# Identifying cattail species and their hybrids using molecular markers: Lessons learned


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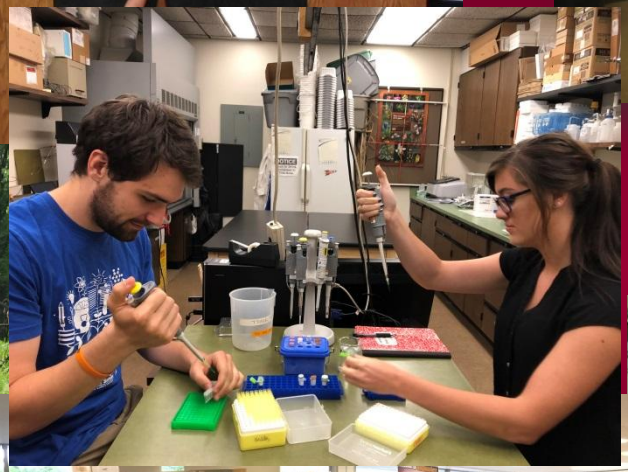
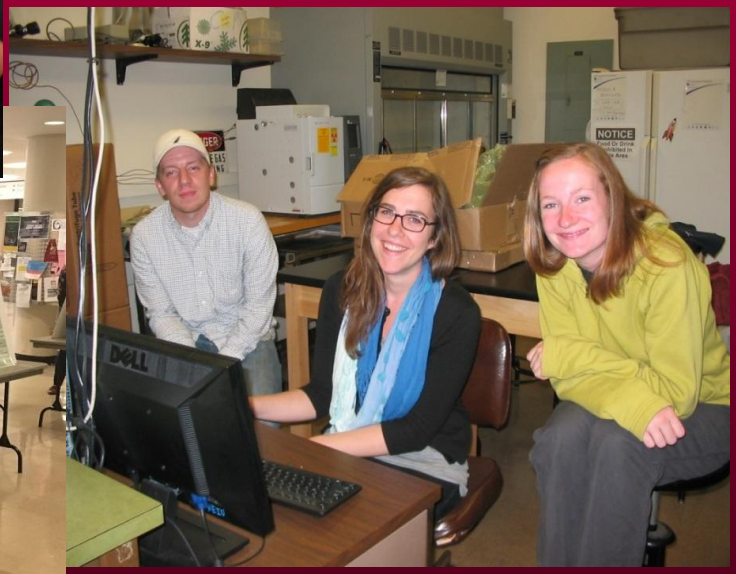



DELL
NOTICE

# Wetlands are threatened by invasive plant species

http://www.fs.fed.us/r9/forests/white_mountain/ecosystems/WMEDN/nnis_plants/nnis_forest.htm

# 37 known cattail species; 4 in U.S.

## Accepted species and natural hybrids [ edit ]

The following names are currently accepted:[14]

1. *Typha albida* – (Afghanistan)
2. *Typha alekseevii* – (Caucasus)
3. *Typha angustifolia* – lesser bulrush, narrow leaf cattail (America), or *jambu* (India)
4. *Typha × argoviensis* – (Germany and Switzerland)
5. *Typha austro-orientalis* – (European Russia)
6. *Typha azerbaijanensis* – (Iran)
7. *Typha × bavarica* – (Germany)
8. *Typha capensis* – (tropical and southern Africa)
9. *Typha caspica* – (Azerbaijan)
10. *Typha changbaiensis* – (northeastern China)
11. *Typha davidiana* – (China)
12. *Typha domingensis* – bulrush, southern cattail (America), narrow-leaved cumbungi (Australia)
13. *Typha elephantina* – (from Algeria to southern China)
14. *Typha × gezei* – (France)
15. *Typha × glauca* (*T. angustifolia* × *T. latifolia*) – hybrid cattail, white cattail (a sterile hybrid)[15]
16. *Typha grossheimii* – (Central Asia)
17. *Typha incana* – (central Russia)
18. *Typha joannis* – (Mongolia, Amur Oblast)
19. *Typha kalatensis* – (Iran)
20. *Typha latifolia* – common cattail – (very widespread)
21. *Typha laxmannii* – Laxman's bulrush – (southern Europe and much of Asia)
22. *Typha lugdunensis* – (western Europe, southwest Asia, China)
23. *Typha minima* – dwarf bulrush – (Europe, Asia)
24. *Typha orientalis* – (East Asia, Australia, New Zealand)
25. *Typha pallida* – (Central Asia, China)
26. *Typha × provincialis* – (France)
27. *Typha przewalskii* – (China, Russian Far East)
28. *Typha shuttleworthii* – (Europe, Iran, Turkey)
29. *Typha sistanica* – (Iran)
30. *Typha × smirnovii* – (European Russia)
31. *Typha subulata* – (Argentina, Uruguay)
32. *Typha × suwensis* – (Japan)
33. *Typha tichomirovii* – (European Russia)
34. *Typha turcomanica* – (Turkmenistan)
35. *Typha tzvelevii* – (Primorye)
36. *Typha valentinii* – (Azerbaijan)
37. *Typha varsobica* – (Tajikistan)

