Using genetics to inform invasive weed management

Tales from flowering rush, common mullein, and a new thistle species

John Gaskin
Botanist
Sidney, MT

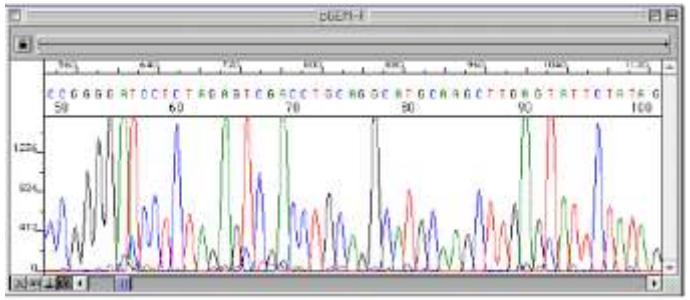
USDA Agricultural Research Service

Questions that DNA can help answer

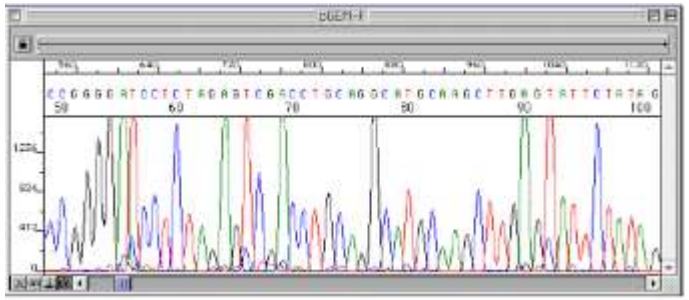
- What species is this weed?
  - Know your enemy
- How does it reproduce?
  - Helps us design control options
- Are there different genotypes?
  - Differences in resistance or tolerance to control
- How are genotypes distributed?
  - What control to use where
- Where did the invasion come from?
  - Finding host-specific enemies

Which DNA method/marker?

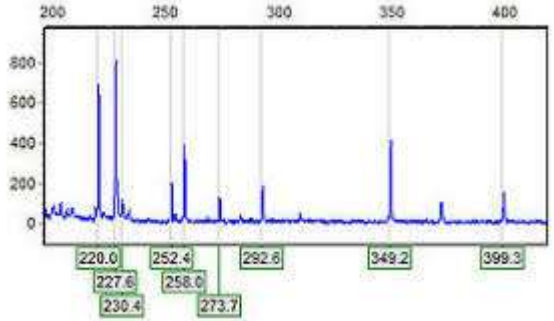
Slowly evolving DNA

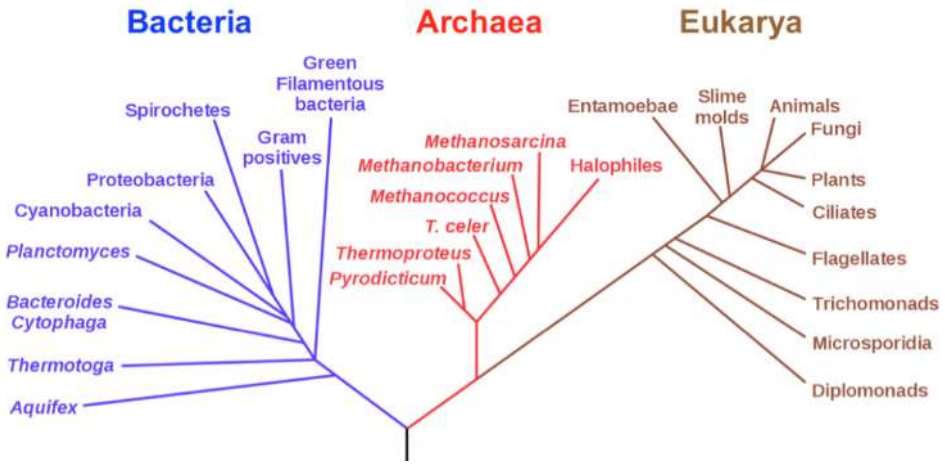


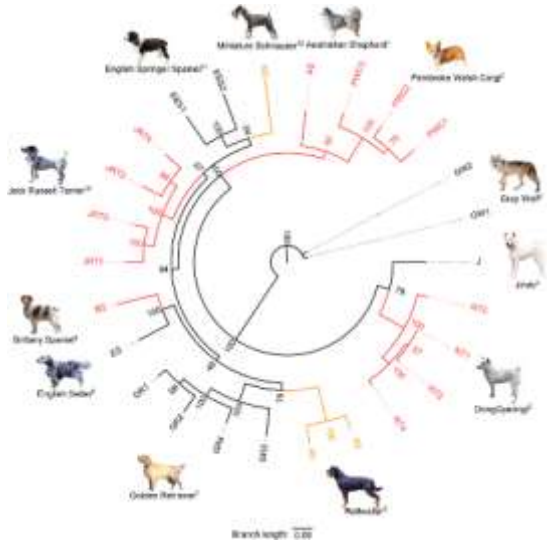
Moderately fast evolving DNA

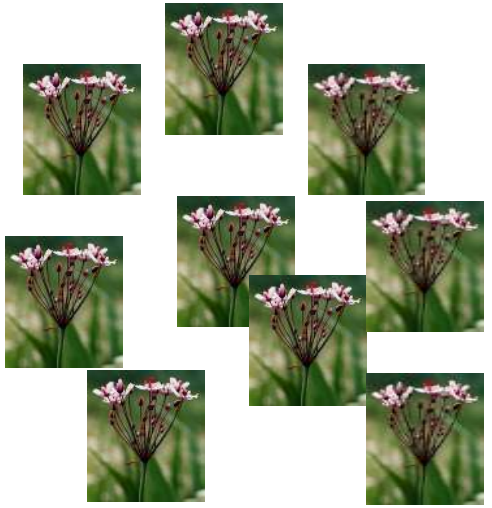


Really fast evolving DNA
Microsatellites
RadSeq
AFLP

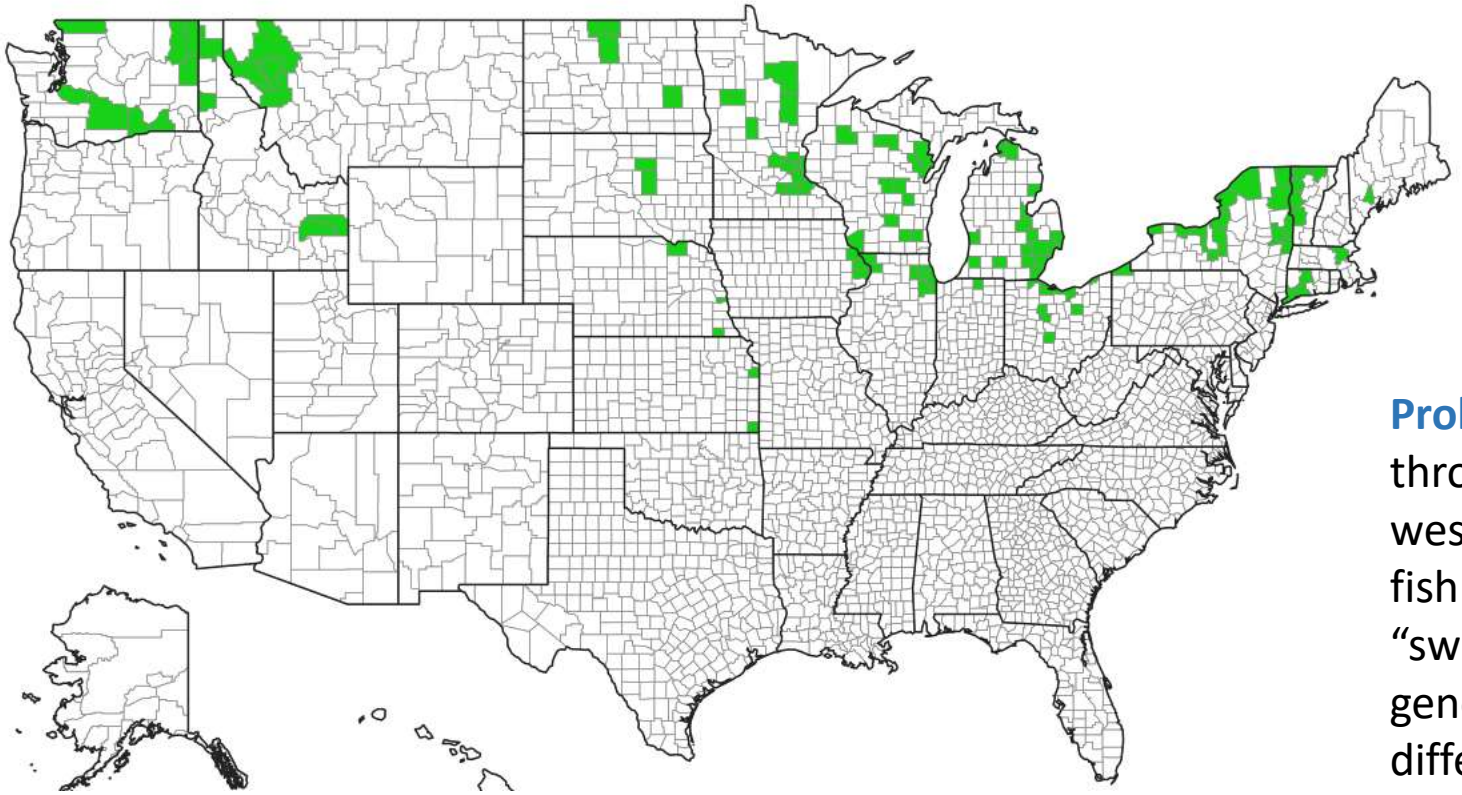








Flowering rush (Butomus)



Problem: Flowering rush is now spreading throughout river and lake systems in western North America, eliminating native fish habitat and enhancing diseases such as “swimmers itch”. Different flowering rush genotypes are invading that behave differently in terms of reproduction and management needs, such as potential biological control.

