

# Habitat distribution, population status, and genetic diversity of American ginseng



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# Project Objectives

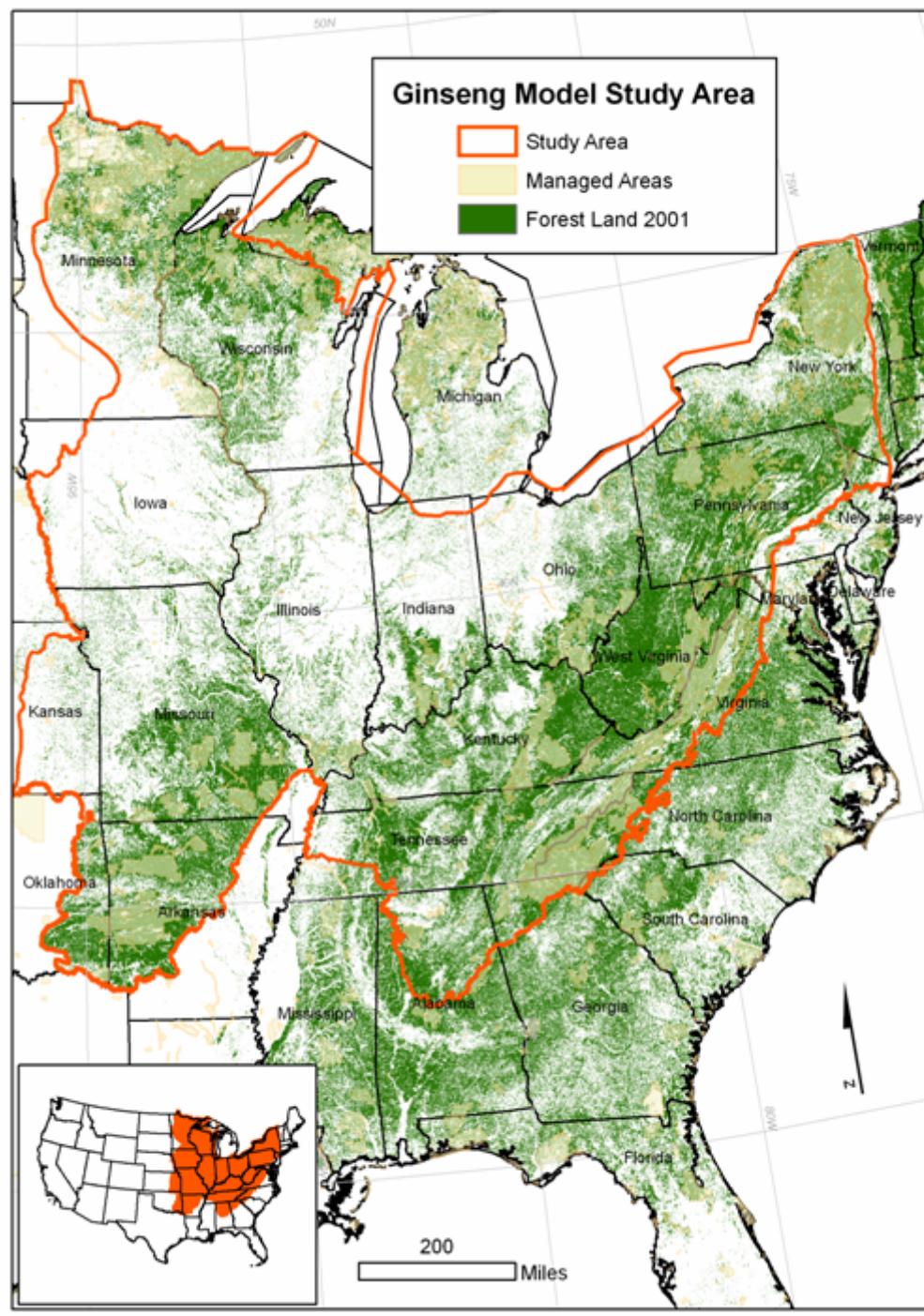
1. Develop habitat distribution models for American ginseng for portions of 18 states.
2. Determine demographic parameters and population characteristics.
3. Determine genetic diversity and structure within and among field sampled populations.