# 3 *Typha* species in the Midwest

***Typha latifolia***

**Common or broad-leaved cattail**

**NATIVE**

***Typha x glauca***

**Hybrid cattail (F1)**

**INVASIVE**

***Typha angustifolia***

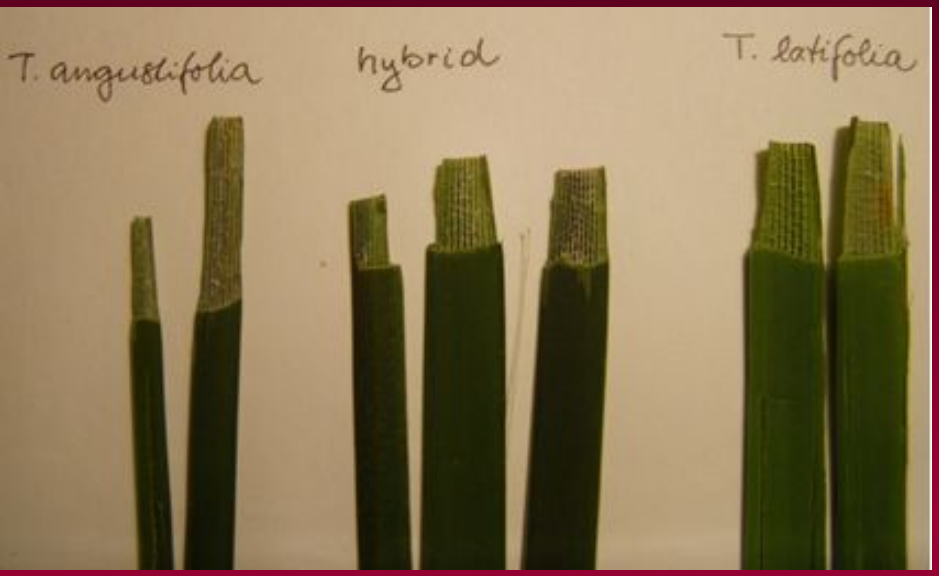
**Narrow-leaved cattail**

**EXOTIC?**

**INVASIVE**

# *Typha x glauca* is degrading wetlands due to aggressive growth

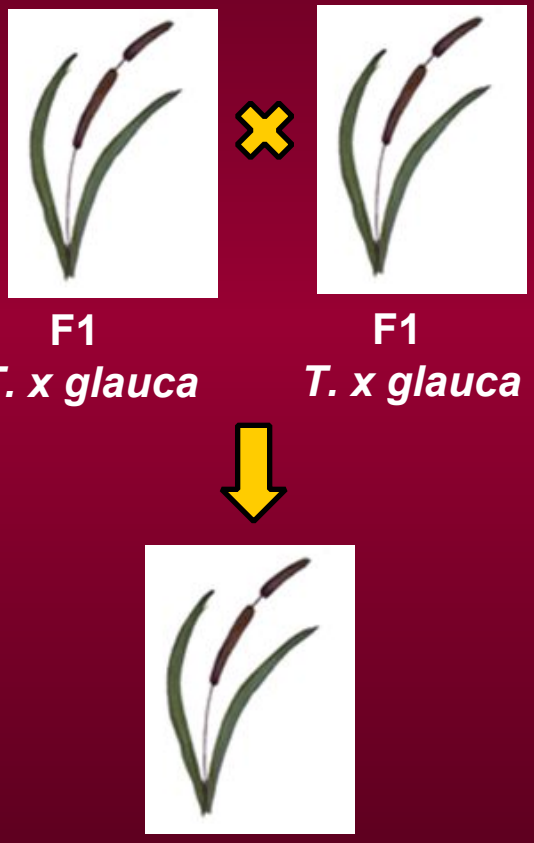
# How do we identify *Typha* species?