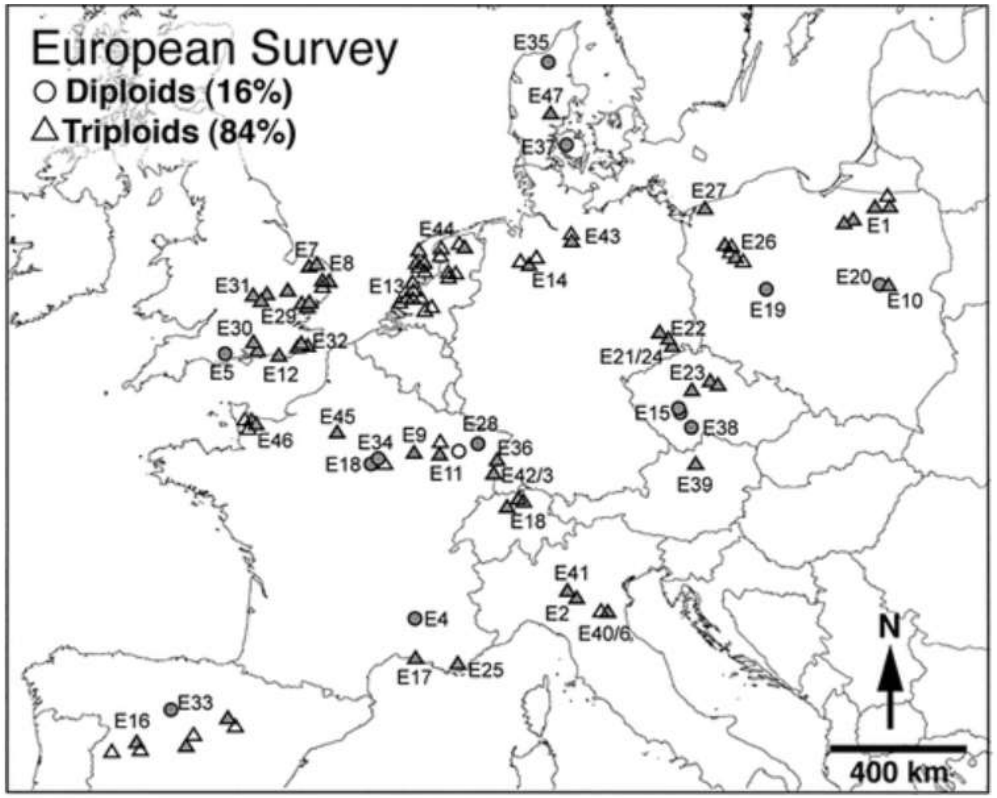
eddmaps

DNA analysis questions

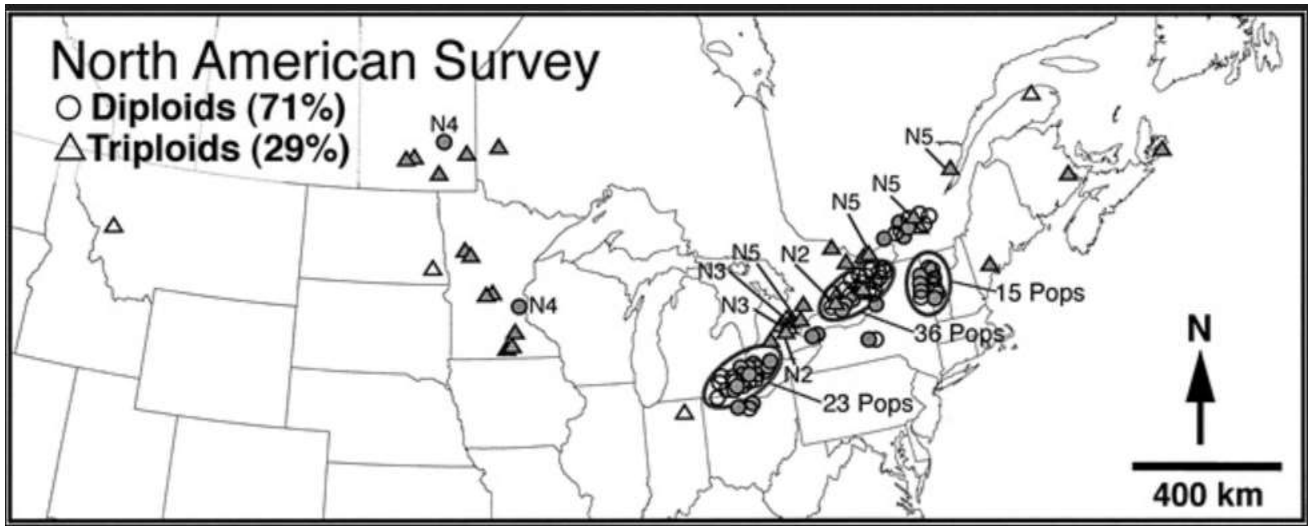
- What ploidy is the invasion (2x-diploid/3x-triploid)?
- What genotypes exist in N. America?
- Where are genotypes distributed?
- Where did genotypes come from in Eurasia?
  - Native to: Afghanistan, China, India, Kashmir, Kazakhstan, Kyrgyzstan, Mongolia, Pakistan, Russia, Tajikistan, Uzbekistan; SW Asia, Europe. More?

We already know some stuff:

- Most studies focused on eastern North America
- Changes from diploid to triploid may be historical and recent and recurring in Europe (some diploid and triploid plants genetically similar). Enzyme study. Kirschner et al. 2002.
- Most natives triploid, most invasives diploid. Kliber and Eckert 2005.
- Both diploid and triploid NA pops usually clonal, even though diploid seed viable. Eckert et al. 2003.

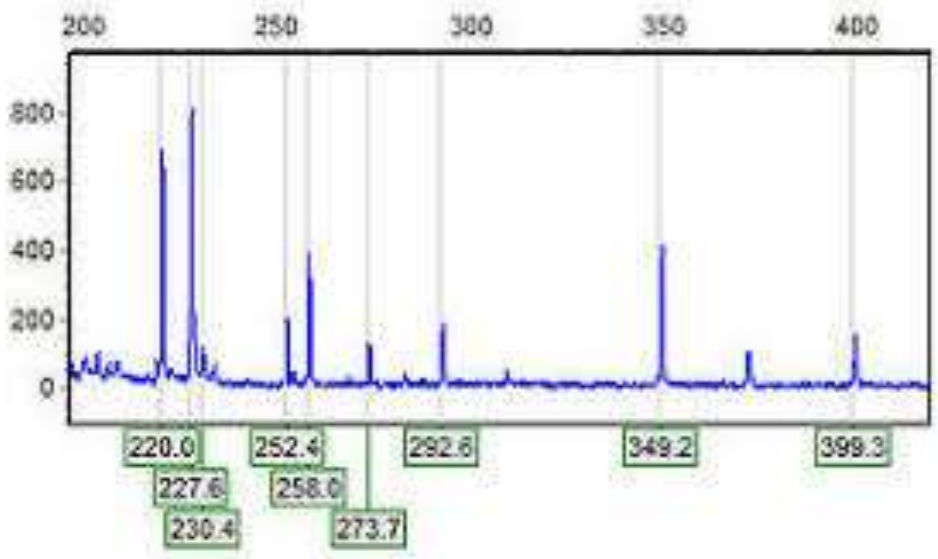


Kliber and Eckert 2005.

