



Plant Barcoding Initiatives in Canada: *Challenges and New Directions*

Canadian Plant Barcoding Group

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Research Progress

- Evaluative stage
- Criteria
 - a) suitable variability for species discrimination
 - b) Relative ease of sequencing



Current Projects

	Candidate Regions	Taxonomic Scale	Geographic Scale
1. Ontario Report Card	7	land plants	regional
2. Grasses	4	family	regional/ national
3. Local flora (KSR)	---	land plants	local



Challenges to Plant Barcoding

- Implementation (e.g. application to pollen, seeds, roots, seedlings)
- Taxonomic ambiguities
 - a) species homogeneity
 - b) blurred species boundaries



Barcoding Roots

Ecology of root profiles:

- a) diversity
- b) vertical structure, biomass distribution
- c) ecosystem function

Challenge for barcoding:

- a) protoplastids, less abundant
- b) quantity of root, limited





Barcoding Roots at KSR

- Sequencing roots
 - a) Amplify cpDNA from 24 species
- Sample plots in old-field, forest ecosystems
 - a) Sample and map above-ground diversity
 - b) Sample/map roots from soil profiles
 - c) Identify roots to species using KSR plant barcode database





Taxonomic Ambiguities

Solidago

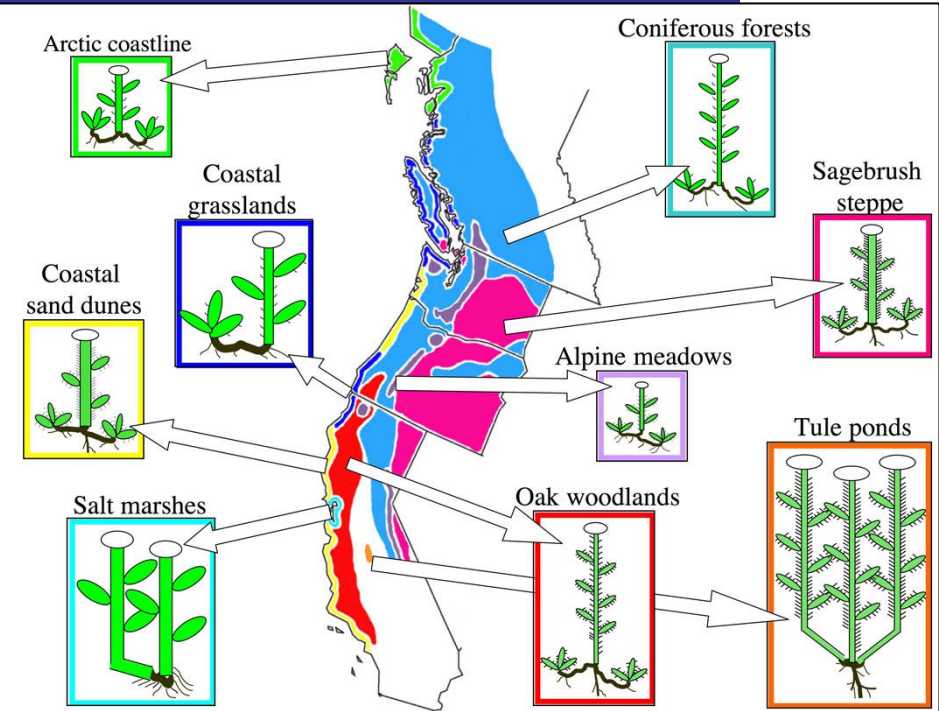
matK sequence

<i>S. canadensis</i>	CCAGGAAGGATTTATATAAACCAATTATCCAATCATTCCCT
<i>S. canadensis</i>	CCAGGAAGGATTTATATAAACCAATTATCCAATCATTCCCT
<i>S. caesia</i>	CCAGGAAGGATTTATATAAACCAATTATCCAATCATTCCCT
<i>S. caesia</i>	CCAGGAAGGATTTATATAAACCAATTATCCAATCATTCCCT
<i>S. nemoralis</i>	CCAGGAAGGATTTATATAAACCAATTATCCAATCATTCCCT
<i>S. nemoralis</i>	CCAGGAAGGATTTATATAAACCAATTATCCAATCATTCCCT
<i>S. nemoralis</i>	CCAGGAAGGATTTATATAAACCAATTATCCAATCATTCCCT
<i>S. altissima</i>	CCAGGAAGGATTTATATAAACCAATTATCCAATCATTCCCT
<i>S. altissima</i>	CCAGGAAGGATTTATATAAACCAATTATCCAATCATTCCCT
<i>S. altissima</i>	CCAGGAAGGATTTATATAAACCAATTATCCAATCATTCCCT



