Nuclear, chloroplast, and mitochondrial data of a US cannabis DNA database

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Paper Presentation

NIJ Disclosure

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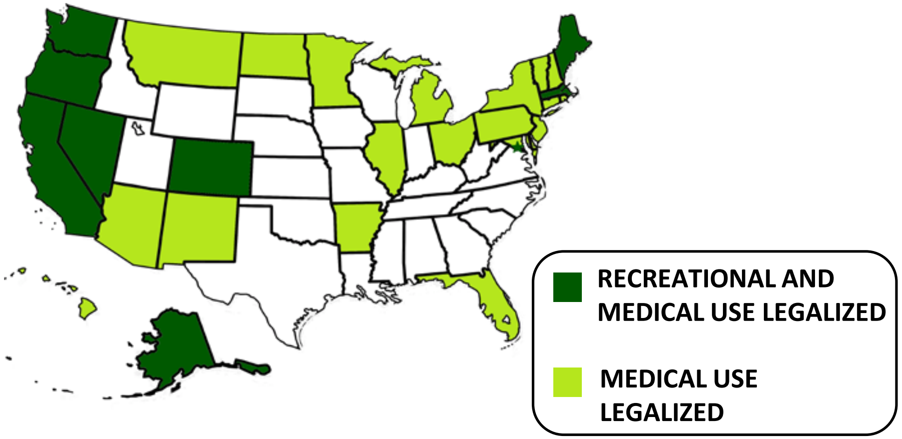
Disclosure

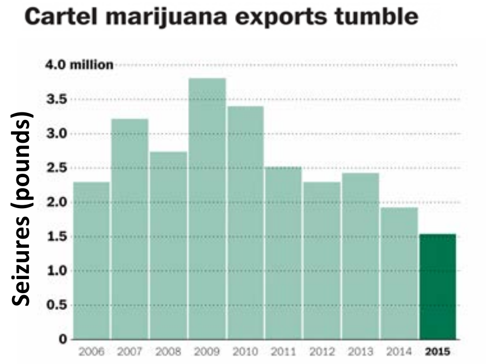
There is no real or apparent conflicts of interest related to the content of this presentation.

Statement of the Problem

Marijuana legalization

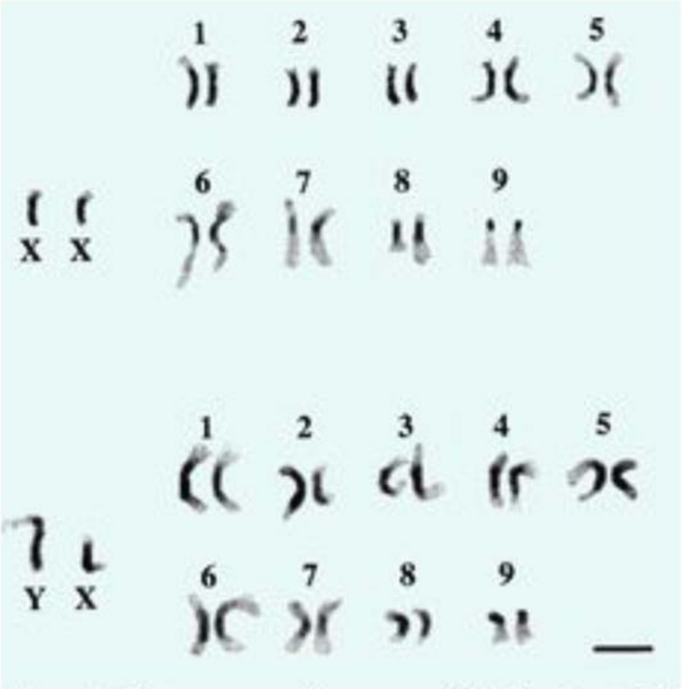


Illegal trafficking



Marijuana Background

- Family: Cannabaceae
- Dioecious plant
- Diploid genome: 2n = 20
    - 9 pairs of autosomes
    - Pair of sex chromosomes
- Draft genome complete[1]
    - 818 Mb for female (haploid)
    - 843 Mb for male (haploid)