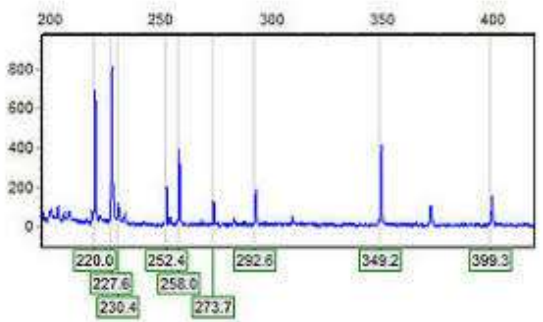


Now, let's use AFLPs to try to answer questions about western North America invasion.

- We have samples from:
  - PNW
  - Europe (looking for matches for biocontrol)
  - Midwest and Eastern USA



Butomus (flowering rush) AFLP genotype similarity - PCOA

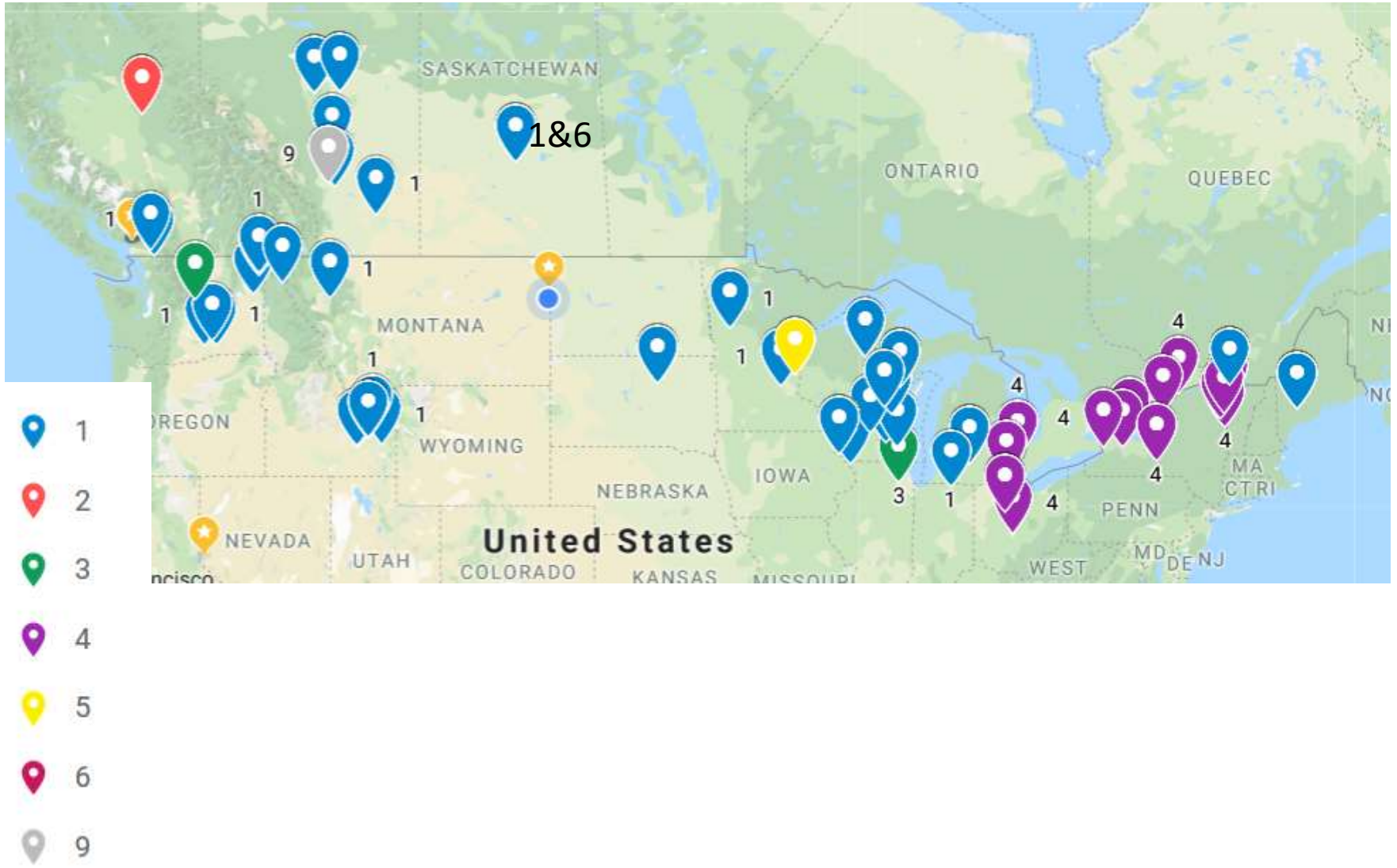


Slovakia n=7, Euro nursery n=2
Slovakia n=9
0.40
Serbia n=2
Serbia
Germany n=5
Serbia n=2
Slovakia n=5
Slovakia n=10
Euro seed
Slovakia
Slovakia n=2
Slovakia
Hungary
Slovakia
Czech Rep. n=10
Slovakia n=8
Euro seed/nursery n=5
0.18
Germany/Poland n=4
Germany/Poland n=16
Hungary
Hungary
Germany n=10
Hungary n=2
Poland
BC n=8
Slovakia n=4
Slovakia
Poland n=4
Hungary
Hungary
Serbia n=4
Serbia n=4
Hungary n=3
-0.04
Serbia n=4
Euro seed
Euro nursery n=1, IL n=9
Georgia n=2
12.1%
Serbia
Georgia
Euro seed
Euro seed
Euro seed
-0.26
Georgia
Georgia n=2
Euro seed
Georgia n=2
Georgia
Georgia
OH, MI, NY n=30
MI n=10
MN n=10
-0.48
-0.56
-0.30
-0.04
0.21
0.47
46.1%

AB
BC
WA
ID
MT
MN
WI
MI
n=159

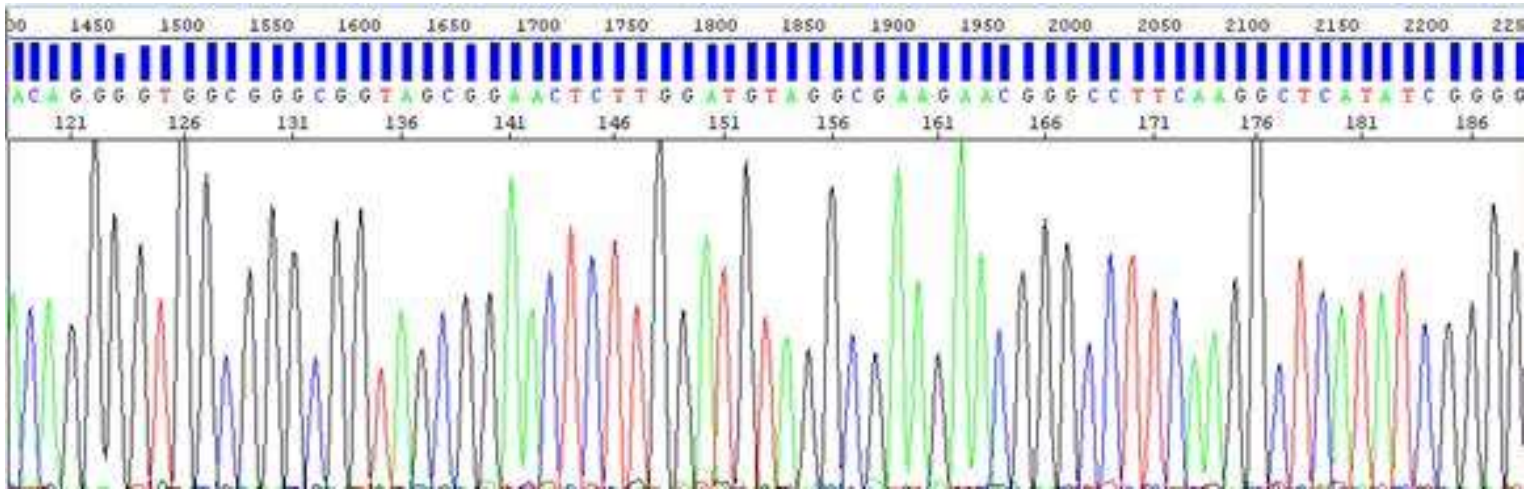
n=1 unless stated otherwise
= North America (non-native)
= Europe (native)

How are they distributed?



There were some unexpected genotypes; are they Butomus?