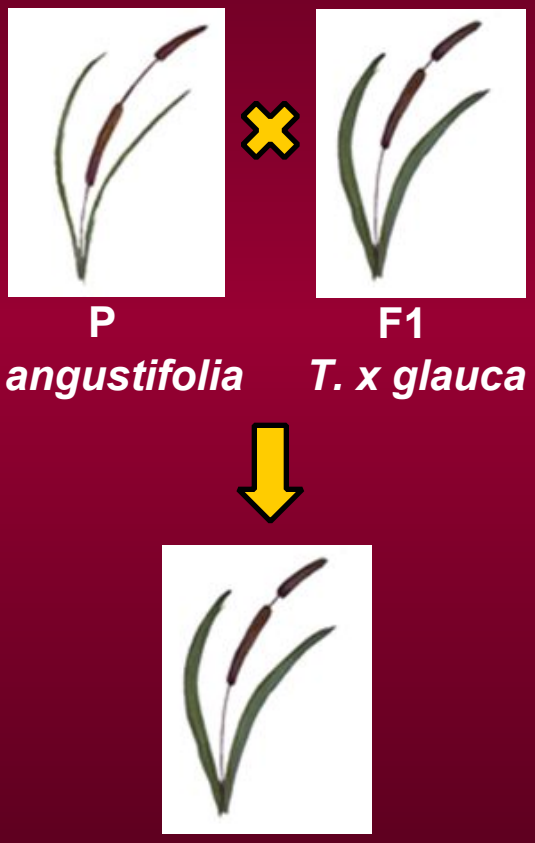


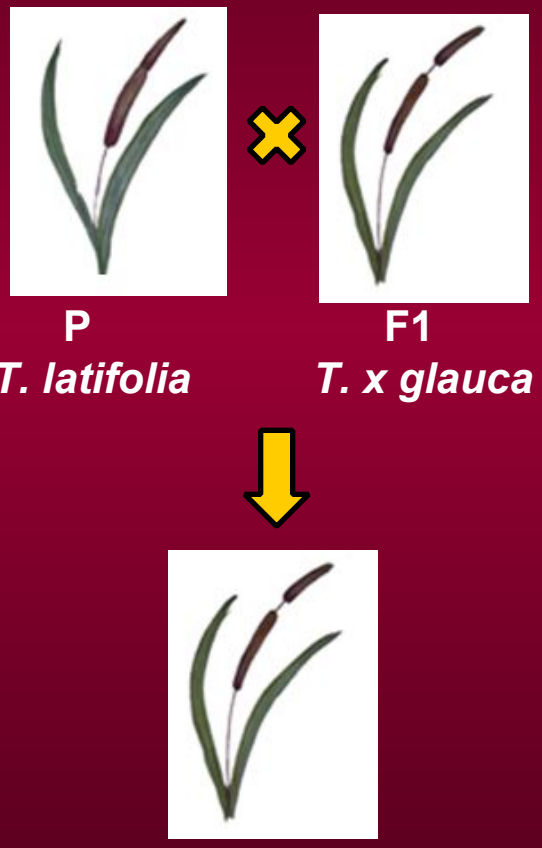



# Introgression: Complex mixture of parental genes that leads to "hybrid swarms"

## Advanced Generation Hybrid (AGH) (≥ F2)



# Research questions

1. What is the relative abundance of parental versus hybrid cattails in the Midwest?

2. Do we detect fertile hybrids that backcross to parental species? (i.e., evidence of introgression?

3. How accurate is our morphological identification?

# Molecular markers: Microsatellites or SSRs (Simple Sequence Repeats)

ATATATATATAT

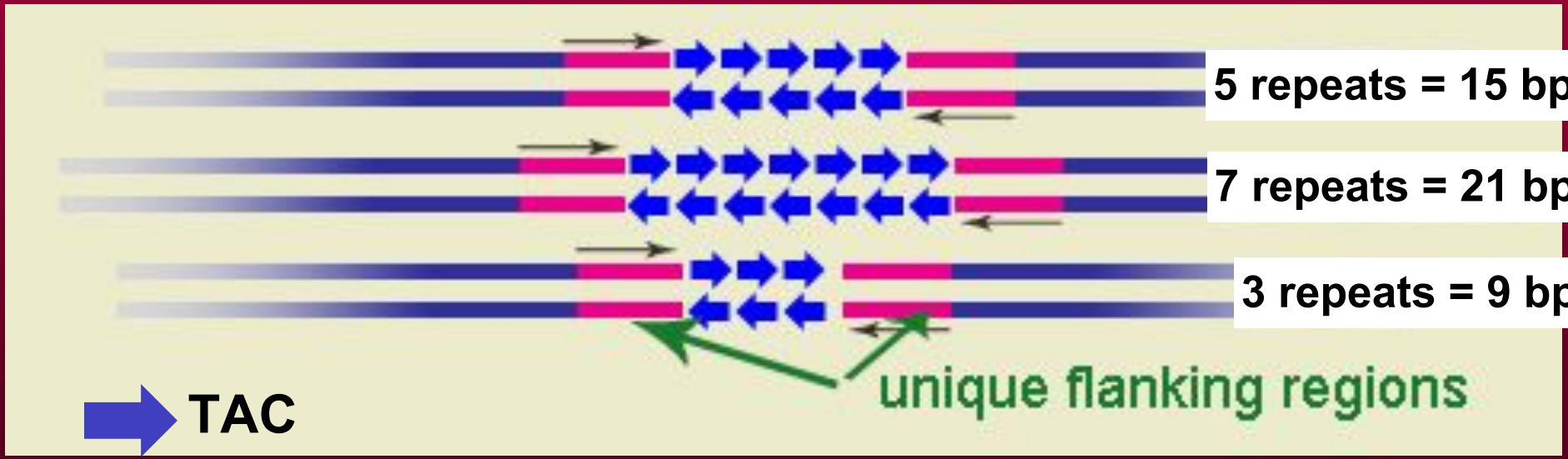
GATGATGATGAT

TAACTAACTAAC

# Microsatellite markers

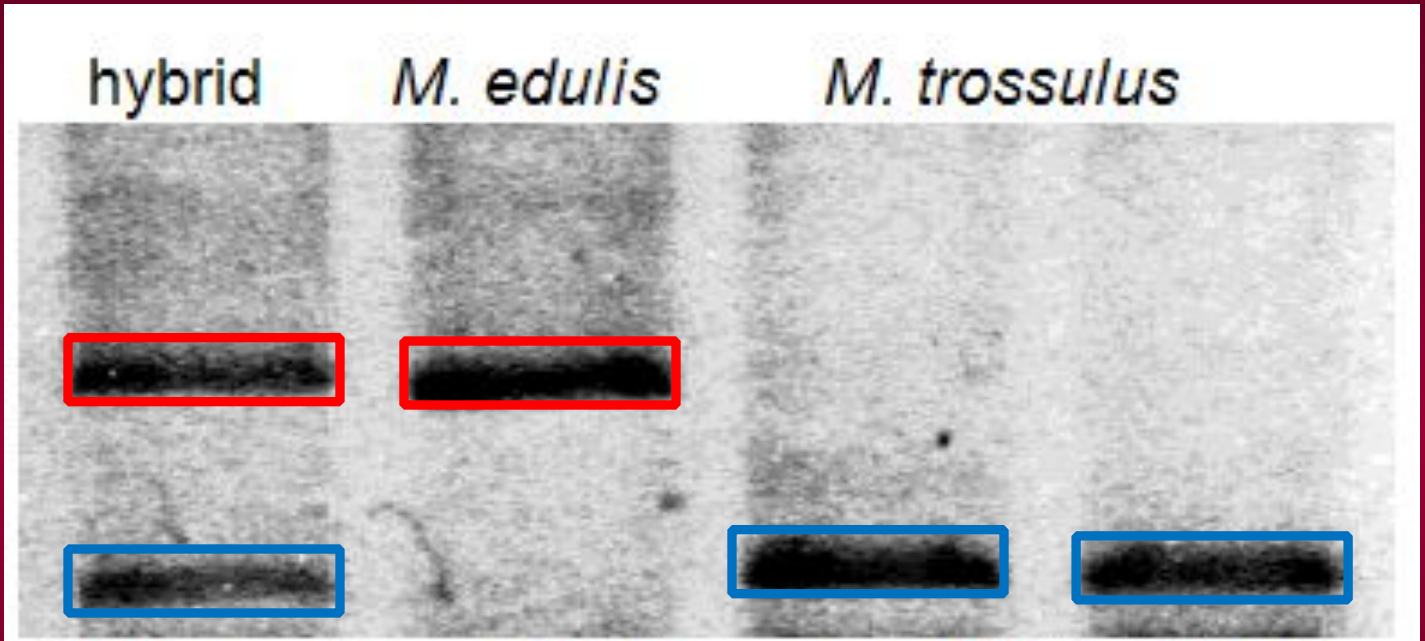
-Length of microsatellite sequence or number of repeats ➡ may be different among species

