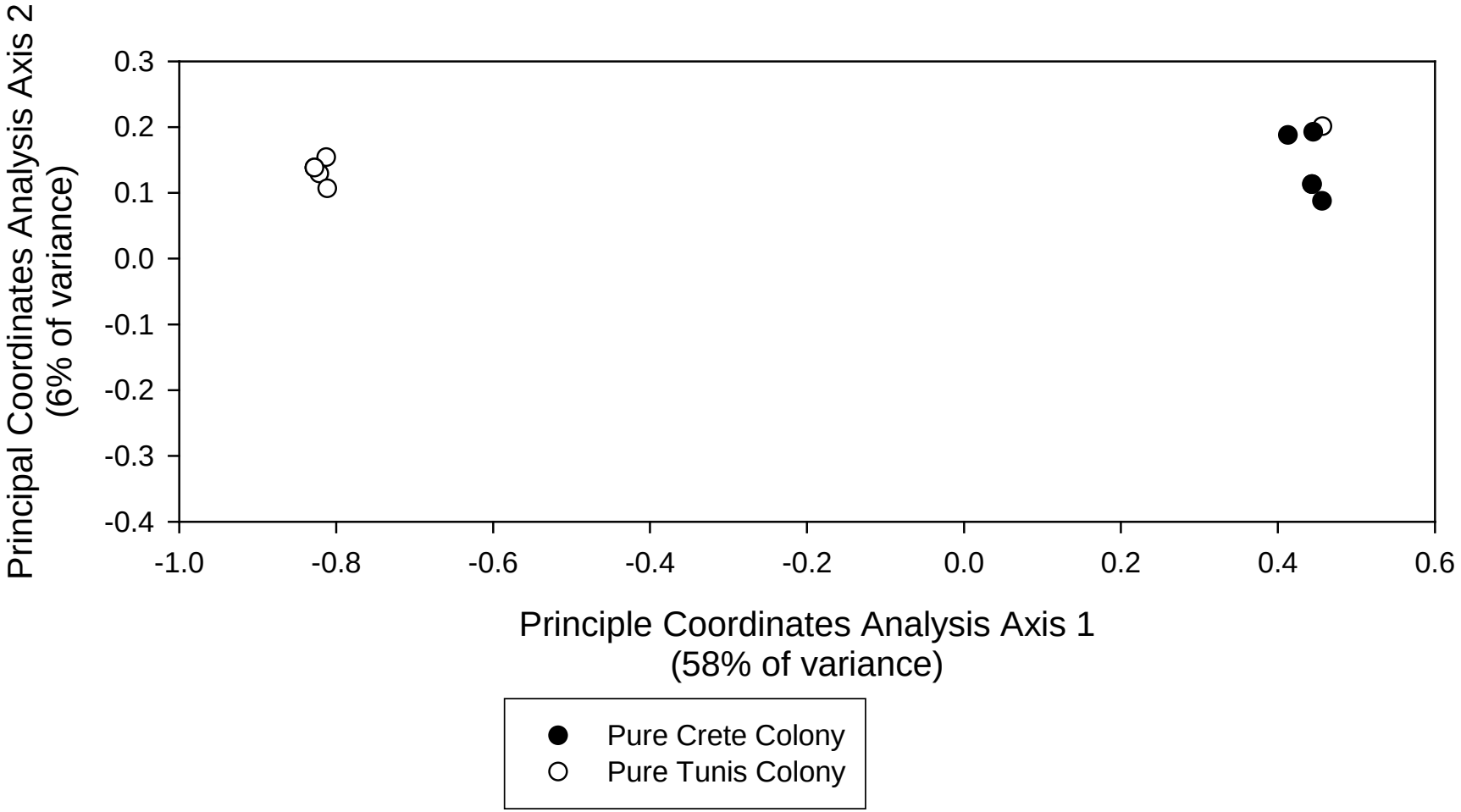
AFLP/PCOA Analysis of Hybridization: An Example Using Crete and Tunis Ecotypes