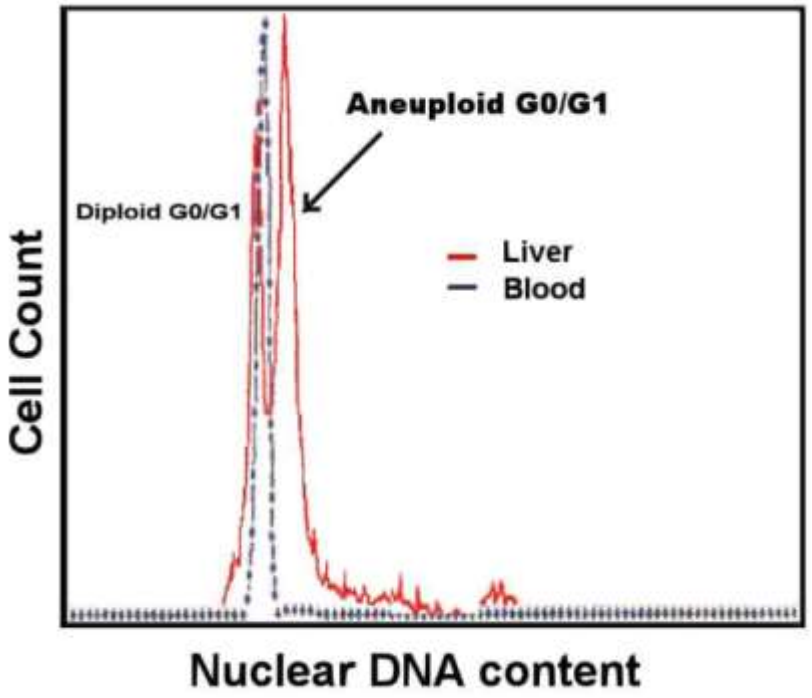
- Some AFLP genotypes are unique and rare (6,7,8,9)
- DNA sequenced (chloroplast marker) them and others (1,2,3,4,5,6,9) identical match to Butomus in GenBank
- (7,8) *Sparganium* (aquatic bur-reed)



Diploid or triploid?

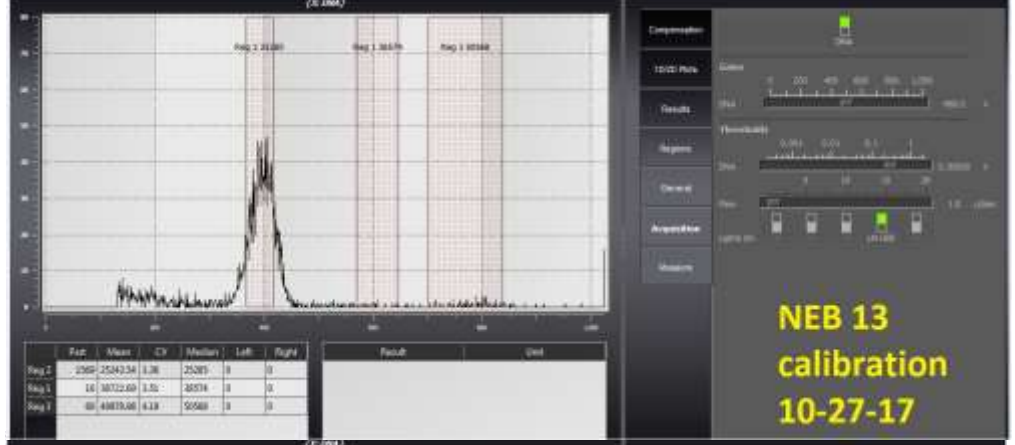
Fresh tissue

Can also tell by stomata

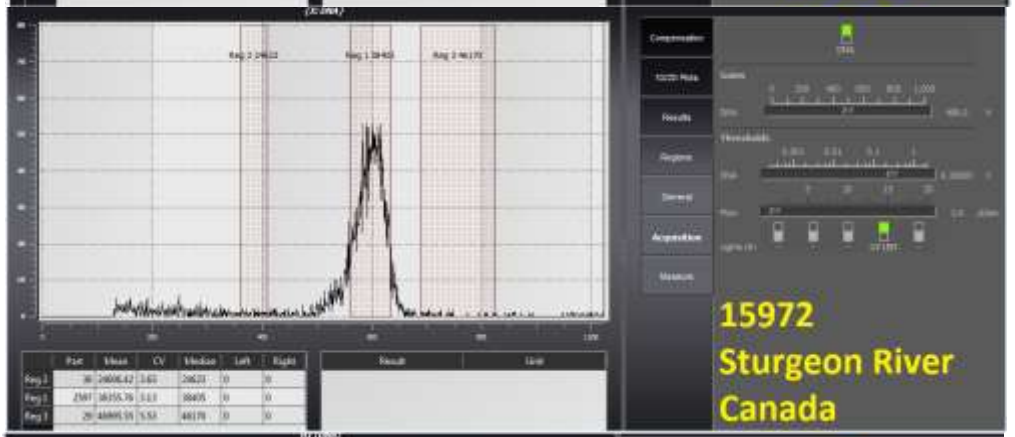


2x= 400
3x=600

Fresh leaf material

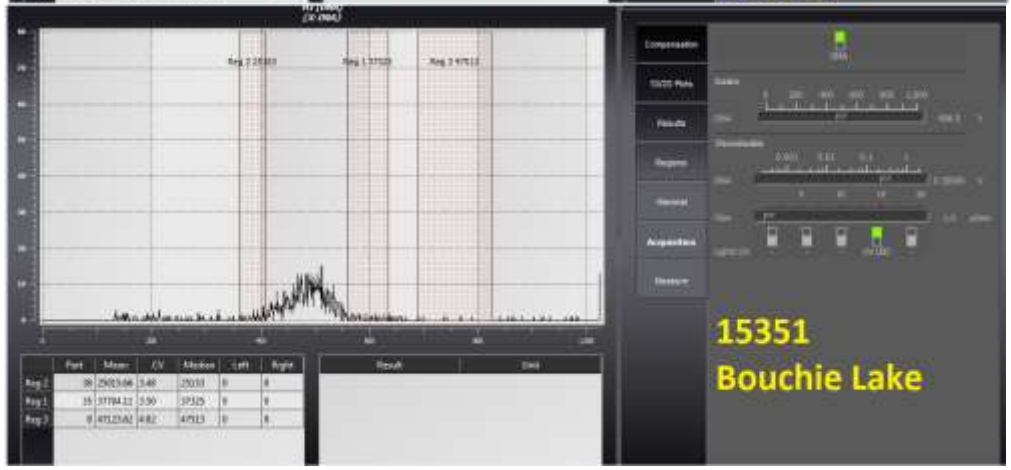


Genotype 4 VT



Genotype 1

Grrr. Stringy tissue?



Genotype 2

Species Code	ARS PMRU DNA Sample #		# Root Tip Evaluations	# Cell Evaluations	Chromosome Count (*2n*=)
BUUM	15348.00	Yak-Pross	2	15	26
BUUM	15349.00	Yak-Pross	2	15	26
BUUM	15350.00	Yak-Pross	2	15	26
BUUM	15351.00	Bouchie	2	16	39

3-D analysis

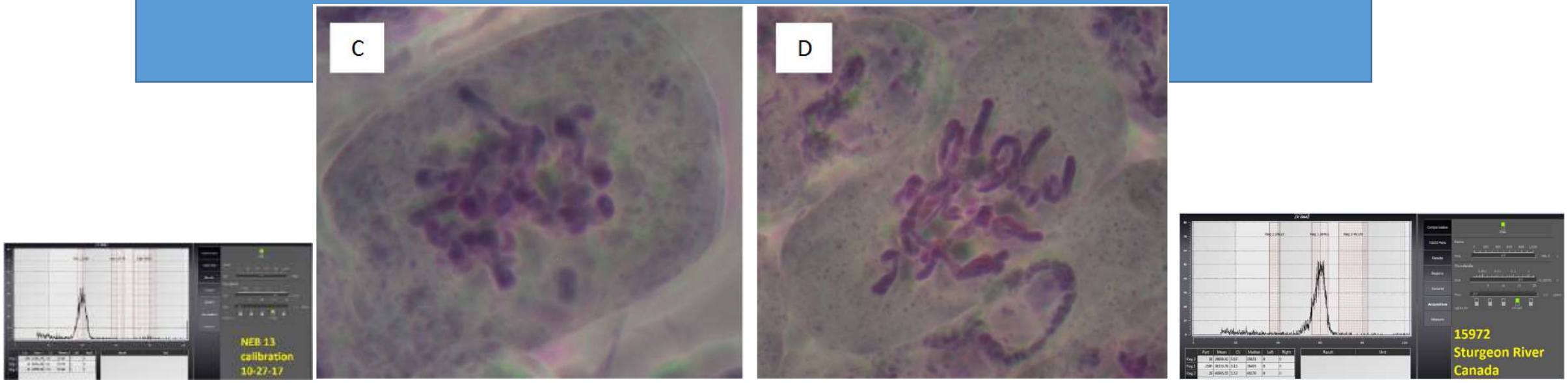


Figure 1. Composite cell images from *Butomus umbellatus* with A-C) 2*n*=26 chromosomes, D) 2*n*=39 chromosomes.