-Flanking regions ▬ are conserved, so they can be used to develop primers



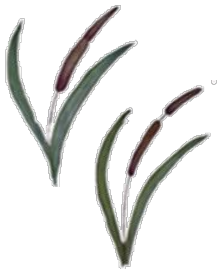

http://www.ncbi.nlm.nih.gov/genome/probe/doc/TechSTS.shtml

# Detecting hybrids in mussels

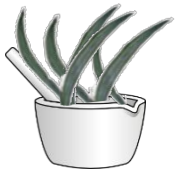



(*ITS* fragments separated in an agarose gel. Photo: J. Toro)

# Methods

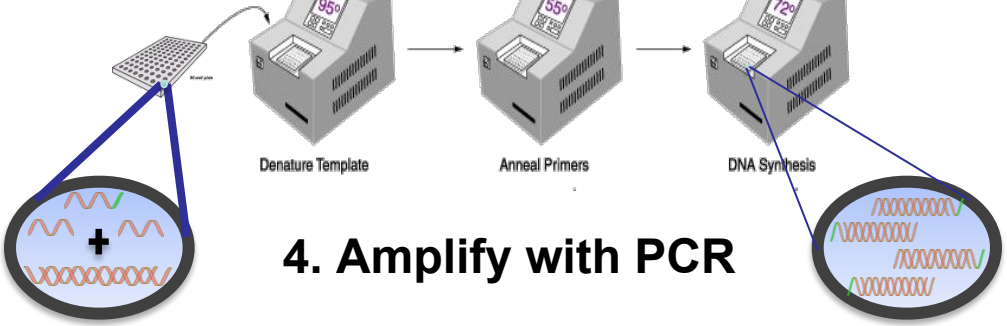


1. Collect & ID morphologically

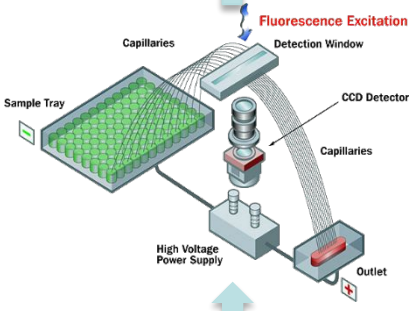


2. Grind tissue

3. Extract DNA




4. Amplify with PCR




5. Analyze

6. Score & Interpret

# Sampling

39 cattail populations in 7 MW states

~5 individuals per population (n=178)

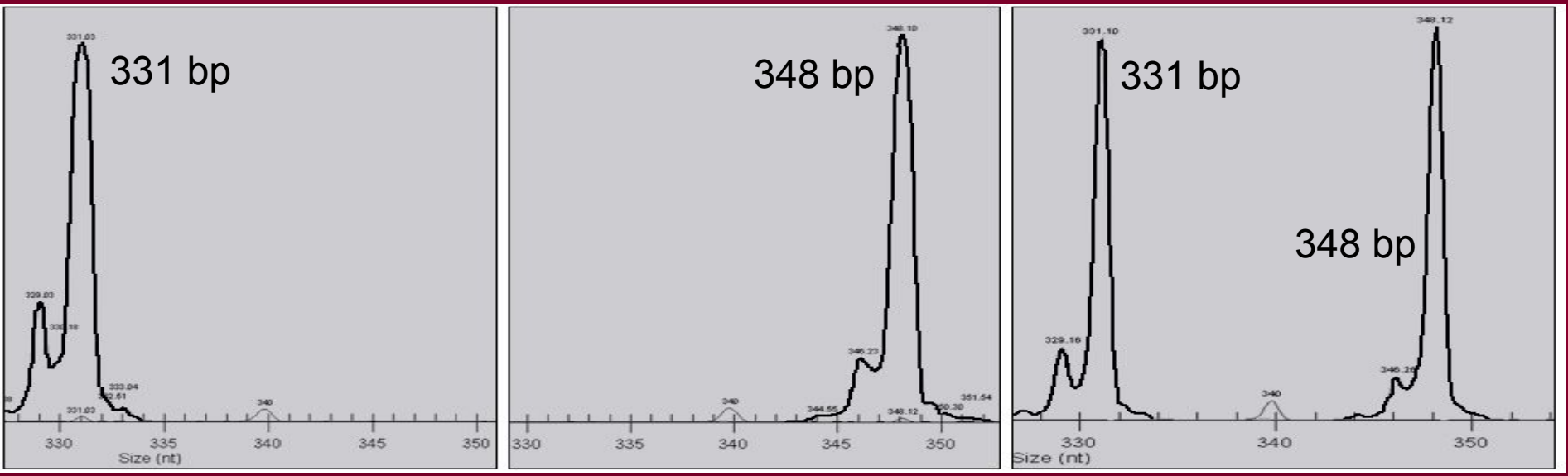
# 35 Microsatellites tested… but only 6 worked well