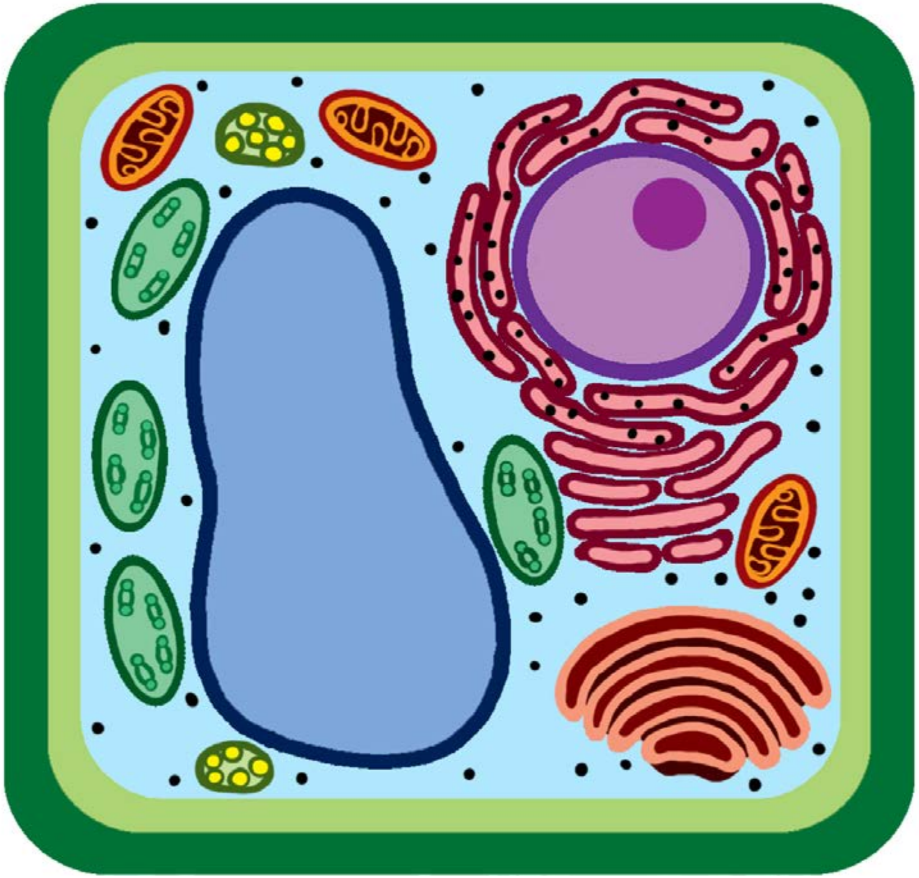


Cannabis Chromosome Karyogram by Sakamoto, et al.
The Japan Mendel Society, 1998

[1] van Bakel, Stout et al. 2011)

Organelle DNA

- Uniparentally inherited
- Circular genome
- Chloroplast
  - 153,871 bp[2]
- Mitochondria
  - 415,499 bp[3]

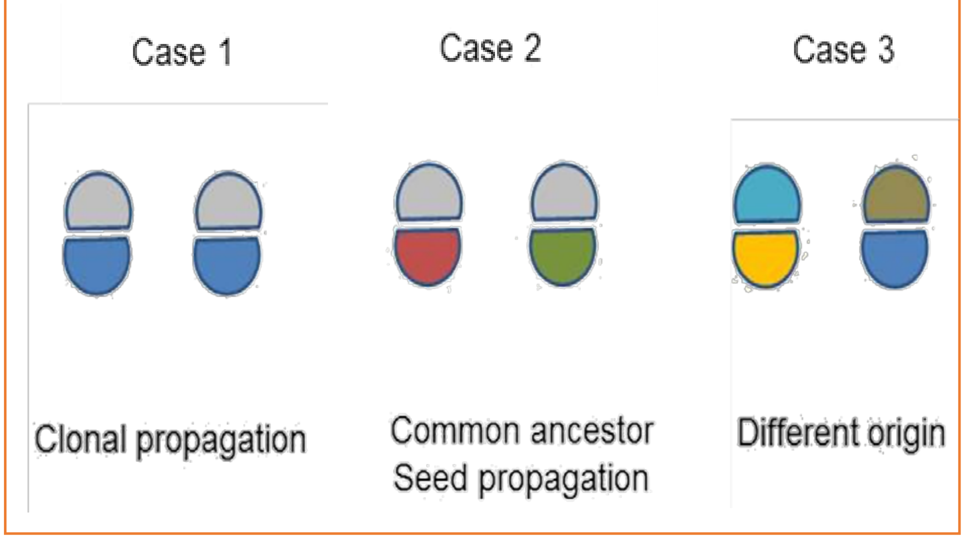


[2] Vergara, White et al. 2016
[3] Vergara, White et al. 2016

Goal of Research

- Build a database of DNA profiles
- Autosomal genotyping
    - Short tandem repeats
    - Individualizing plants
    - Genetic relatedness
- Organelle genotyping
    - Haplotypes
    - Biogeographical origin