# Goals of Analysis

1. Contrast American ginseng distribution, population structure, and genetic diversity by land ownership

<p><u>Public conservation</u></p> <p>- National &amp; State Parks, Wildlife areas, Wilderness</p>	<p><u>Private conservation</u></p> <p>- Private conservancy lands</p>
<p><u>Public multiple use</u></p> <p>-National and State Forests, Game Lands, etc.</p>	<p><u>Other private</u></p>

2. Assess relationship between habitat suitability, connectivity, and genetic diversity



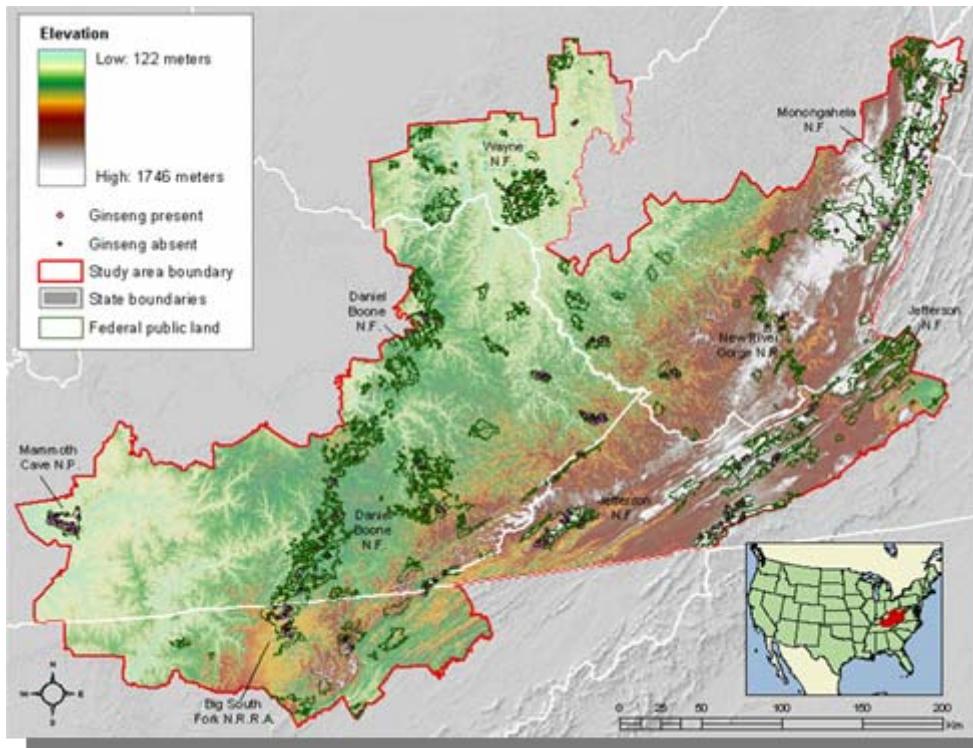
# Habitat modeling (previous study)

Regression equation to predict relative probability of occurrence:

$$\begin{aligned} & -1.2931 \text{ (intercept)} + (\text{slope} * 0.0589) \\ & - (\text{elevation} * 0.0012) + (\% \text{ deciduous forest} * 0.0181) - (\text{average solar insolation} * 0.0129) \end{aligned}$$

Probability of occurrence increases with:

- Decreasing elevation
- Increasing slope
- Deciduous forest cover
- Low sun exposure