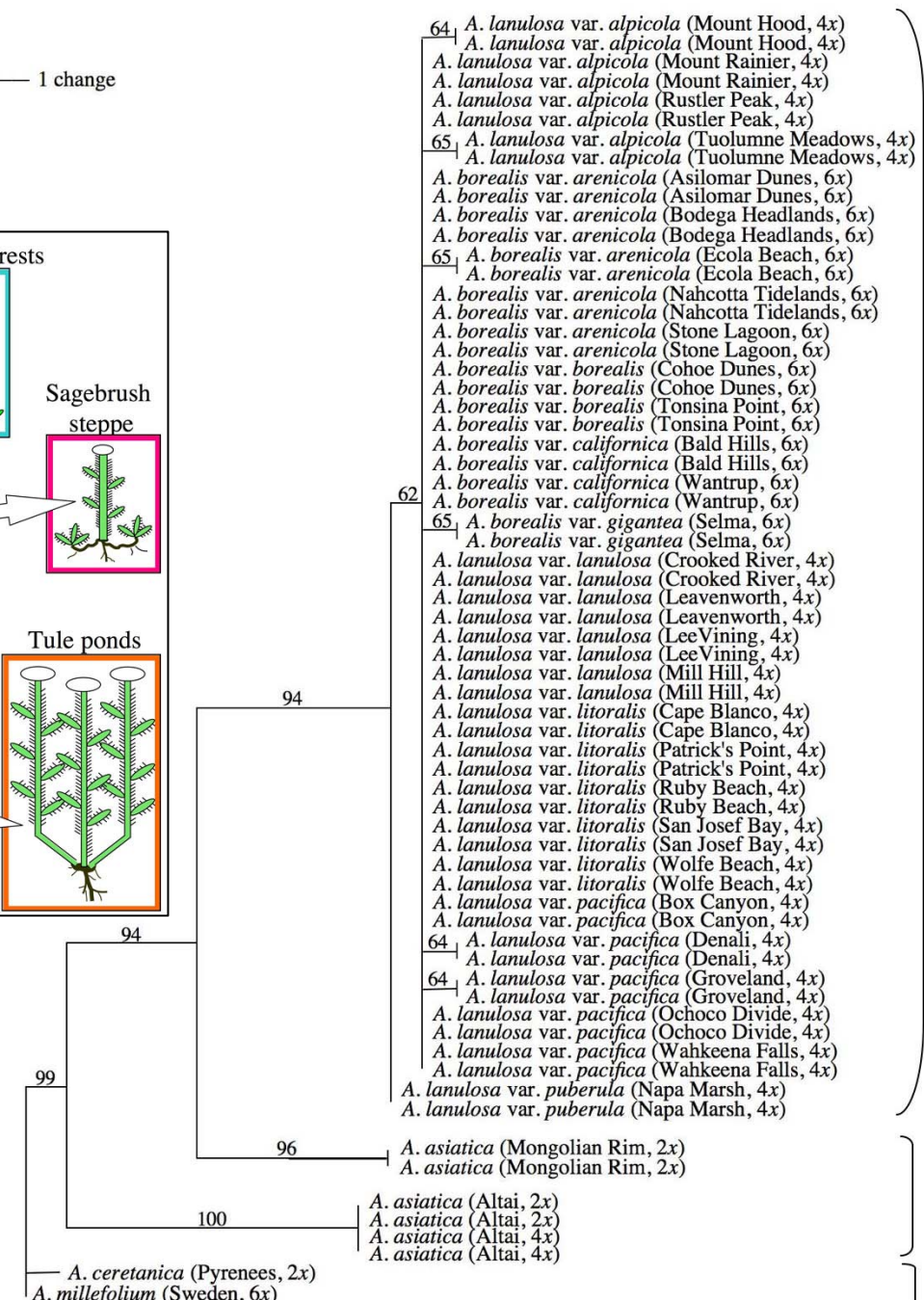
Recent, rapid diversification

— 1 change



e.g. *Achillea*

Ramsey & Husband unpubl.