DNA typing

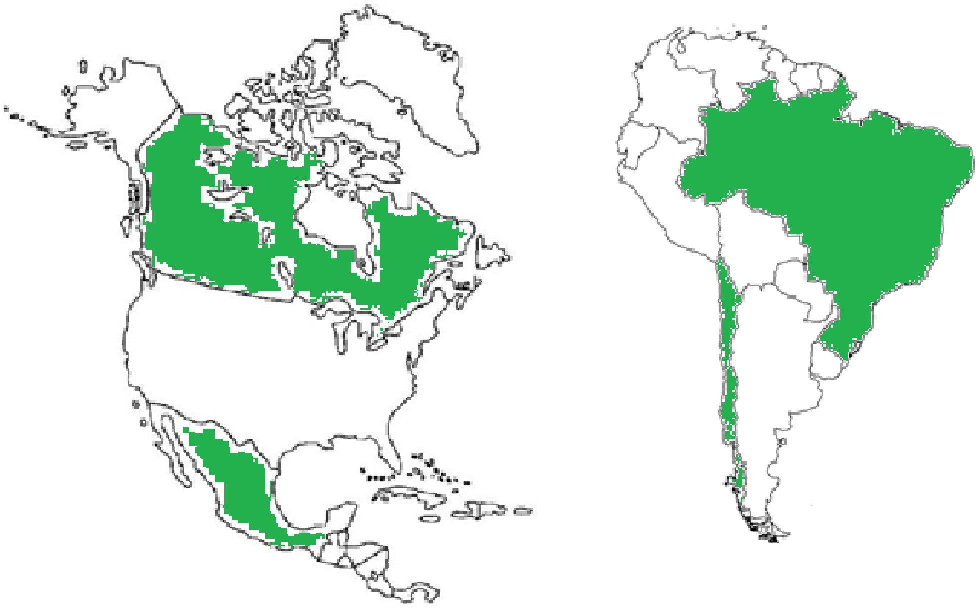


Materials and Methods

- Samples (4 sources)
- DNA Extraction (DNeasy® Plant Mini Kit)
- Autosomal DNA Typing
- Organelle DNA Typing
- Statistical Analysis

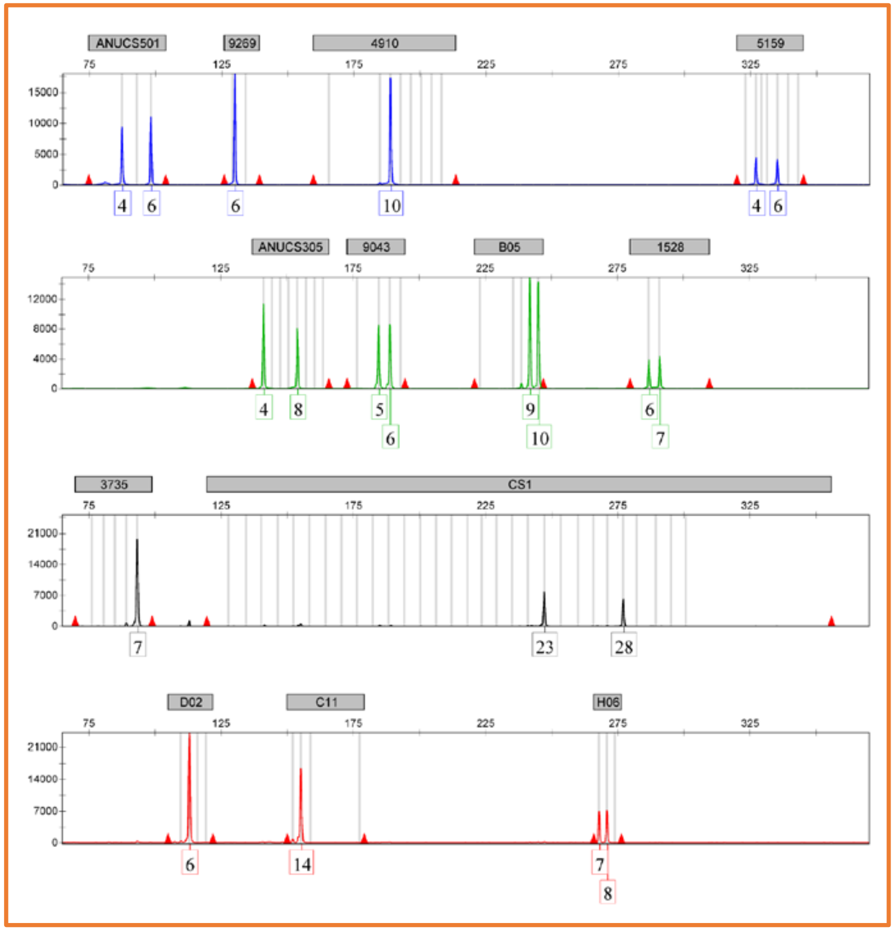
Samples

- US-Mexico Border:
  - 21 seizures
  - *N* = 422 samples
- Chile:
  - 10 seizures
  - *N* = 50 samples
- Brazil:
  - *N* = 8 samples
- Hemp Seeds:
  - Purchased in US (grown in Canada)
  - *N* = 30 samples



Autosomal DNA Typing

- Nuclear quantitation[4]
- Cannabis STR profiling using previously validated STR method[5]



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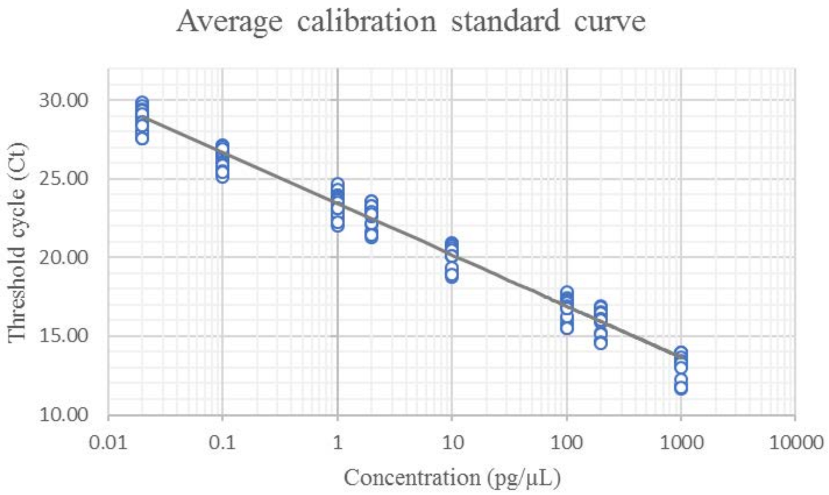
[4] Houston et al. 2016