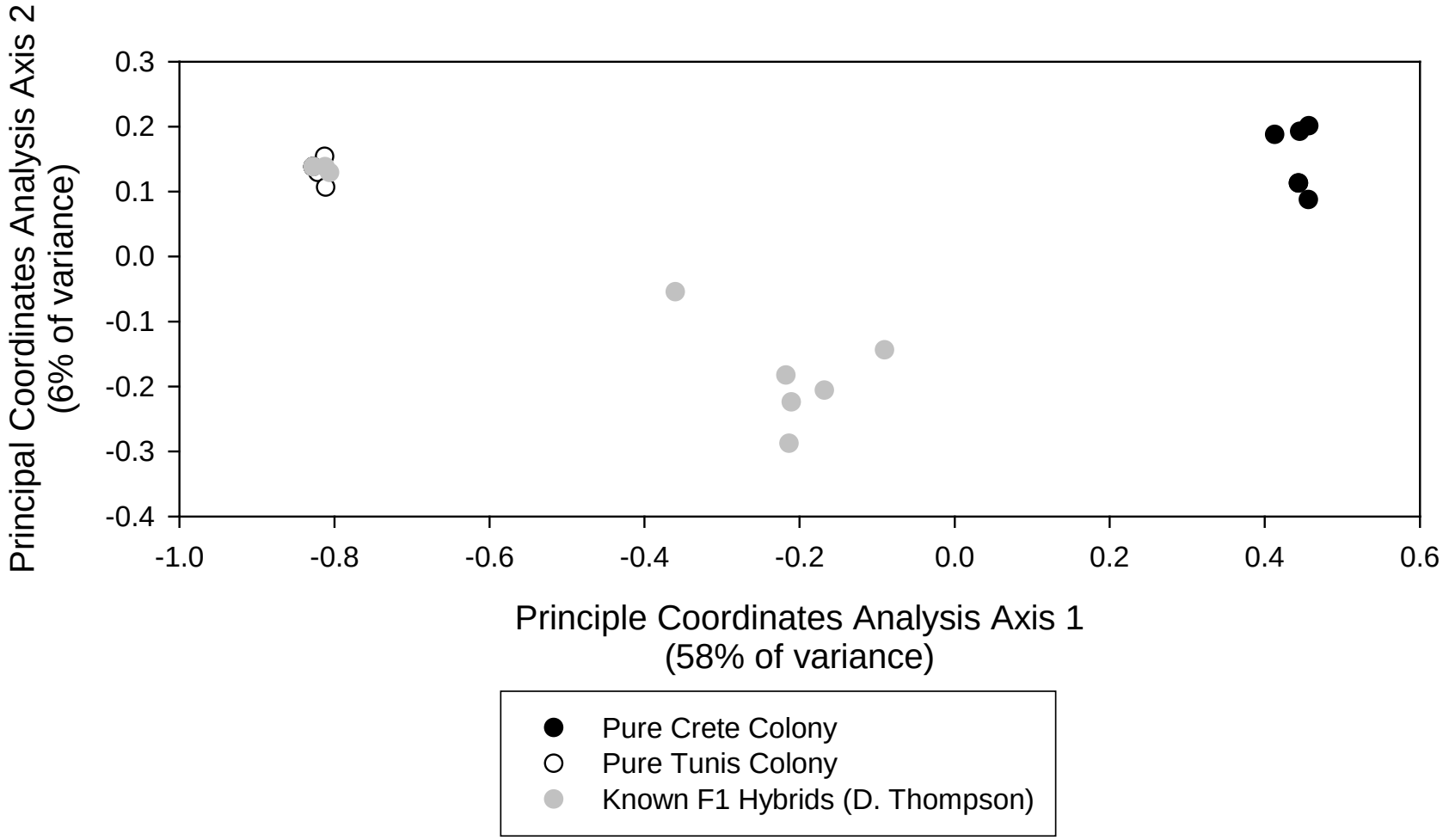
For an example in tamarisk itself see:

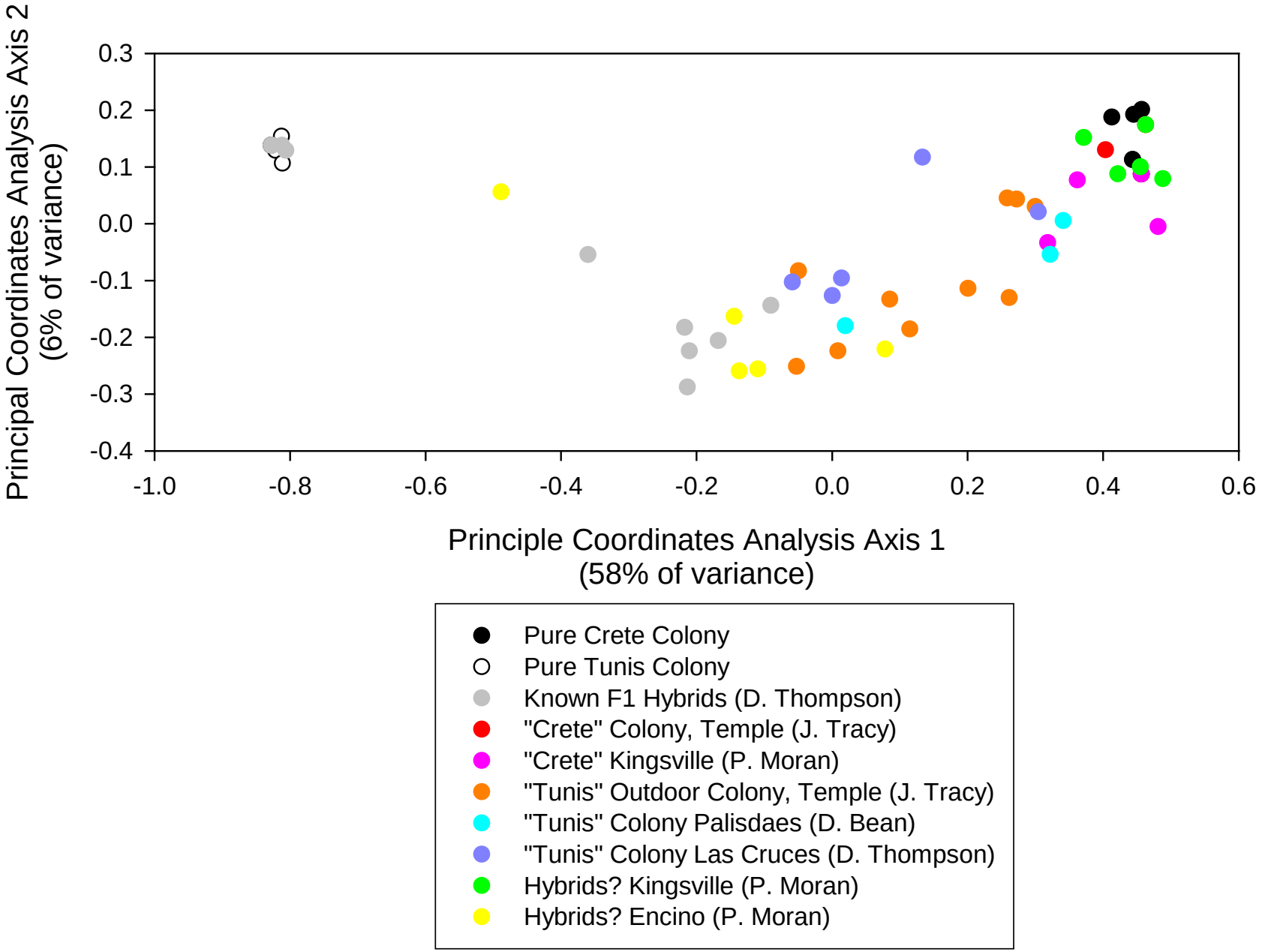
Gaskin, J.F and D.J. Kazmer. (2009) Introgression between invasive saltcedars (*Tamarix chinensis* and *T. ramosissima*) in the USA. Biological Invasions 11:1121-1130

Data and Analysis

- 52 AFLPs in the Crete and Tunis lineages
- 57 Individuals
- Binary AFLP data -> Dice similarity coefficients -> Principal Coordinates Analysis (PCOA)
  - PCOA is an ordination technique similar to Principal Components Analysis (PCA)


Principal Coordinates Analysis Axis 2
(6% of variance)
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-1.0
-0.8
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6
Principle Coordinates Analysis Axis 1
(58% of variance)
Pure Crete Colony
Pure Tunis Colony


Principal Coordinates Analysis Axis 2
(6% of variance)
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-1.0
-0.8
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6
Principle Coordinates Analysis Axis 1
(58% of variance)
Pure Crete Colony
Pure Tunis Colony
Known F1 Hybrids (D. Thompson)


Principal Coordinates Analysis Axis 2
(6% of variance)
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-1.0
-0.8
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6
Principle Coordinates Analysis Axis 1
(58% of variance)
Pure Crete Colony
Pure Tunis Colony
Known F1 Hybrids (D. Thompson)
"Crete" Colony, Temple (J. Tracy)
"Crete" Kingsville (P. Moran)
"Tunis" Outdoor Colony, Temple (J. Tracy)
"Tunis" Colony Palisdaes (D. Bean)
"Tunis" Colony Las Cruces (D. Thompson)
Hybrids? Kingsville (P. Moran)
Hybrids? Encino (P. Moran)

Conclusion

- AFLP/PCOA is a powerful tool for analyzing hybridization between genetically distinct lineages. Hybrids were discovered between *D. sublineata* and *D. elongata*.
  - But note the requirement for genetically distinct lineages

Molecular and morphological analysis show that 4 closely related species in the *D. elongata* complex are currently being used in *Tamarix* biocontrol. These have traits that can play a role in targeting specific *Tamarix* infestations and ecological settings. Molecular techniques allow tracking of species and tracking genetic introgression between species.

Thanks to:

Julie Keller
Tammy Wang
Beth Peterson
Tom Dudley
James Tracy
Tom Robbins
Kevin Gardner