- 11 from *Typha angustifolia* (TA)
  - 4 diagnostic
- 17 from *Typha minima* (TM)
  - 2 diagnostic
- 7 from *Typha latifolia* (TL)
  - None diagnostic

*Typha minima* (European)

# Diagnostic molecular markers are absolutely essential

*Typha angustifolia* *Typha latifolia* *Typha x glauca*

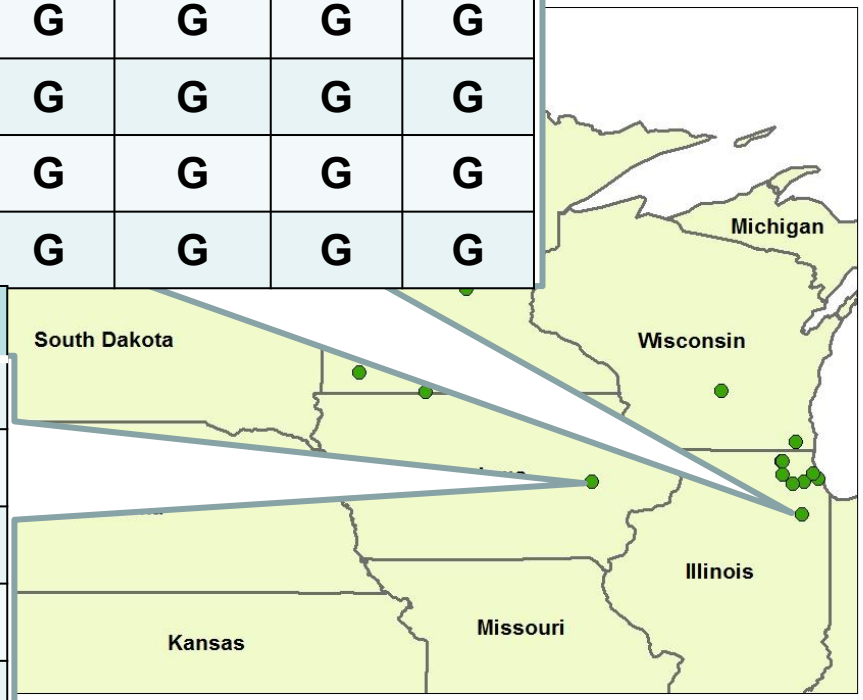



- If peaks are equal for A and L → can't differentiate them or detect hybrids
- Peaks shown are analogous to bands in a gel

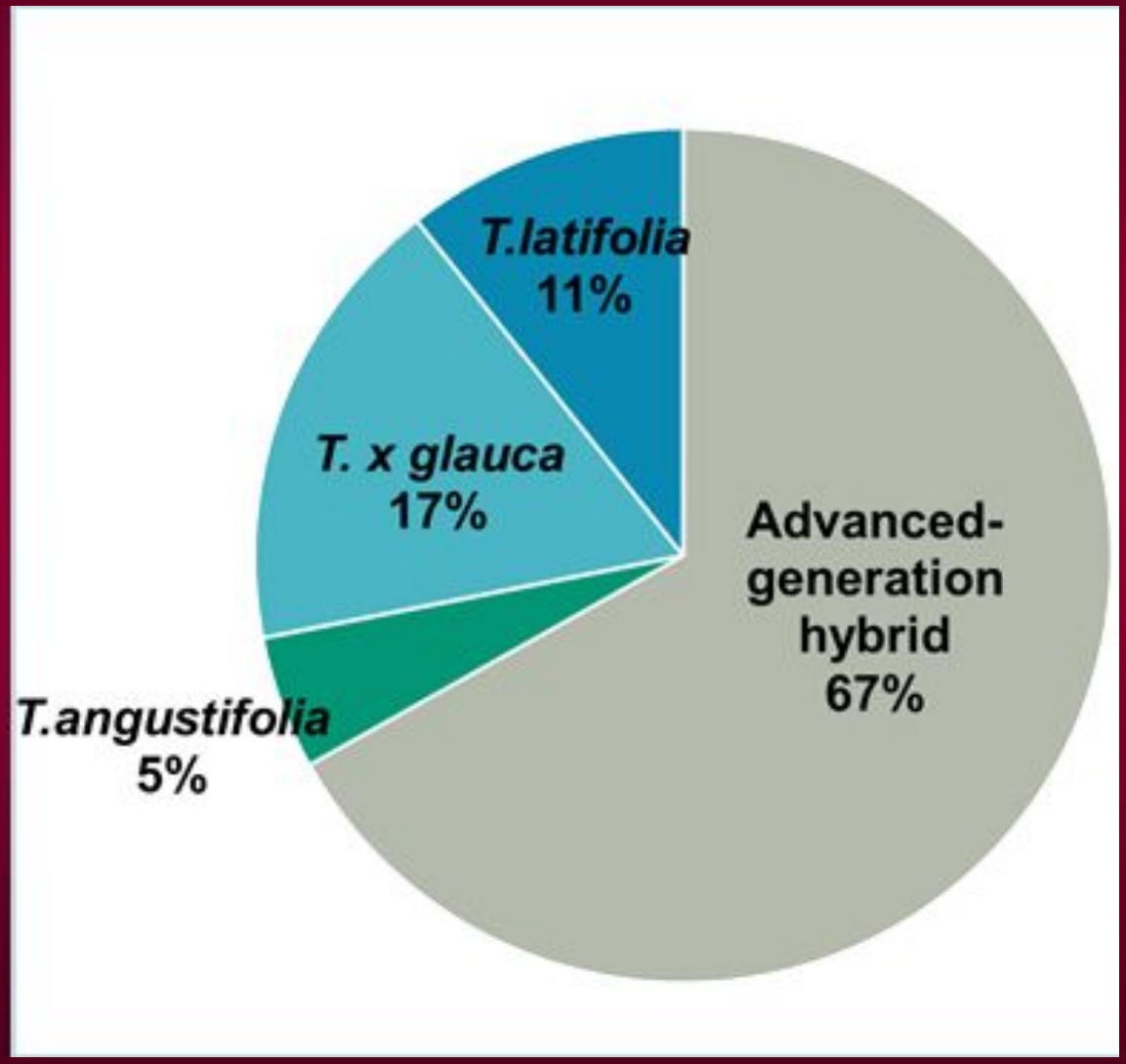
# Set of multiple microsatellites allows identification of AGH