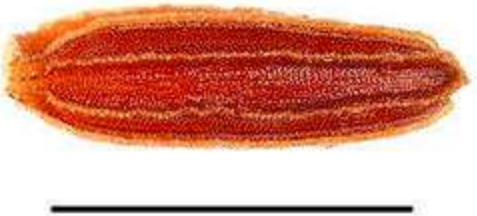
USDA ARS UC Davis Brenda Grewell and Joy Furtrell

1	3x	(2x at Prosser Diversion dam) [Jennifer Parsons]
2	3x	
3	2x	
4	2x	
5	2x	
6	?	
9	?	

yellow star=favorite restaurants

Who cares about ploidy!?

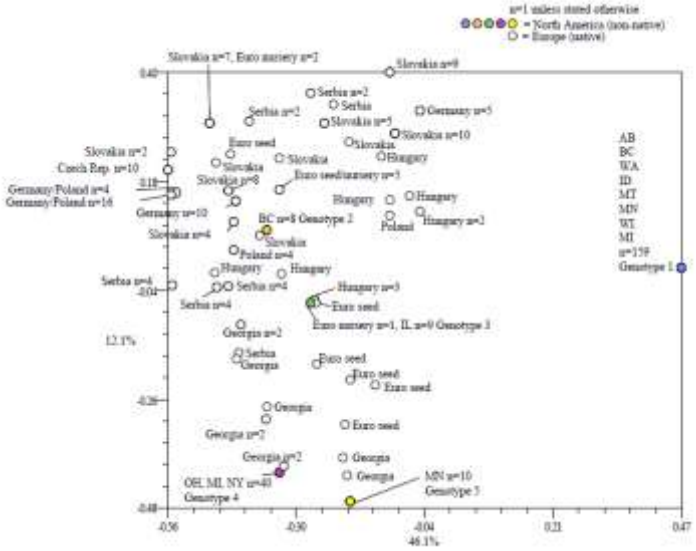
- Reproduction and spread
  - 2x (diploid): seed and bulbil and fragments
  - 3x (triploid): only root fragments?
- Invasiveness?
- Control?
  - Chemical
  - Biological



Results so far

- Europe has many genotypes. NA has few.
- Not all Euro pops clonal. Most NA pops clonal. Why??
- We have both diploid and triploid genotype 1 (mostly triploid so far)
- Diploid and triploid of same genotype…do they act the same when it comes to control?
- We have not found a close match for our very common genotype 1
- CABI and USACE will have or do have all genotypes to use in testing of potential agents.

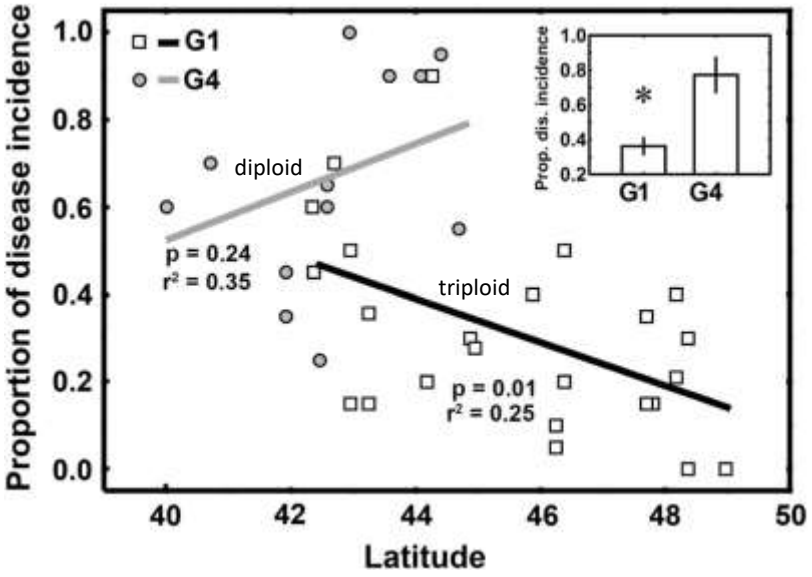
Biocontrol with foreign disease (CABI UK)
Will a disease attack all of our Butomus?



No close genetic match in Eurasia for our genotype 1…yet.

- *Doassansia niesslii*
- Smut
- Does not attack our genotype 1…need to find 1 in Eurasia…collect disease from it

Flowering rush NA diseases. Does genotype matter?



Harms, N., Shearer, J., Cronin, J.T. and Gaskin, J.F., 2019. Geographic and genetic variation in susceptibility of Butomus umbellatus to foliar fungal pathogens. Biological Invasions, pp.1-14.

US Army Corps of Engineers

Side note!

- Plant invasions are rarely homogenous entities.
- Evolutionary processes such as selection, drift, reproductive mode, and founding events shape the population structure and diversity of an invasion, and that information can help guide weed management.

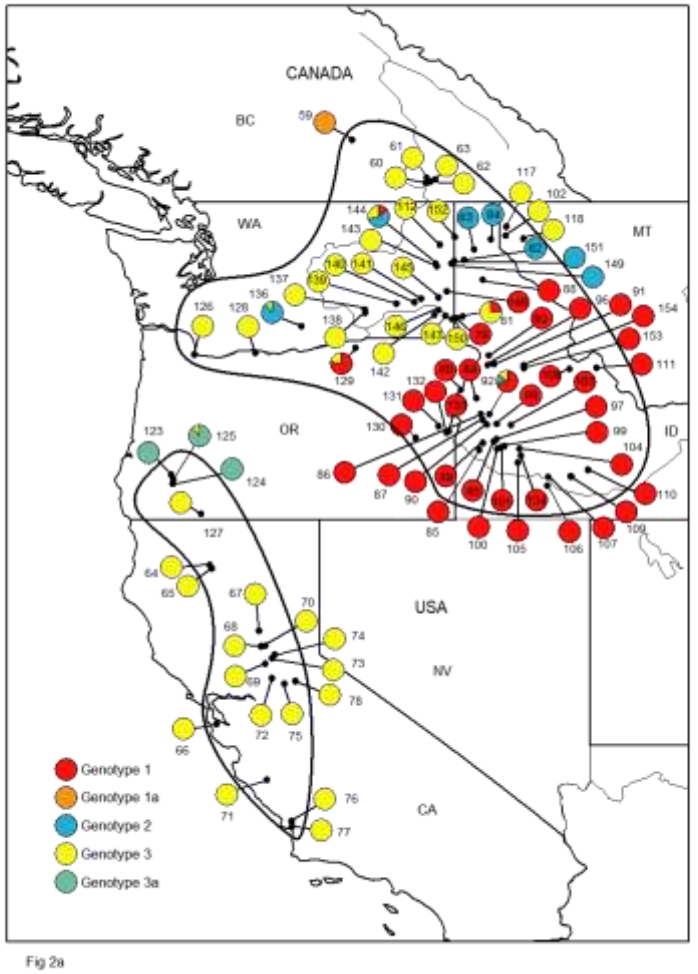


Fig 2a

Rush skeletonweed

Verbascum thapsus; common mullein

John F. Gaskin USDA ARS

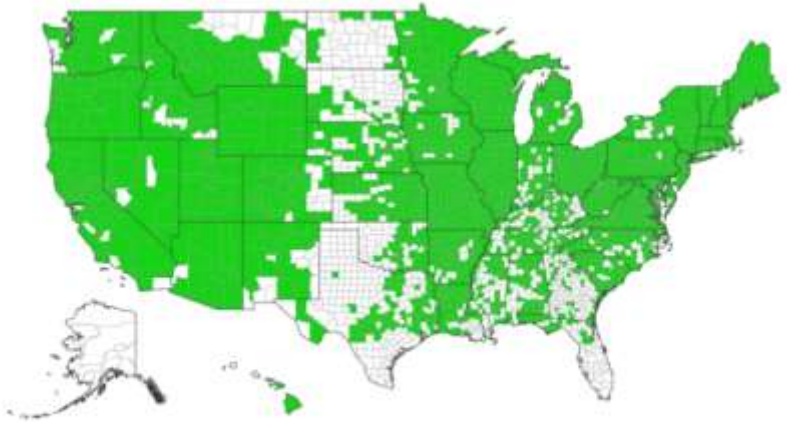
Stacy B. Endriss CSU

Christa Fettig CSU

Ruth Hufbauer CSU

Andrew Norton CSU

René F.H. Sforza USDA ARS EBCL



Verbascum thapsus
Common mullein

How do weeds reproduce? Self? Outcross? Other?
(affects population structure and weed control method)



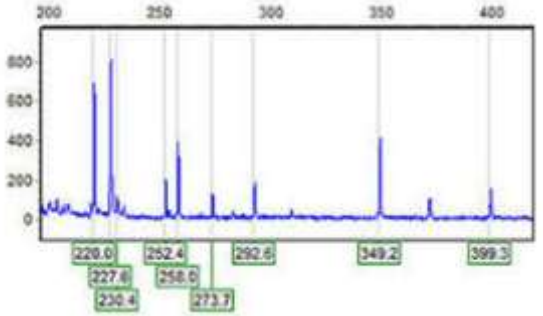
- Donnelly et al. (1998) demonstrated that this species is fully self-compatible without evidence of inbreeding depression, and that it has a mixed mating system with reproductive strategy (selfing or outcrossing) depending on variables that affect pollinator availability (e.g. plant height or population size).
- Donnelly SE, Lortie CJ, Aarssen LW. Pollination in Verbascum thapsus (Scrophulariaceae): the advantage of being tall. American Journal of Botany. 1998 Nov;85(11):1618-25.