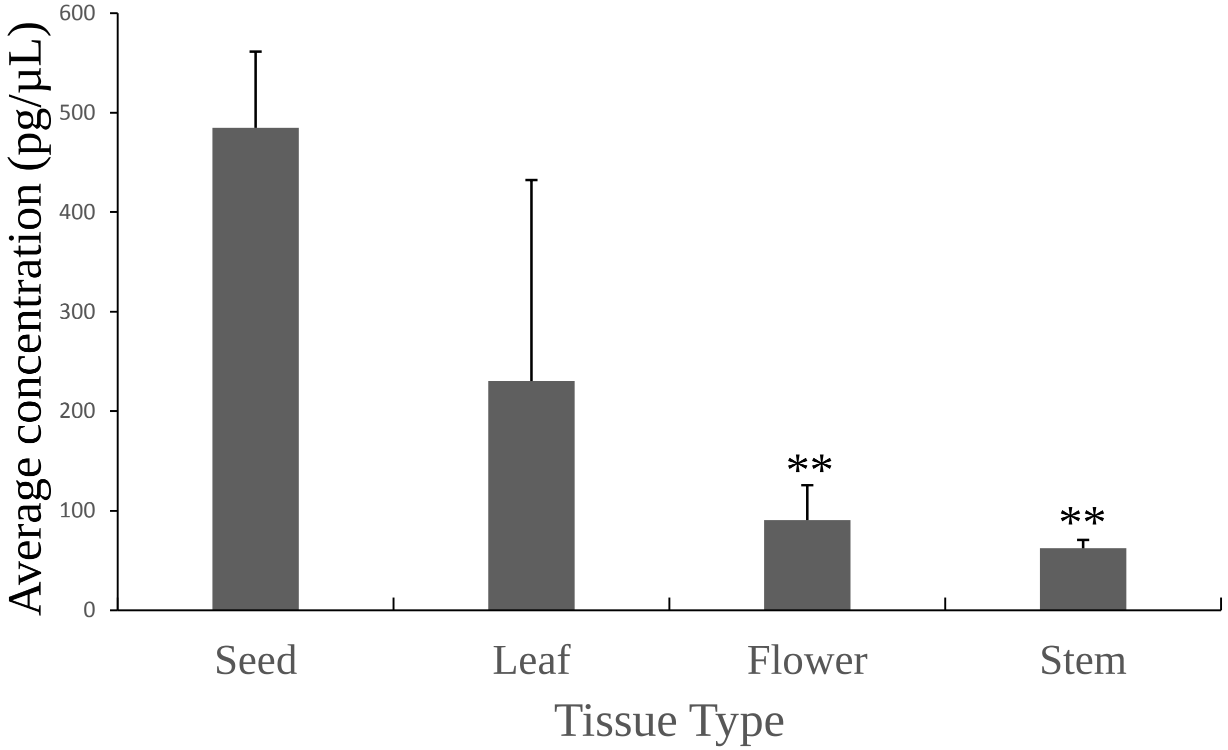
[5] Houston et al. 2017

Organelle DNA Quantitation

- Synthetic DNA standards:
  - Two complementary, PAGE-purified synthetic oligonucleotides
  - Cscp001 region of *C. sativa*
  - Diluted to generate standards 1000 – 0.02 pg/µL
- Quantitation via real-time PCR
- Validation studies:
  - Sensitivity
  - Specificity
  - Precision and accuracy