| | TA 3 | TA 5 | TA 8 | TA 16 | TM 4 | TM 6 |
|---|---|---|---|---|---|---|
| 1 | G | G | G | G | G | G |
| 2 | G | G | G | G | G | G |
| 3 | G | G | G | G | G | G |
| 4 | G | G | G | G | G | G |
| 5 | G | G | G | G | G | G |

| | TA 3 | TA 5 | TA 8 | TA 16 | TM 4 | TM 6 |
|---|---|---|---|---|---|---|
| 1 | G | G | G | L | L | G |
| 2 | G | G | G | G | G | G |
| 3 | G | L | L | L | G | G |
| 4 | G | G | G | G | G | G |
| 5 | G | G | G | G | G | G |

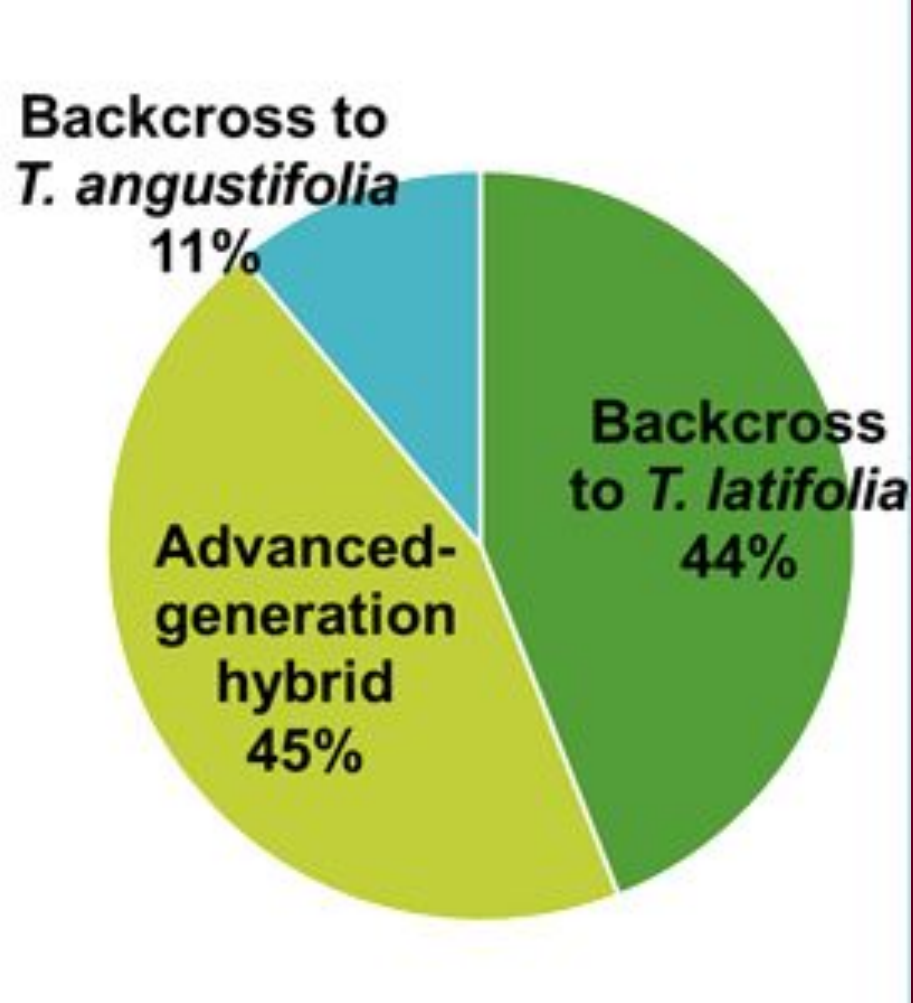


# Q1: What is the relative abundance of parental versus hybrid genotypes?



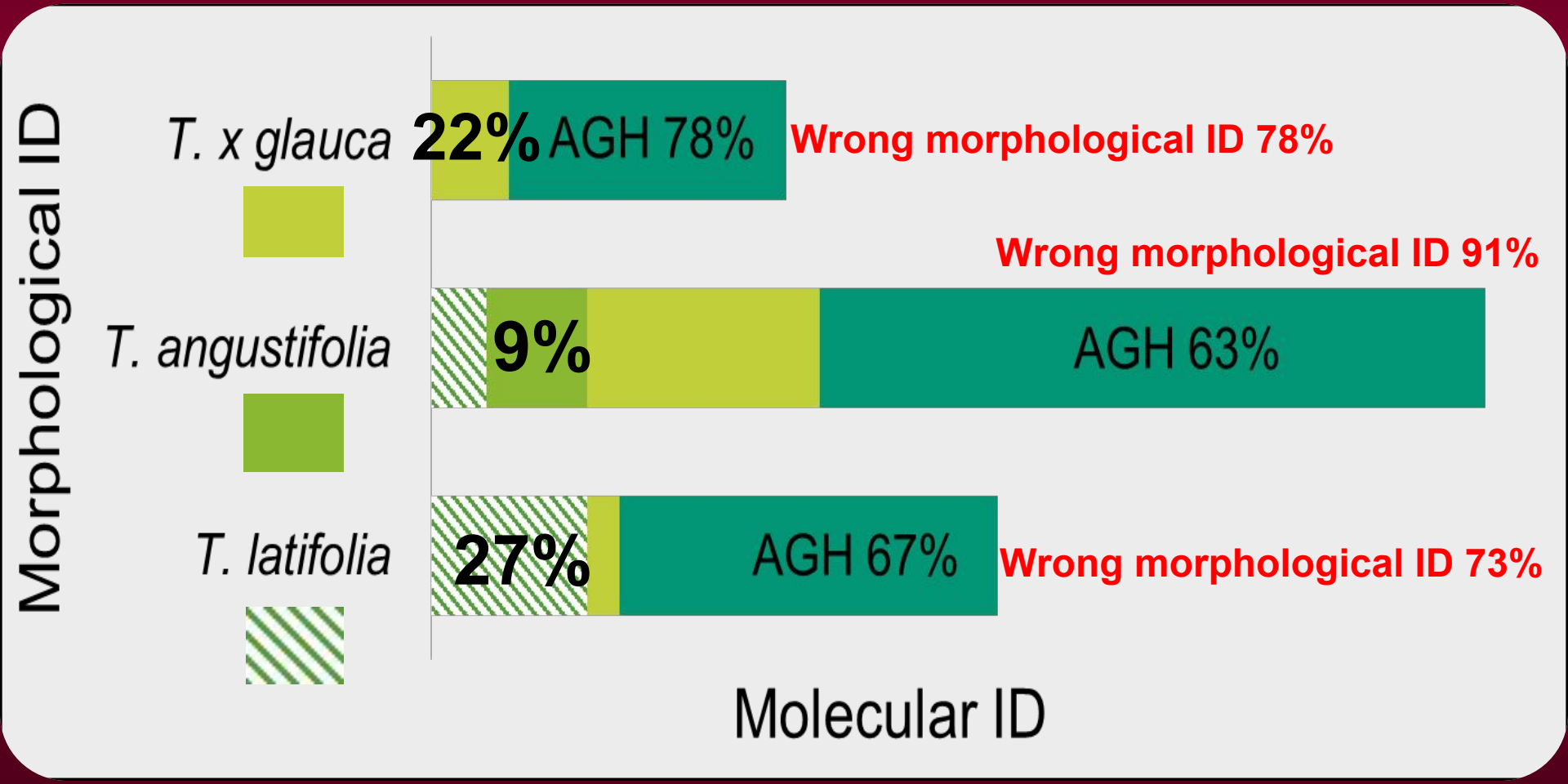

Geddes et al. in prep.

# Q2: Do we detect fertile hybrids that backcross to parental species?




Geddes et al. in prep.

# Q3: How accurate is morphological identification?




Geddes et al. in prep.

# Conclusions

- Morphological ID is not accurate/reliable
- Relative abundance of parental species was low compared to AGH hybrids

  → Introgression is widespread
- Backcrossing of hybrid to native parent (*T. latifolia*) was ~3X greater than to exotic parent
- The hybrid may be replacing both parental species → extinction by hybridization for the native cattail

  - Seed banking for *T. latifolia* to preserve genetic diversity?

# What to do?

- "Management and conservation is easiest when no introgression occurs"
  - Hybrid swarms are a virtually *intractable* problem