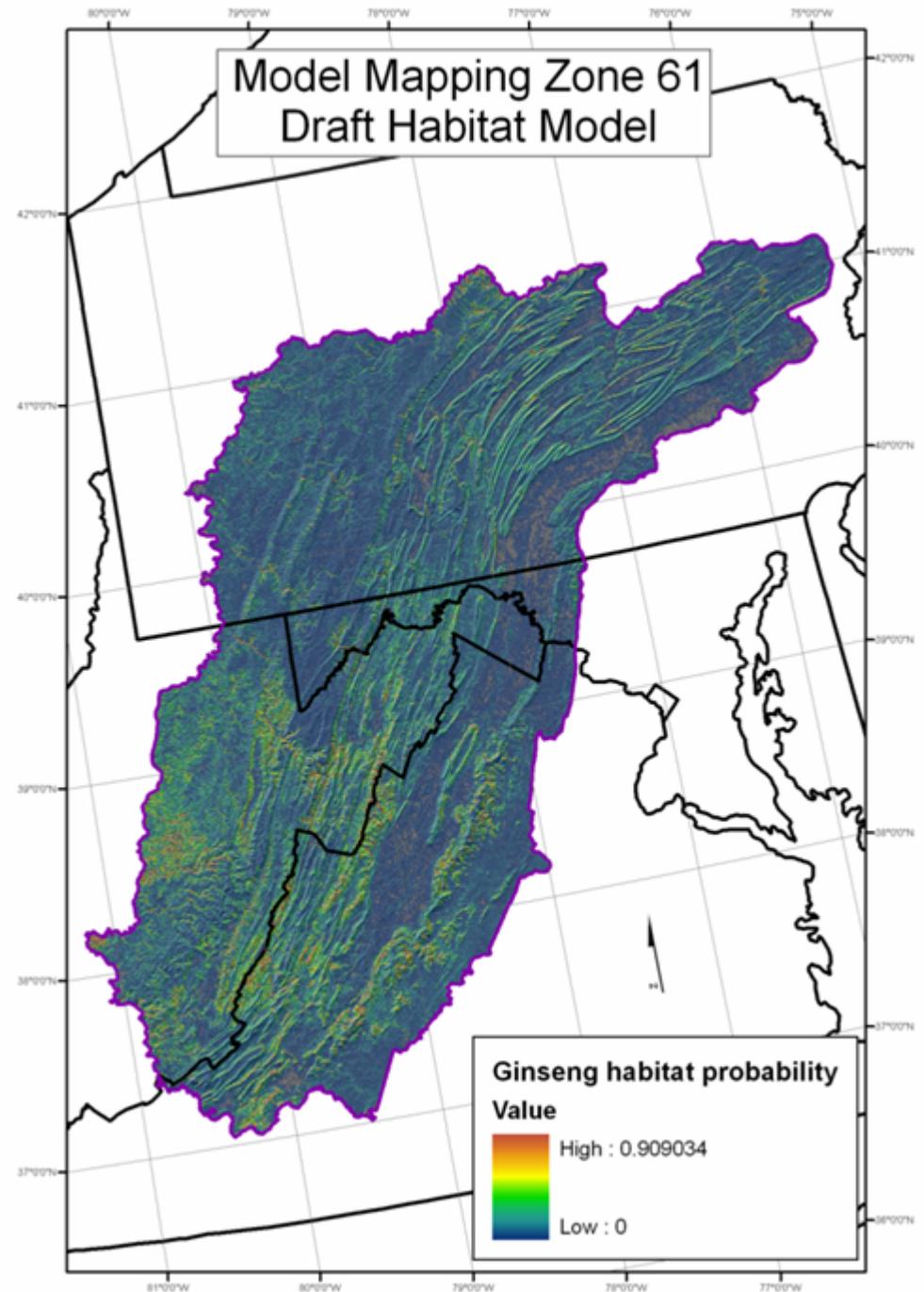


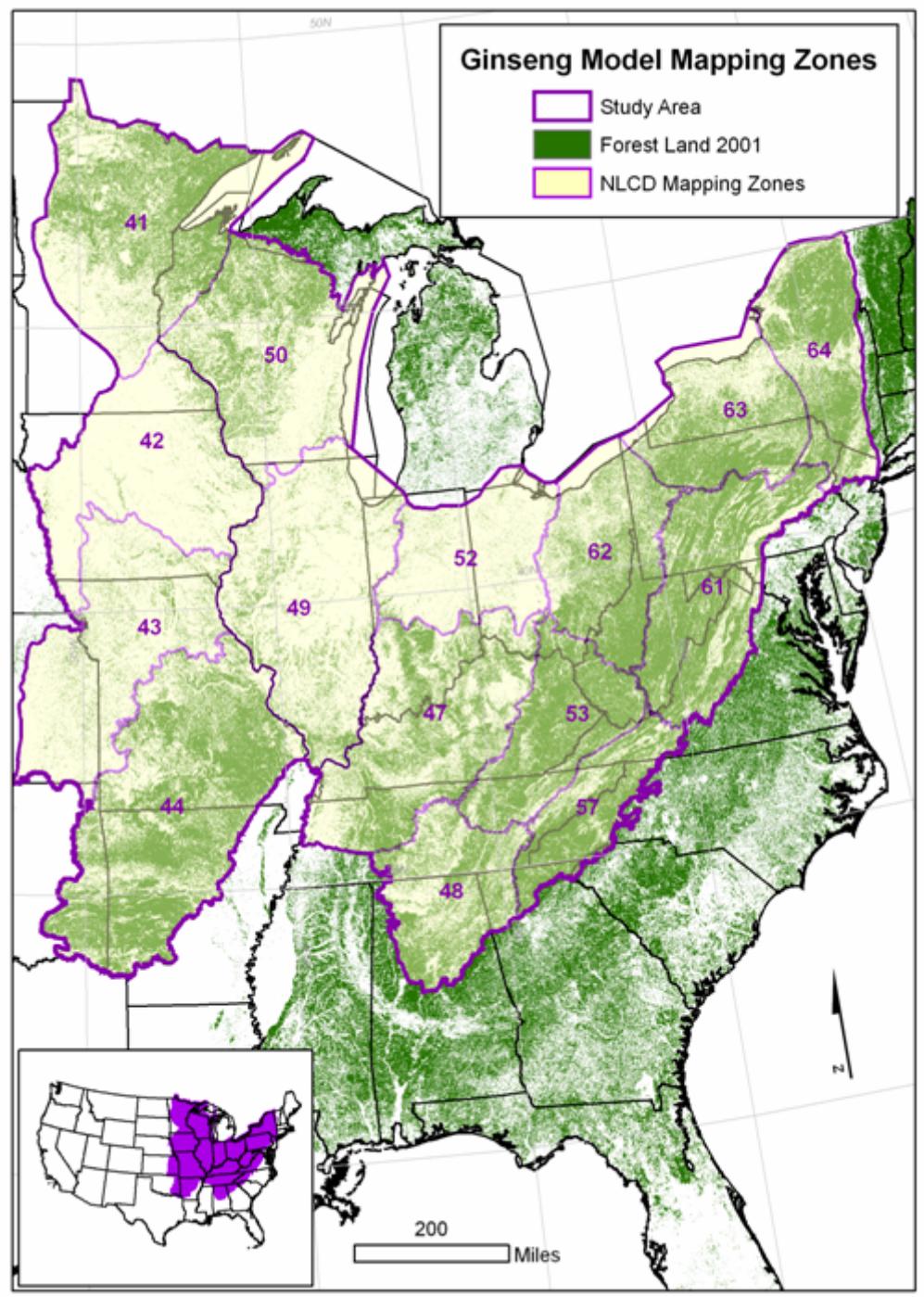
2004-2005 model study area

# Habitat modeling (current study – 1<sup>st</sup> draft)

Extrapolated logistic regression