Why be showy if you don’t need or use sex??

How is it reproducing in the invasion? AFLP study

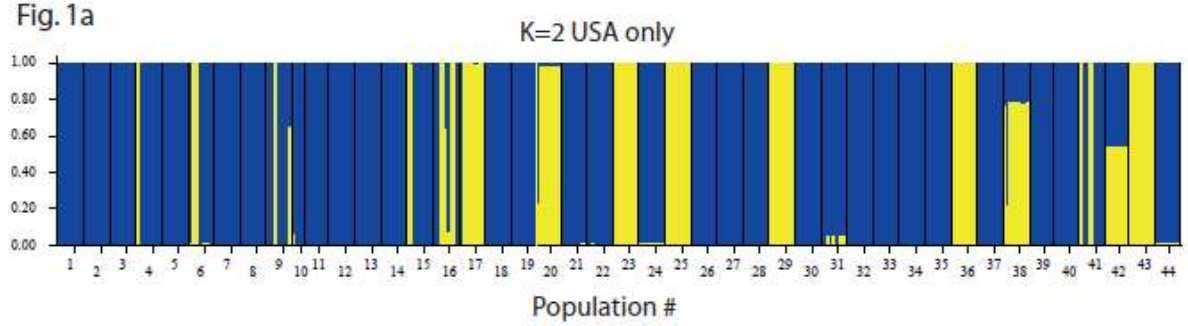
Table 2. Comparison of USA and Eurasian genetic data for Verbascum thapsus.							
Location	N	G	G/N	PLP	Hj	Fst	
USA	431	32	0.0700	12.5%	0.05	0.30	
Eurasia	479	394	0.8200	61.5%	0.20	0.72	

We found much higher diversity in the native Eurasian range, with 394 genotypes found in 479 plants.



- 50% of invasive populations were monotypic, and we found only 32 genotypes in 431 plants.
- Same genotype can be found across range of invasion.
- Why aren't there more genotypes??
  - founding effect
  - selfing

Fig. 1a



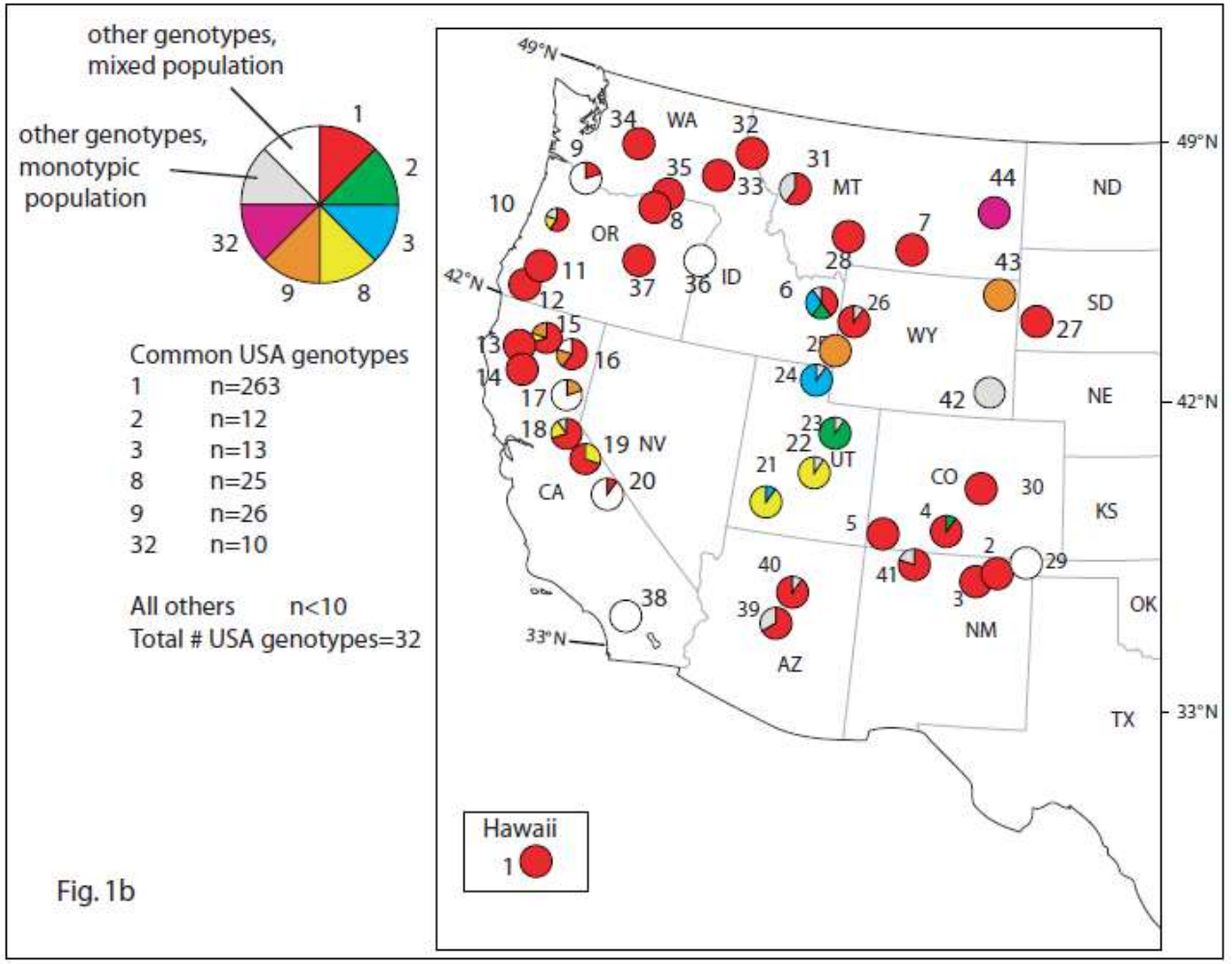


Fig. 1b

Origins

- We found highest genetic similarity between the USA invasion and plants from Belgium, Germany and France.