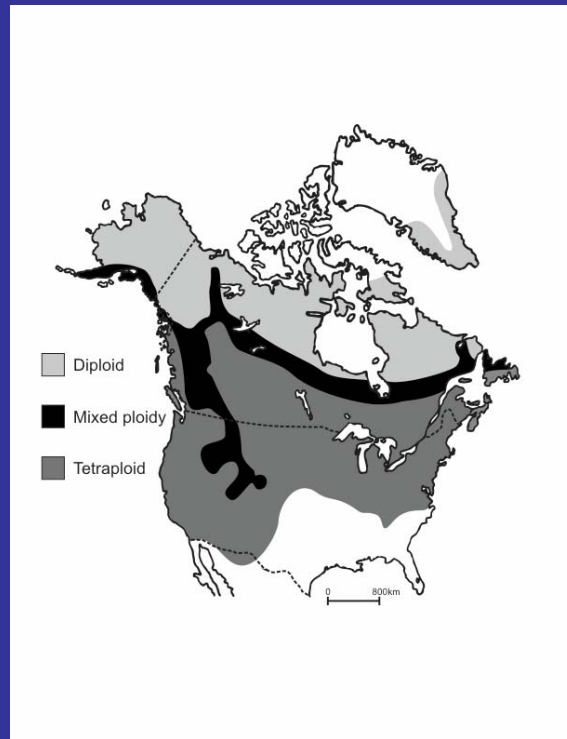
North America

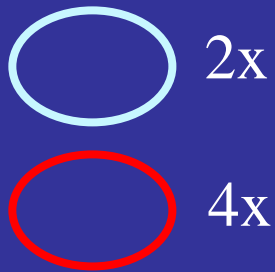
Asia

Europe

3500 bp cpDNA

Genome duplication *e.g. Chamerion angustifolium*





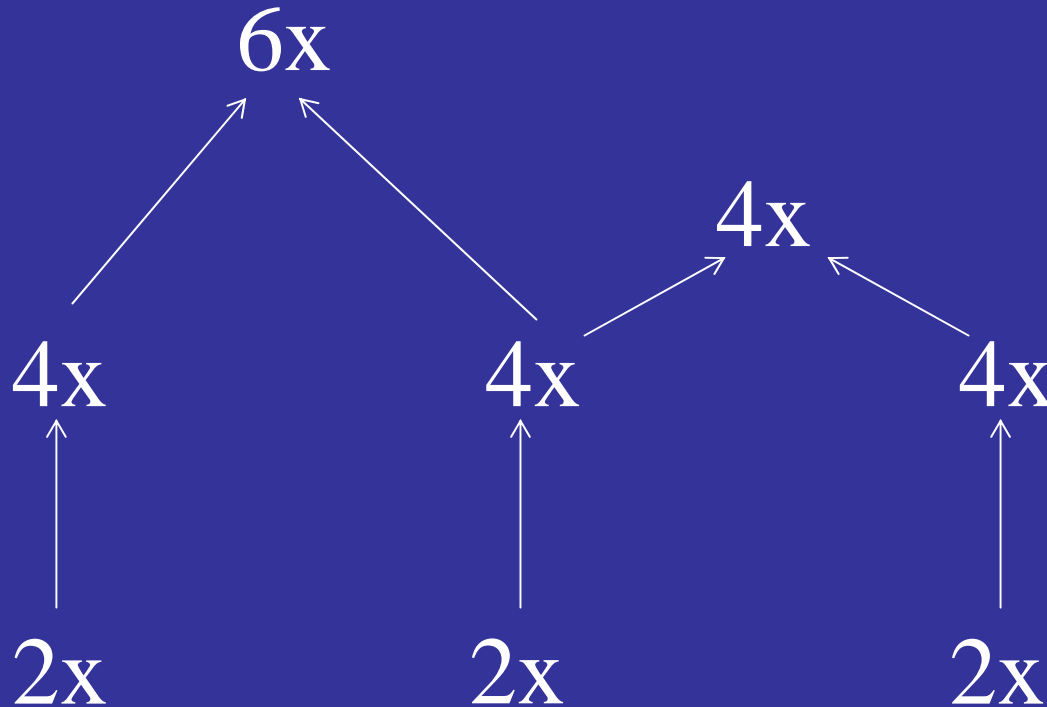
cpDNA haplotype network (2700 bp)

Hybridization

e.g. Solidago

matK

<i>S. canadensis</i>	CCAGGAAGGATTTATATAAAACCAATTATCCAATCATTCCCT
<i>S. canadensis</i>	CCAGGAAGGATTTATATAAAACCAATTATCCAATCATTCCCT
<i>S. caesia</i>	CCAGGAAGGATTTATATAAAACCAATTATCCAATCATTCCCT
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Role of low-copy Nuclear Genes in Plant Barcoding

Advantages

- a) rapid evolution
- b) biparental inheritance

Challenges

- a) lack of info on universal nuclear genes
for plants
- b) lack of universal primers



Selection of Nuclear Genes for Plant Barcoding

Survey taxonomically diverse, genomic
databases for single copy nuclear genes

180 candidate nuclear genes

Exclude genes
with introns

6 genes



Alignment of nuclear gene sequences of *Solidago*

S.canadensis	ACT T TCTTA A GGTGGATGAAAT-ATCCCTTTC A CTTTTCTTTTT
S.canadensis	ACT T TCTTA A TGGTGGATGAAATTATCCCTTTC G CTTTTCTTTTT
S.caesia	ACT T TCTTA A GGTGGATGAAATTATCC A TT C ACTTTTCTTTTT
S.caesia	ACT G TCTTA C GGTGGATGAAAT-ATCCCTT TG ACTTTTCTTTTT
S.nemoralis	ACT T TCTTA A TGGTGGATGAAAT-ATCCCTTTC A CTTTTCTTTTT
S.nemoralis	ACT T TCTTA A NGGTGGATGAAATTATCCCTT TN ACTTTTCTTTTT
S.nemoralis	ACT T TCTTA A NGGTGGATGAAATTATCCCTT TN ACTTTTCTTTTT
S.altissima	ACT G TCTTA A TGGTGGATGAANTTATCCCTT TC ACGTTTCTTTTT
S.altissima	ACT G TCTTA A TGGTGGATGAA-TTATCCCTT TC ACNTTTCTTTTT
S.altissima	ACT T TCTTA A GGTGGATGAA-TTATCCCTT TC ACTTTTCTTTTT
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Summary

- Continue to complete the evaluation of candidate barcodes at several taxonomic and geographic scales
- Explore ecological applications where quality and quantity of tissue is suboptimal.
- Explore candidate nuclear genes for taxonomically complex groups.