- Work on rapid detection of hybridization and fast response → TOUGH and TOO LATE, but maybe we can try with other species?
  - 2 new cases of *Typha* invasion

http://labs.russell.wisc.edu/peery/extinction-of-species/

# *Typha laxmannii* (Graceful cattail)

Found in Milwaukee, WI – Classified as prohibited



http://alienplantsbelgium.be/content/typha-laxmannii

Photo courtesy of Kelly Kearns, WI-DNR

# 20 primers tested → 8 supported the identification of the species as different from Midwest cattails

**(3 overlap, 6 need to be retested, 3 did not work)**

| | | *T. laxmannii* | *T. latifolia* | *T. angustifolia* |
|---|---|---|---|---|
| | Primer derived from: | Size of microsatellite (bp) | | |
| TA 3 | *T. angustifolia* | 241-245 | 191-193 | 224-228 |
| TA 5 | *T. angustifolia* | 350-352 | 295-298 | 303-306 |
| TA 8 | *T. angustifolia* | 293-294 | 288-292 | 304-308 |
| TM 2 | *T. minima* | 340 | | |
| TM 3 | *T. minima* | 296-297 | | |
| TM 4 | *T. minima* | 186-187 | 189 | 194 |
| TM 5 | *T. minima* | 152-153 | | |
| TM 7 | *T. minima* | 301 | | |
| TM 9 | *T. minima* | 162 | | |
| TM 11 | *T. minima* | 302-305 | 305-307 | 302-304 |
| TM 14 | *T. minima* | 347-348 | | |

# *Typha laxmannii*

- Given morphological characteristics and our molecular results → samples belong to either *T. laxmannii* or *T. minima*

- Both species have been thought to be synonymous, with very similar morphology

The Plant List → *Angiosperms* → *Typhaceae* → *Typha* → *Typha laxmannii* Lepech.