Fig. 2a

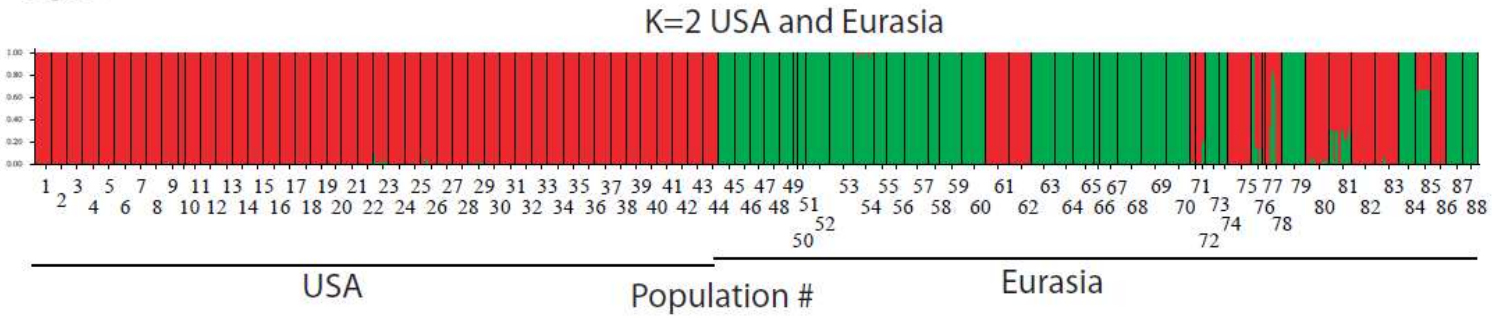
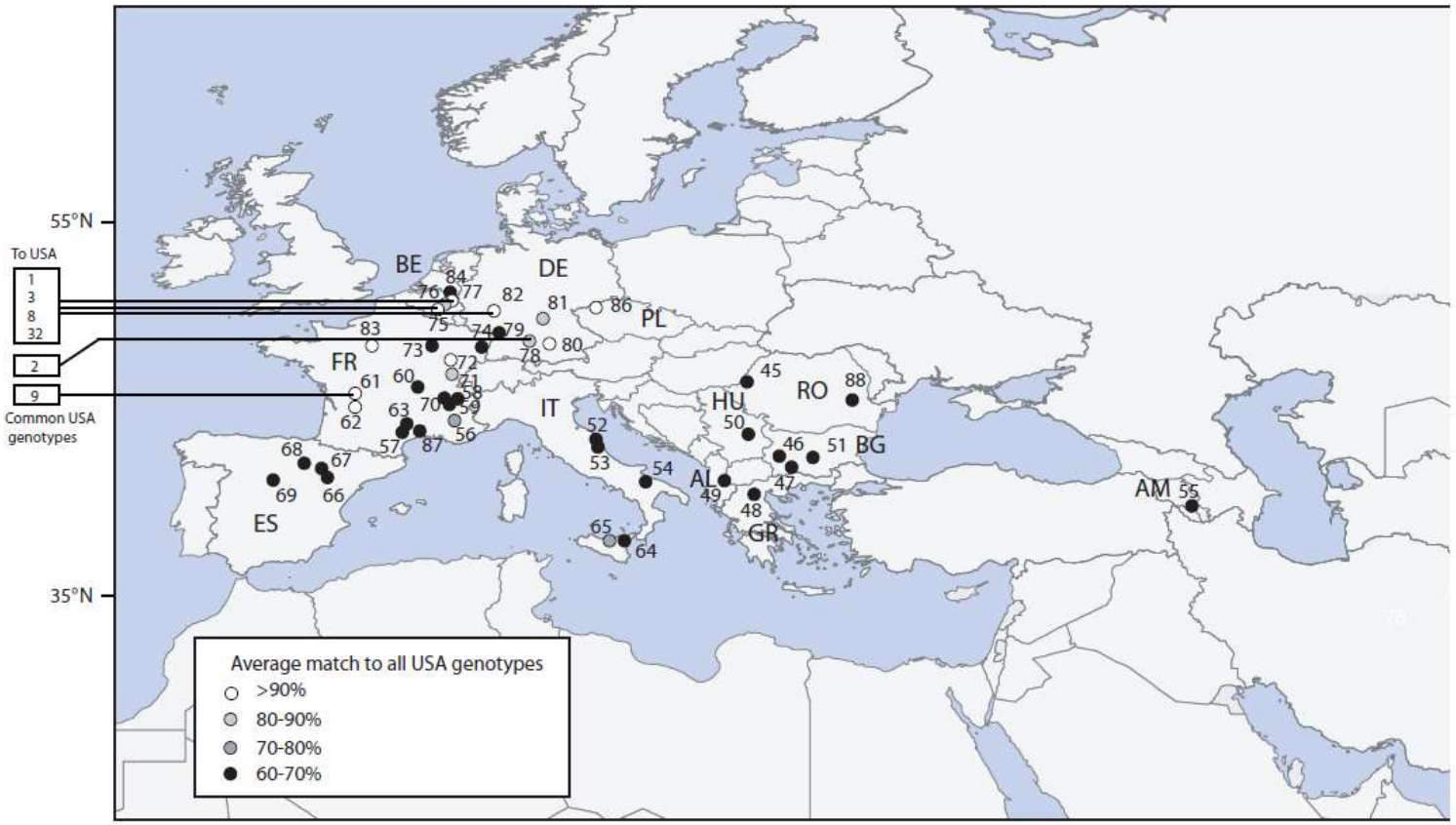


Fig. 2b



Verbascum

There is an unsupported suggestion that common mullein has some herbicide resistance or tolerance (Burton 1964 in Gross and Werner 1978).

- With this DNA information we can assist common mullein management by
  - identifying and supplying the common, or all, invasive genotypes for herbicide resistance research
  - identification of specific origins for searching for co-evolved biological control agents in the native range.

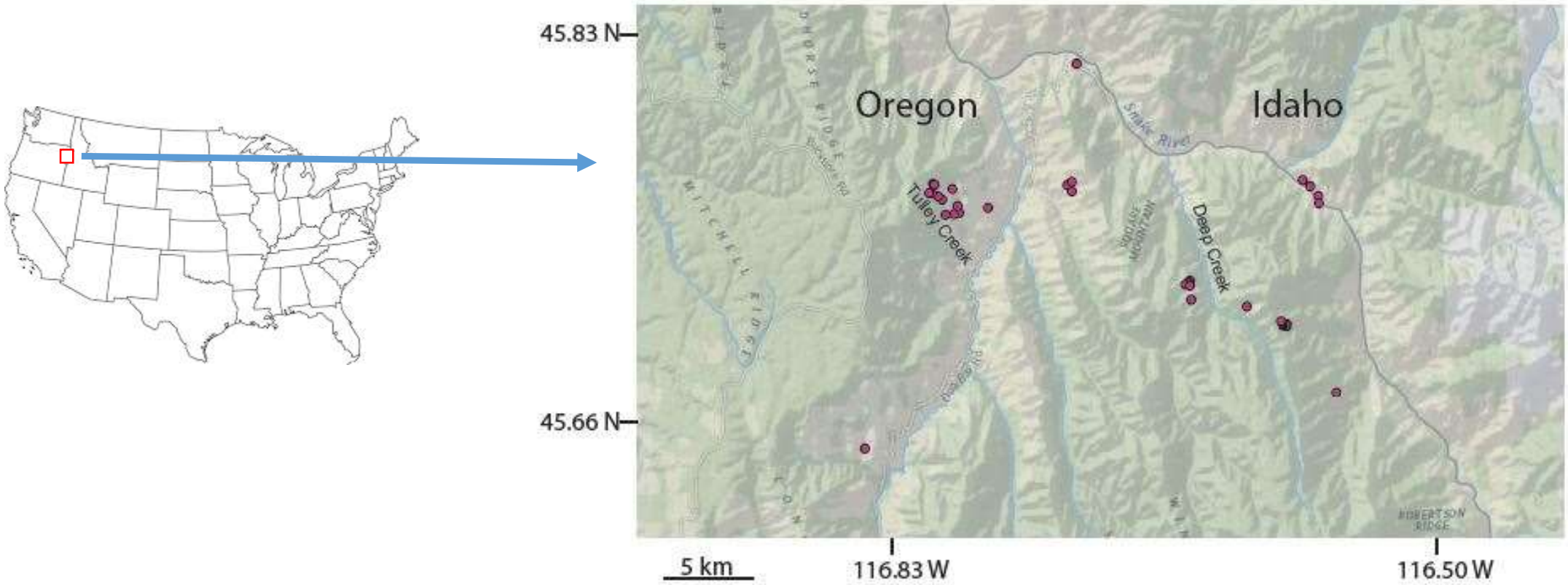
What thistle is this?

John F. Gaskin	USDA
Eric Coombs	OR Dept. Ag
Mark Porter	OR Dept. Ag
Dean G. Kelch	UC Berkeley, California Department of Food and Agriculture
David J. Keil	California Polytechnic State University
Alfonso Susanna	Botanic Institute of Barcelona