parameters from previous models to 15 “mapping zones” using GIS

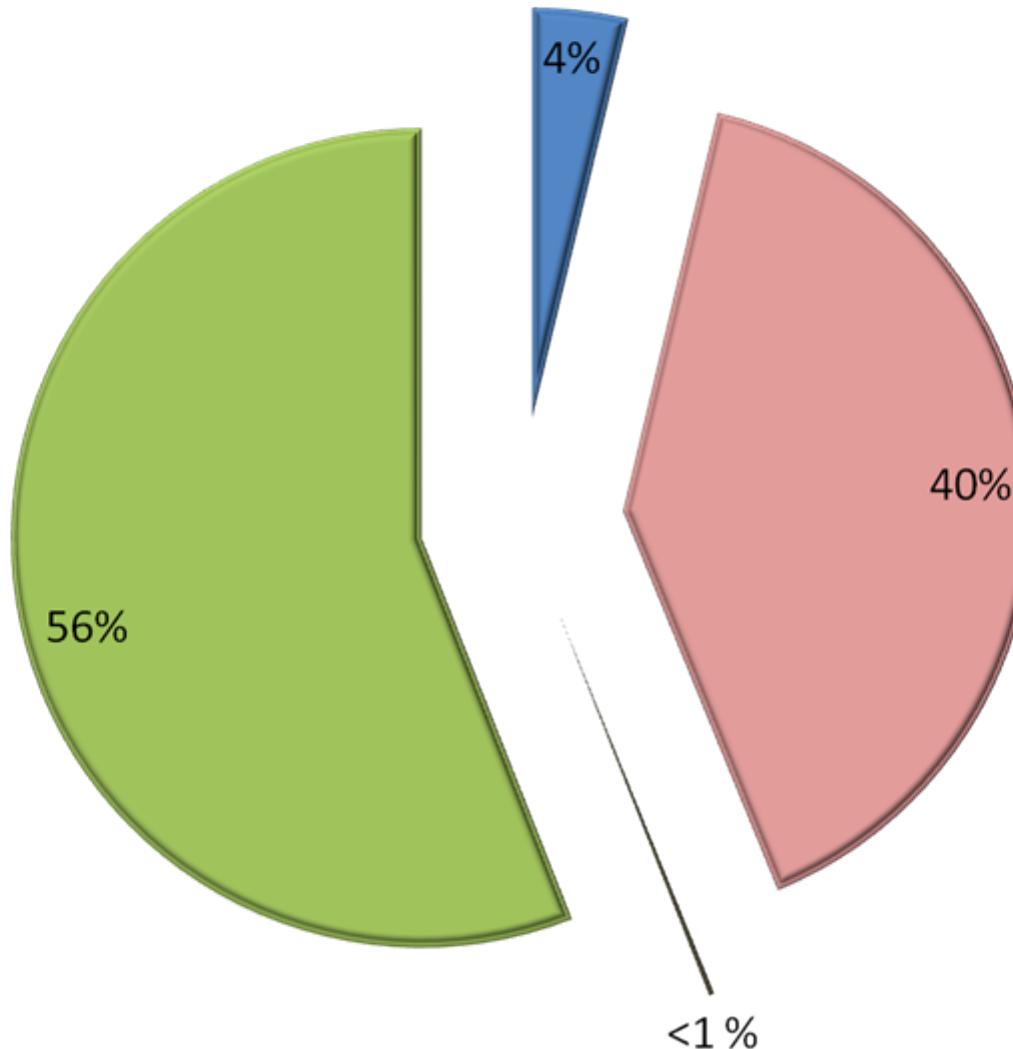
Zones correspond to regional land cover mapping area of the National Land Cover Database (USGS/EPA)