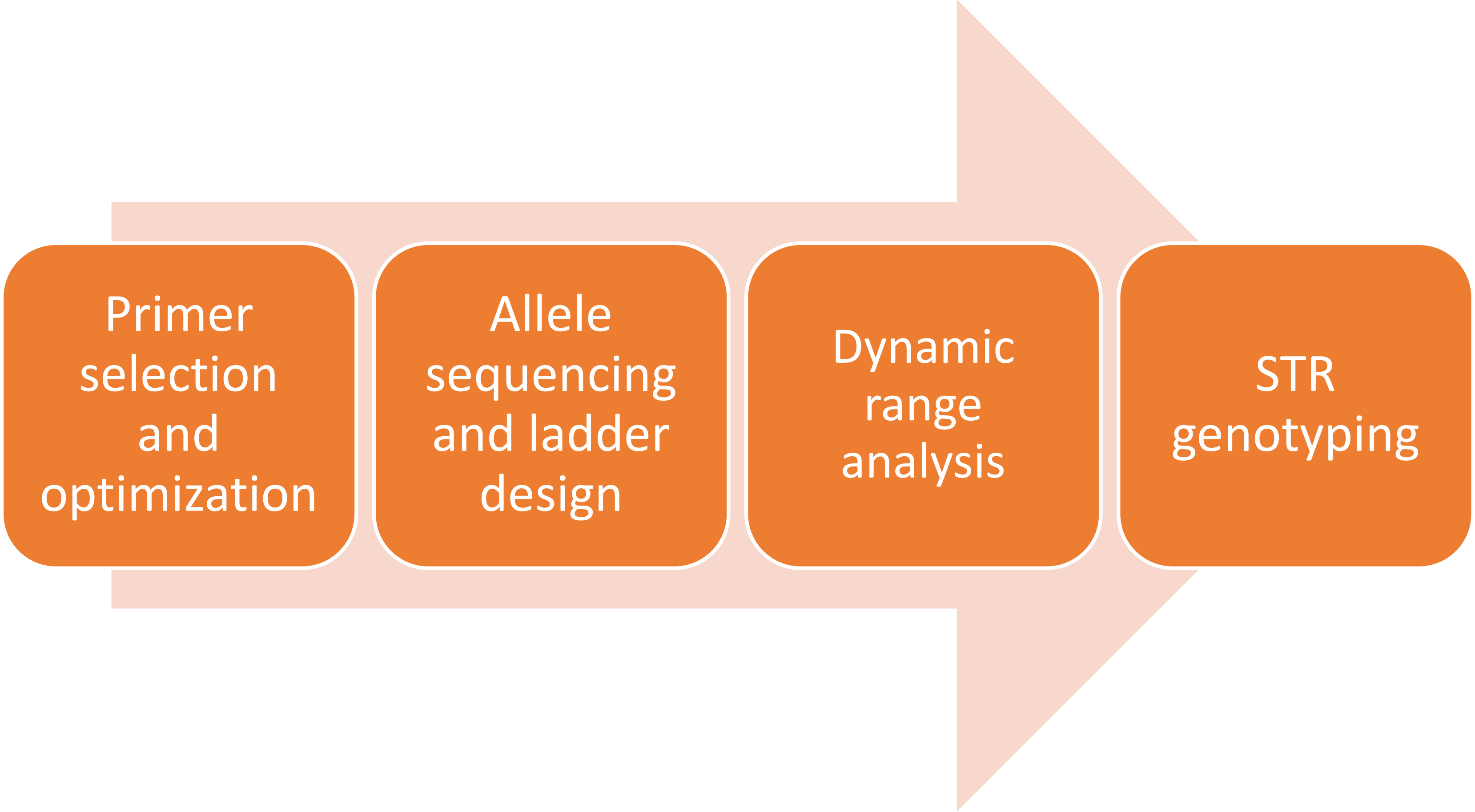
Relative cpDNA Quantitation



Error bars represent standard deviations.

** p-value < 0.01 when compared to seed tissue.

Organelle STR Typing

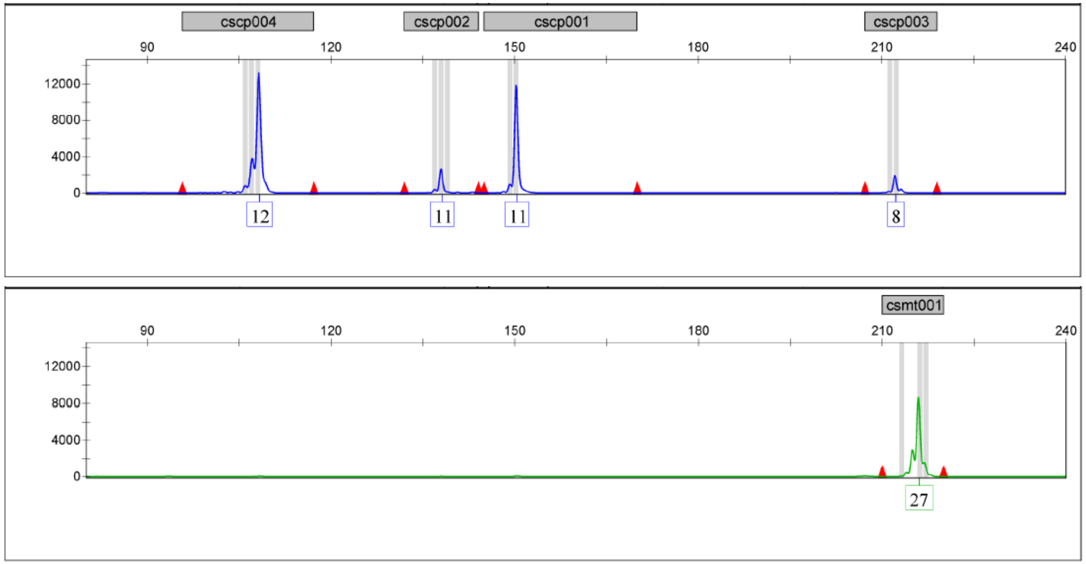


- Primer selection and optimization
- Allele sequencing and ladder design
- Dynamic range analysis
- STR genotyping

**** Modified from a previous study Gilmore et al. 2007**

Organelle STR Typing

Locus	Primer Reference	Region of DNA
STR based		
Cscp001	[6]Gilmore et al. 2007	*trn*L – *trn*F
Cscp002	[6]Gilmore et al. 2007	*rbc*L – orf106
Cscp003	[7]Weising et al. 1999	ccmp2 (5’ to *trn*S)
Cscp004	[7]Weising et al. 1999	ccmp6 (orf77 – orf82)
Csmt001	[6]Gilmore et al. 2007	*nad* 4 exon 3 to exon 4