Refresher course: *Carduus* in N. America

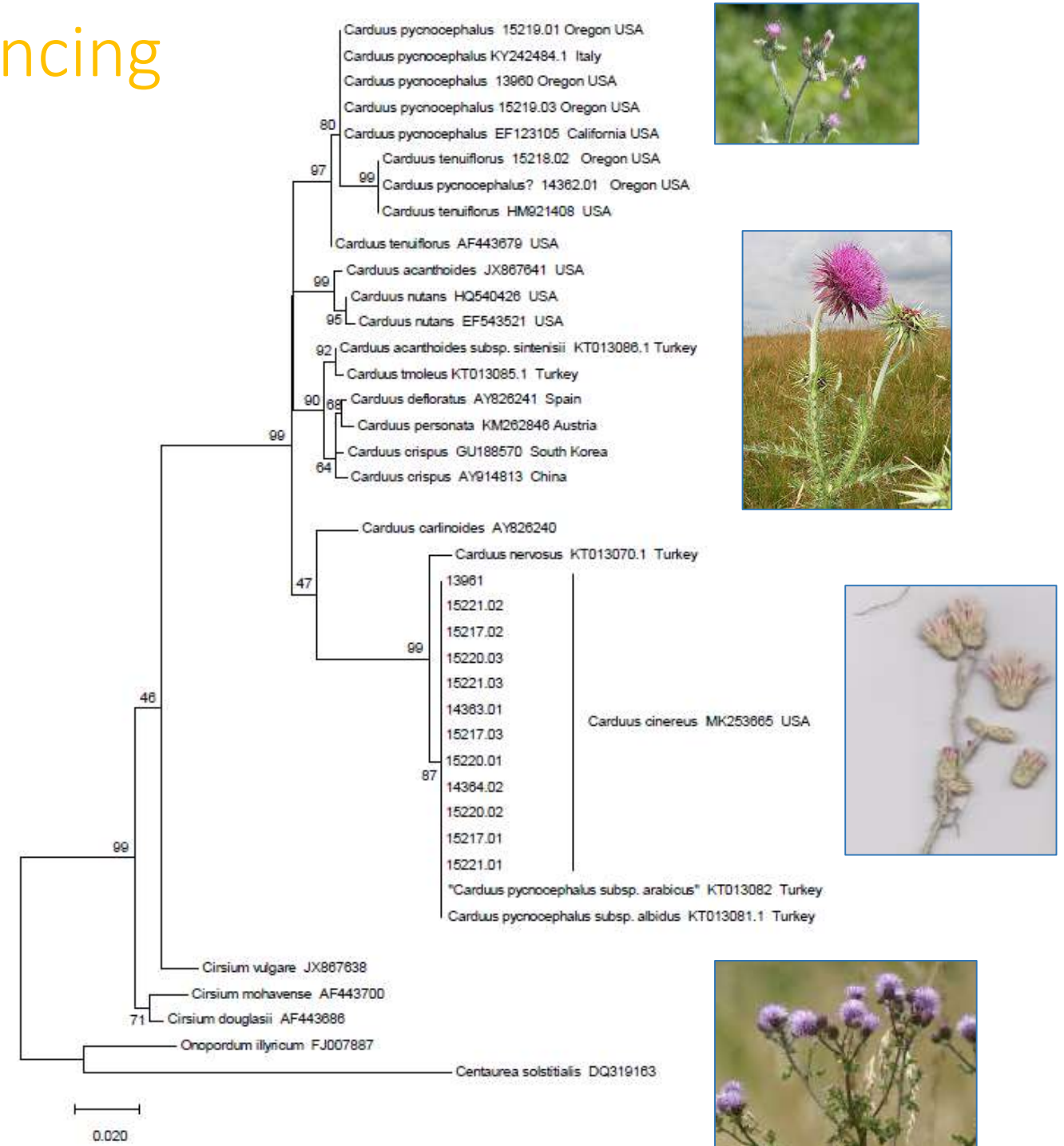
All non-native, state noxious

- *Carduus nutans*
- *Carduus acanthoides*
- *Carduus crispus*
- *Carduus tenuiflorus*
- *Carduus pycnocephalus*


45.83 N
45.66 N
Oregon
Idaho
Snake River
Tulley Creek
Deep Creek
Mitchell Ridge
Horse Ridge
Robertson Ridge
5 km
116.83 W
116.50 W

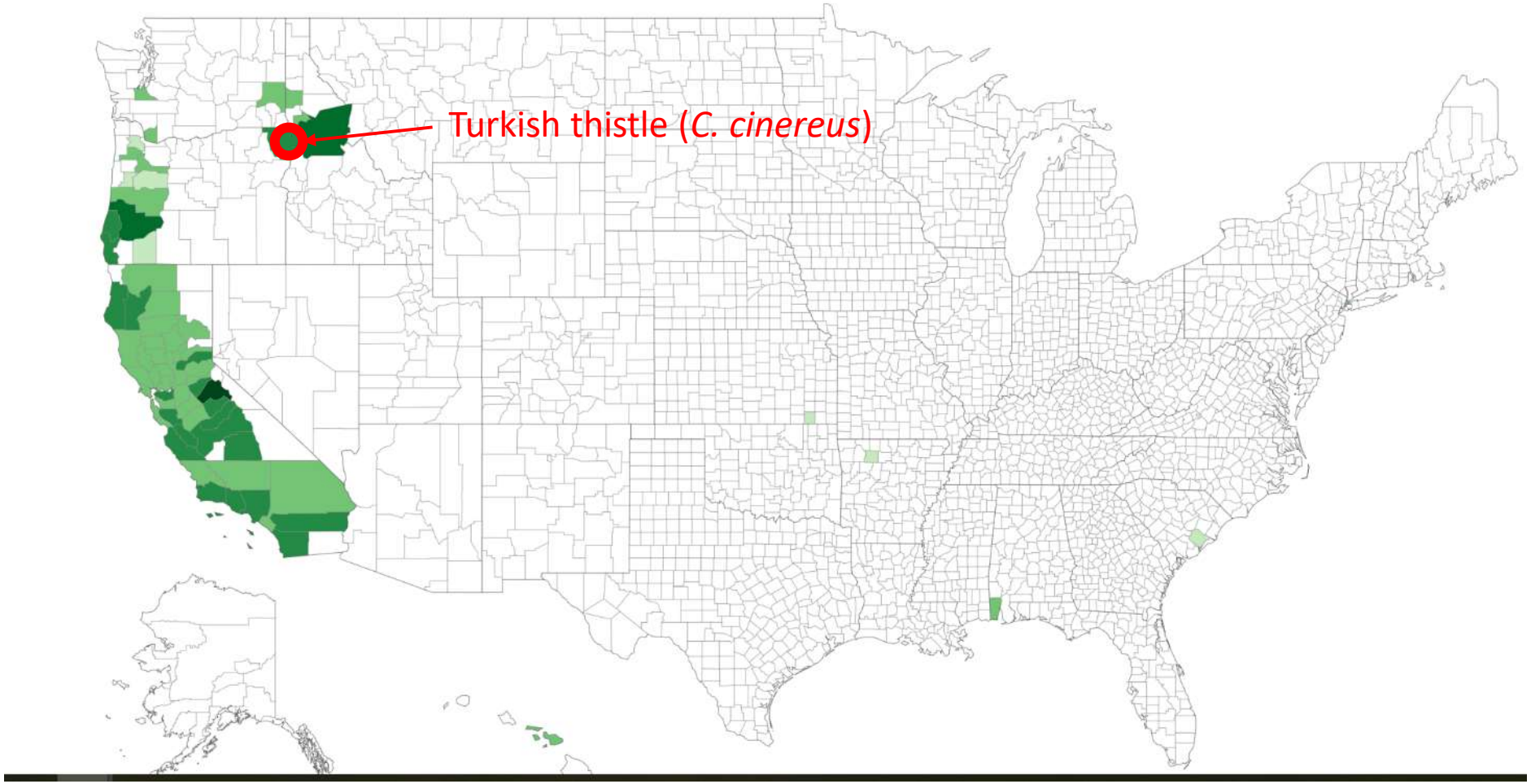
- Carduus nutans
- Carduus acanthoides
- Carduus crispus
- *Carduus tenuiflorus*
- *Carduus pycnocephalus?*
- *Carduus? sp.???*

DNA sequencing



- *Carduus pycnocephalus*??
  - Garbage can… No.
- Hybrid?
  - Nuclear DNA. No.
- Eastern Euro *C. pycnocephalus?*
- *Revise the genus!*
- *Carduus cinereus!!*
- Turkish thistle
- Is it invasive?

Italian thistle (*C. pycnocephalus*)



Modified Key to *Carduus* species in North America

Gaskin et al. Madrono. In Press. *Carduus cinereus*; a new species to the Americas

1.Phyllaries 2–7 mm wide, usually wider than the appressed bases; peduncles often elongate, distally wingless; heads often nodding, usually borne singly or in leafy corymbiform arrays; involucres 20–70 mm diam — *Carduus nutans*

1'Phyllaries 0.5–2.0 mm wide, usually narrower than the appressed bases; peduncles short, if present, usually winged throughout, less often sparsely winged or unwinged; heads erect, 1–many, often clustered or loosely clustered at branch tips or upper axils; involucres 7–30 mm diam

2. Involucres spheric or hemispheric

3.Corollas 13–20 mm long; heads 18–25 mm long; involucres 14–20 mm long; abaxial leaf faces glabrate except for long, curled, septate hairs along veins — *Carduus acanthoides*

3'Corollas 11–16 mm long; heads 15–18 mm long; involucres 12–17 mm long; abaxial leaf faces sparsely to densely tomentose with fine, non-septate hairs and often with curled, septate hairs along veins as well — *Carduus crispus*

2‘ Involucres cylindric or narrowly ellipsoid

4. Heads 5–20 at ends of branches; phyllaries glabrous or sparingly tomentose, distally ciliolate or glabrous — *Carduus tenuiflorus*

4' Heads 1–5 at ends of branches; phyllaries ± persistently tomentose, distally scabrous on margins and faces

5. Heads usually pedunculate and loosely clustered; phyllaries scarious-margined — *Carduus cinereus*

5‘. Heads usually sessile and tightly clustered; phyllaries not scarious-margined — *Carduus pycnocephalus*

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What are your needs or questions?

John Gaskin
Botanist
Sidney, MT

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