## *Typha laxmannii* Lepech. is an accepted name

This name is the accepted name of a species in the genus *Typha* (family *Typhaceae*).

The record derives from WCSP (data supplied on 2012-03-26) which reports it as an accepted name (record 271021) with original publication details: *Nova Acta Acad. Sci. Imp. Petrop. Hist. Acad. 12: 84 1801.*

Full publication details for this name can be found in IPNI: http://ipni.org/urn:lsid:ipni.org:names:836872-1.

## Synonyms:

See "Status", "Confidence level", "Source" for definitions.

Sort the name records using the ▲▼ buttons.

24 synonyms . . .

| | | | | |
|---|---|---|---|---|
| *Typha angustissima* Griff. ex Rohrb. | Synonym | ★★★ | WCSP | 2012-03-26 |
| *Typha balansae* Reut. ex Rohrb. | Synonym | ★★★ | WCSP | 2012-03-26 |
| *Typha bungeana* C.Presl | Synonym | ★★★ | WCSP | 2012-03-26 |
| *Typha caucasica* Lehm. ex Rohrb. | Synonym | ★★★ | WCSP | 2012-03-26 |
| *Typha elliptica* C.C.Gmel. | Synonym | ★★★ | WCSP | 2012-03-26 |
| *Typha juncea* Steven ex Rohrb. | Synonym | ★★★ | WCSP | 2012-03-26 |
| *Typha juncifolia* Celak | Synonym | ★★★ | WCSP | 2012-03-26 |
| *Typha juncifolia* Montando | Synonym | ★★★ | WCSP | 2012-03-26 |
| *Typha laxmannii* var. *bur* ei Krasnova | Synonym | ★☆☆ | WCSP | 2012-03-26 |
| *Typha laxmannii* var. *mongolica* Kronf. | Synonym | ★☆☆ | WCSP | 2012-03-26 |
| *Typha laxmannii* var. *planifolia* Kronf. | Synonym | ★☆☆ | WCSP | 2012-03-26 |
| *Typha laxmannii* var. *turczaninovii* Krasnova | Synonym | ★☆☆ | WCSP | 2012-03-26 |
| *Typha media* Barbieri ex Rohrb. [Illegitimate] | Synonym | ★★★ | WCSP | 2012-03-26 |
| *Typha minima* Hoffm. [Illegitimate] | Synonym | ★★★ | WCSP | 2012-03-26 |
| *Typha minima* subsp. *laxmannii* (Lepech.) Nyman | Synonym | ★☆☆ | WCSP | 2012-03-26 |
| *Typha minima* var. *nana* (Avé-Lall.) Nyman | Synonym | ★☆☆ | WCSP | 2012-03-26 |
| *Typha minor* Sm. | Synonym | ★★★ | WCSP | 2012-03-26 |
| *Typha minuta* Schrenk ex Rohrb. | Synonym | ★★★ | WCSP | 2012-03-26 |
| *Typha nana* Avé-Lall. | Synonym | ★★★ | WCSP | 2012-03-26 |
| *Typha poitiaei* Poit. ex Rohrb. | Synonym | ★★★ | WCSP | 2012-03-26 |
| *Typha stenophylla* Fisch. & C.A.Mey. | Synonym | ★★★ | WCSP | 2012-03-26 |
| *Typha veresczaginii* Krylov & Schischk. | Synonym | ★★★ | WCSP | 2012-03-26 |
| *Typha zerovii* Klokov f. & Krasnova | Synonym | ★★★ | WCSP | 2012-03-26 |
| *Typha zerovii* Klok. fil & A. Krasnova | Synonym | ★★☆ | TRO | 2012-04-18 |

***T. minima***

# *Typha domingensis* (Southern cattail)

https://pfaf.org/USER/Plant.aspx?LatinName=Typha+domingensis

- Native to temperate and tropical regions (FL, Caribbean)
- Problem: Found in IL, WI, and OH
- Morphologically ≈ Midwestern cattails
- Can hybridize with *T. latifolia* and *T. angustifolia* (Smith 1967, 2000)
- Analyzed WI samples using our primers

☞ Find out what happened in next talk by Alice Thompson!

# Lessons learned

1. Cattail morphological ID based on leaf width and inflorescence gap is not accurate
2. *Typha* taxonomy needs revision
   - Incorporate new info from molecular analysis
3. More Eurasian cattails will spread and presumably hybridize with Midwestern cattails, given globalization and climate change, and genus characteristics
4. Rapid detection and response is critical
   -But hybridization makes this even harder than it already is (**cryptic invasions**)
   -Research-wise, we lag behind the invasions

# Thanks!


- USDA CREAR Award #2010-38422-21240
- NEIU COR Grant, Research Communities Grant, and SCSE Summer Research Grants
- NIH MARC grant (Award T34 GM105549)
- Chicago Botanic Garden (J. Fant, L. Steger, H.. Noble)
- Jennifer Richards, Florida International University, for *T. domingensis* samples from FL, and Alice Thompson for WI samples
- Amy Kretlow, Maureen Ferry, and Kelly Kearns from WI-DNR for *T. laxmannii* samples
- NEIU Biology Dept.