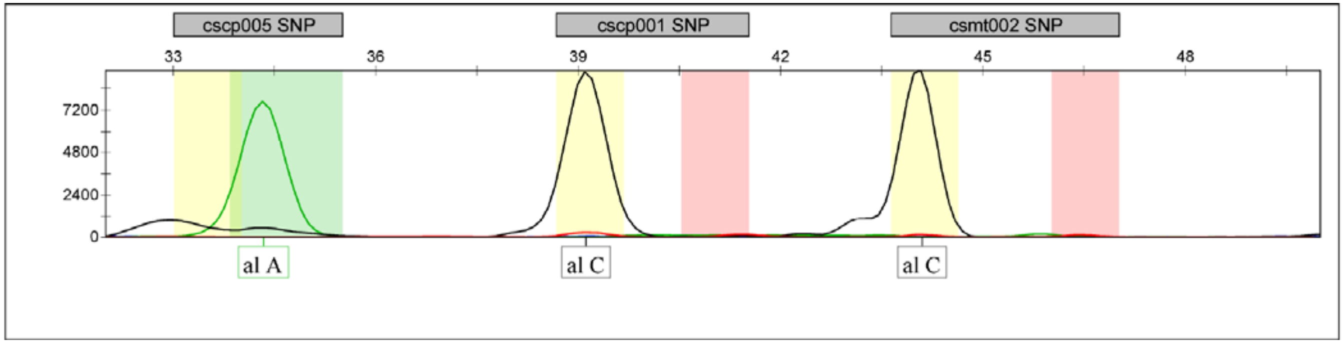
Organelle SNP Typing

- Primer design and optimization
- Allele confirmation via sequencing
- SNP genotyping (SNaPshot)

** Modified from a previous study Gilmore et al. 2007

Organelle SNP Typing

Locus	Primers	Primer Reference	Region of DNA
SNP based			
Cscp001	F: 5’ – tccctctatccccaaaaagg – 3’ R: 5’ – attgcacattgggattcctt – 3’ SBE F: 5’ – ttttttttttttacaaccggacctgaatgacc – 3’	This study	*trnL* – *trn*F
Cscp005	F: 5’ – tccactgccttgatccactt – 3’ R: 5’ – ccctctagacttagctgctct – 3’ SBE R: 5’ – cttttatcttgtctaaaattgaaat – 3’	This study	*trn*H – *trn*K
Csmt002	F: 5’ – tgtgcgaagagtgcgttatg – 3’ R: 5’ – acttcactcgctaggggatg – 3’ SBE F: 5’ – tttttttttttttttttttatgacctgtggccgcctg – 3’	This study	*nad* 5 exon 4 to exon 5



Statistical Analysis – Autosomal

- Number of multi-locus genotypes and genotype sharing determined
- Determine reference population
  - Phylogenetic analysis (GDA) and parsimony analysis (*PAUP*4.0a*)
  - Case-to-case comparisons with F_{ST}
- Phylogenetic analysis between reference population, Brazil, Chile, and hemp
  - Neighbor Joining method (GDA) and parsimony analysis (*PAUP*4.0a*)
  - Bayesian clustering with *STRUCTURE* software
  - Principal component analysis with *R* package, *Adegenet*

Statistical Analysis – Organelle

- Due to less variability, subsampling of 134 samples
- Number of haplotypes and haplotype sharing
- Phylogenetic analysis between reference population, Brazil, Chile, and hemp
  - Neighbor Joining method (GDA)
  - Parsimony analysis (*PAUP*4.0a*)

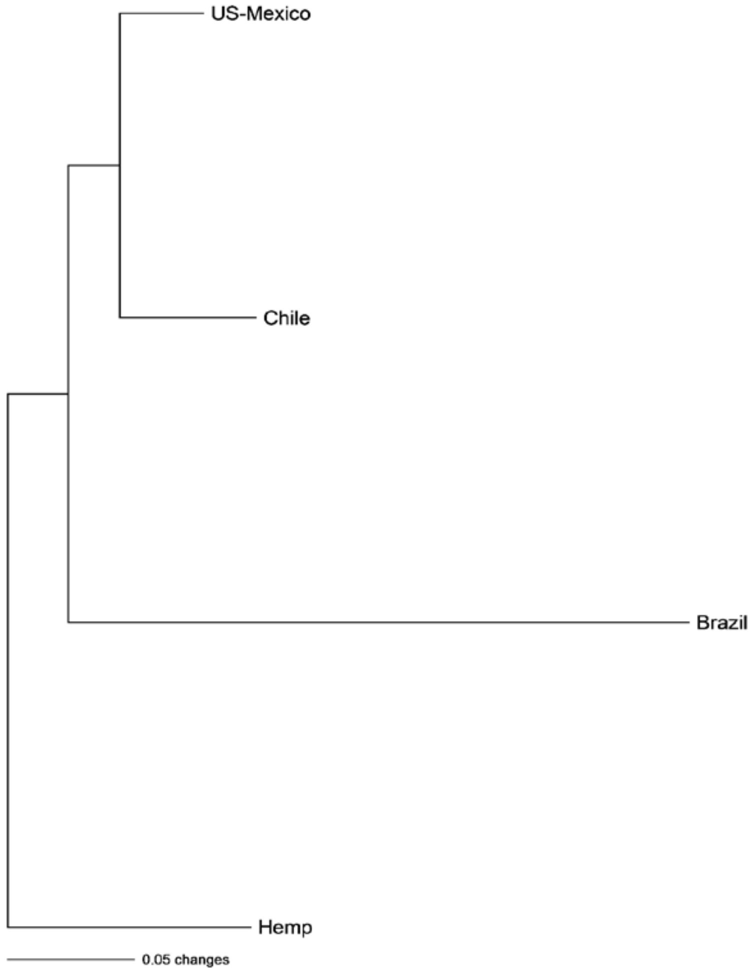
Autosomal – Genotypes

Source	Sample Number	Partial Profiles	Mixed Profiles	Full Profiles	Unique Genotypes	Duplicate Genotypes
US-Mexico	422	23	32	367	326	18 (41 samples)
Brazil	8	2	0	6	6	0
Chile	50	18	0	32	4	7 (28 samples)
Hemp	30	9	1	20	20	0
	510	52	33	425	356	25 (69 samples)

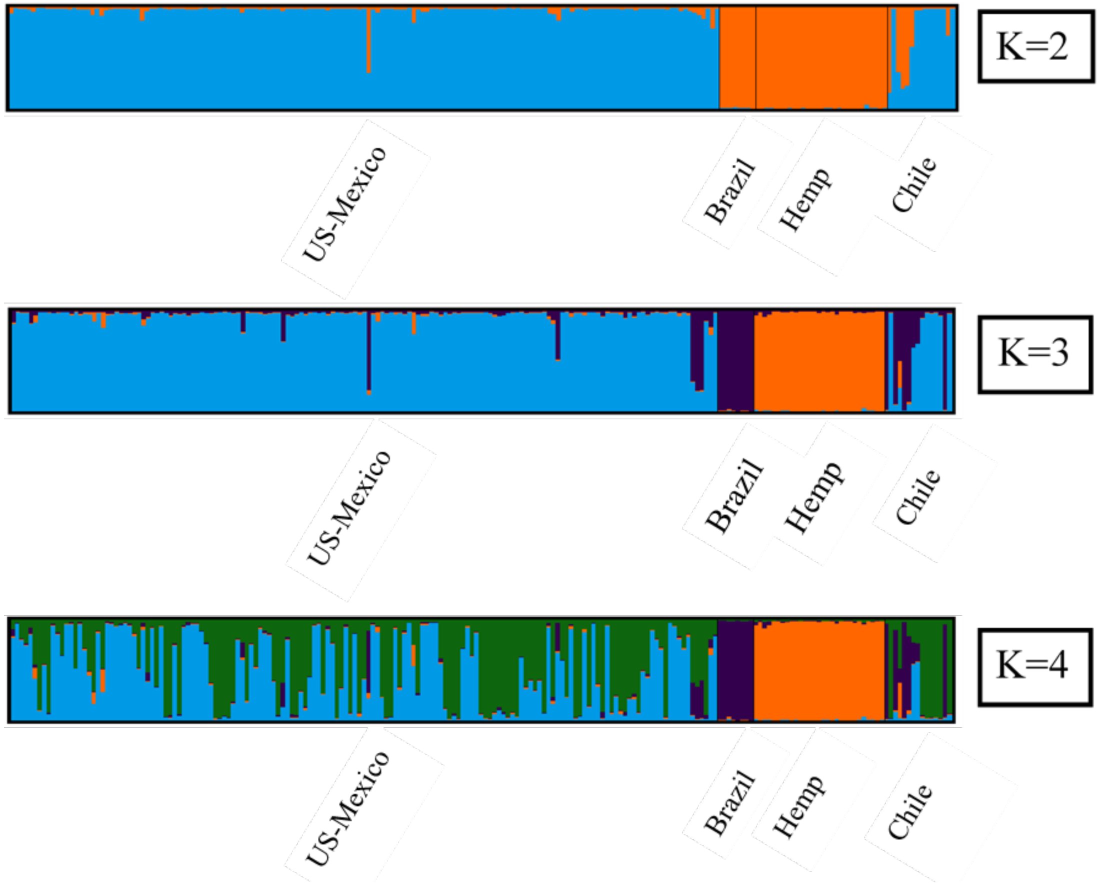
- Duplicates from same seizure or same plant
- Hypothesized that 9 out of 10 Chilean seizures clonally propagated

Autosomal – Phylogenetic Analysis

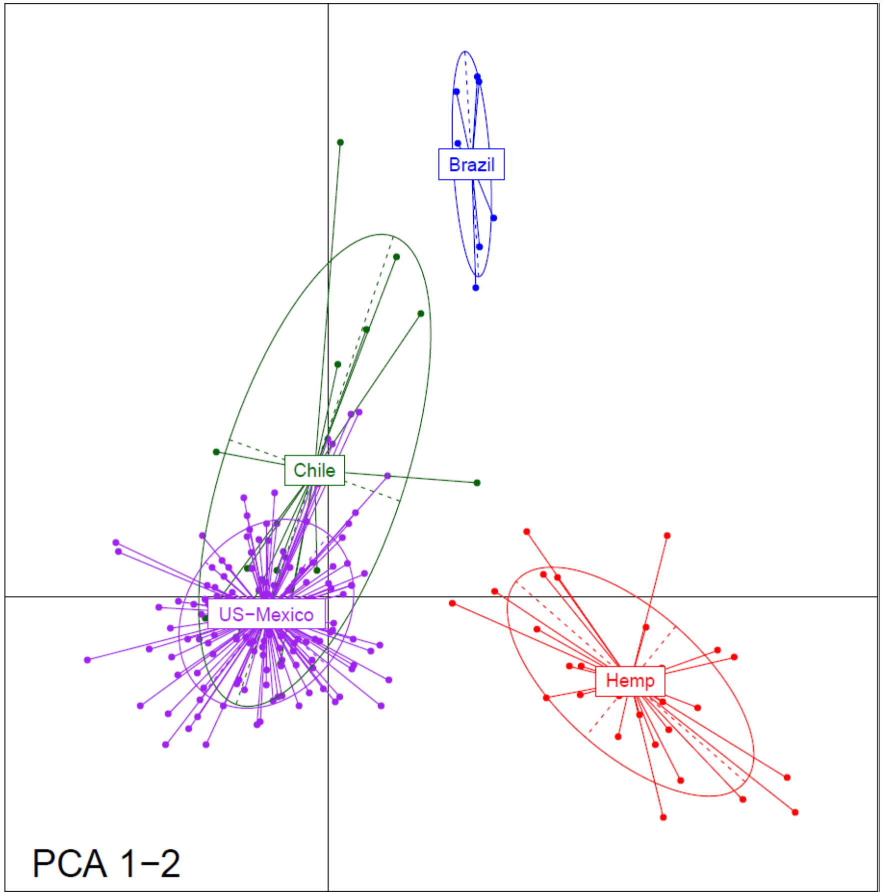
- 9 seizures (*N*=157 samples) formed a reference population for US-Mexico
  - $F_{ST} = 0$



Autosomal – Bayesian Clustering



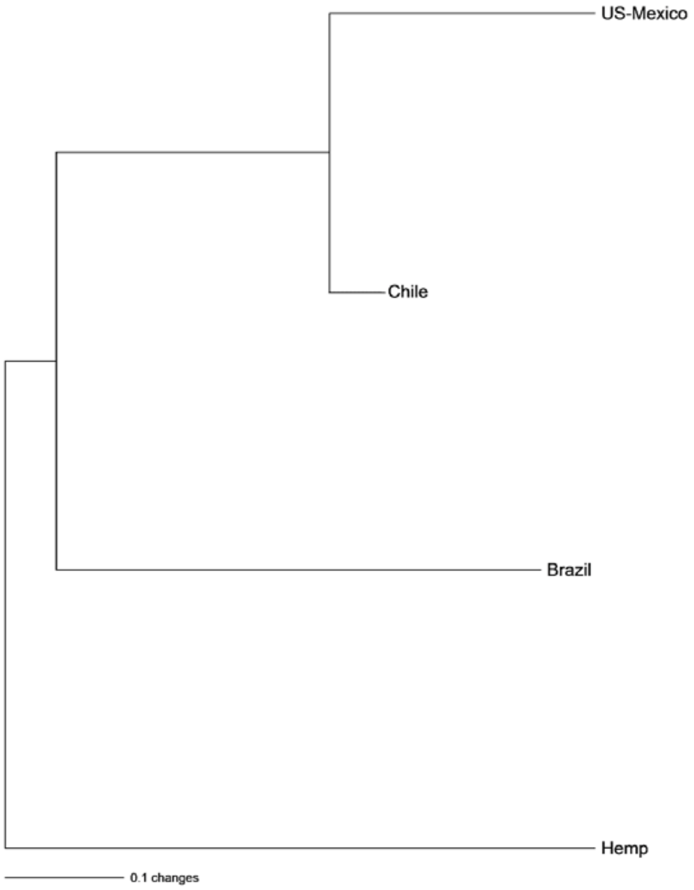
Autosomal – PCA



- Genetic relatedness between drug types
- Distinction from fiber type

Organelle – Statistical Analysis

- 127 out of 134 samples yielded full haplotypes
- Extensive haplotype sharing
- Five distinguishable haplotypes
- Haplotype sharing between US border seizures, Brazil, and Chile
- Distinct haplotype for hemp



Conclusions

1. Statistical analysis
   - Reference population of 9 seizures (*N*=157)
   - Both autosomal and organelle markers could discern population sub-structure
   - Clear distinction between drug and hemp type samples
2. Novel real-time PCR method for quantifying cannabis cpDNA using synthetic standards developed and validated
3. Organelle typing: two analytical methods were modified and optimized
   - Homopolymer STR pentaplex
   - SNP triplex with SNaPshot™ (Thermo Fisher Scientific)

References

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Acknowledgements

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Questions

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