# Prime Habitat by Land Ownership

■ Public protected ■ Public ■ Private protected ■ Private



\* Preliminary, for Mapping Zone 61 only, using prime habitat cutoff from 2004-2005 study

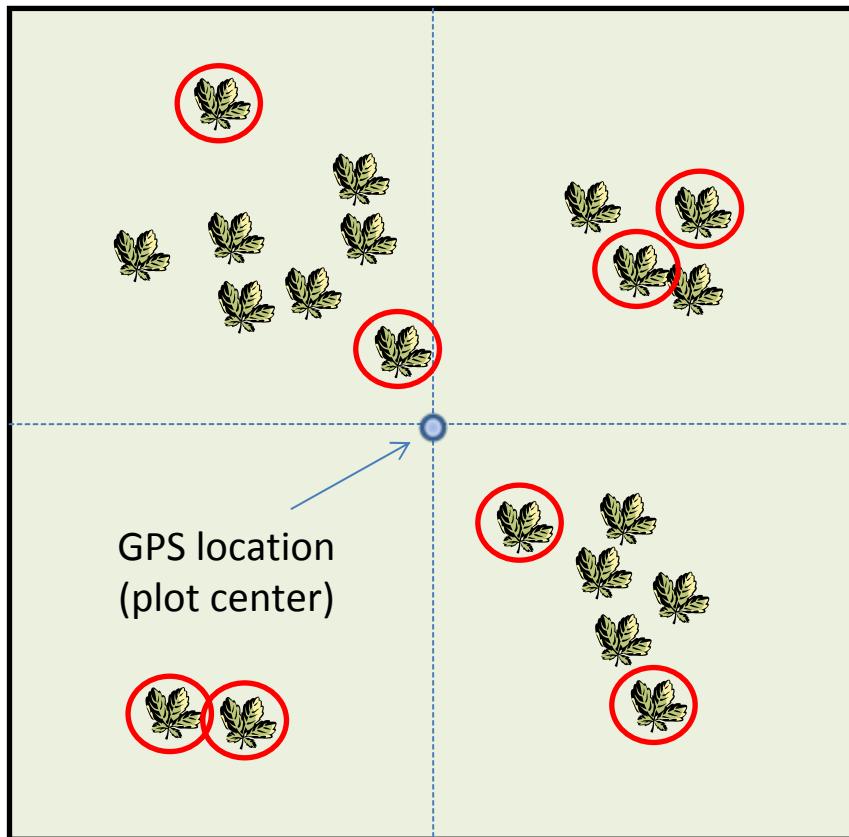
# Modeling issues

- Draft regional models used to guide field sampling
  - Models based on topography, solar radiation, and forest cover only
  - Models did not extrapolate well outside of previous study area
- New model development
  - Additional variables (i.e., geology [soils], precipitation)
  - Additional data (known plant locations) from 2007-2008 field surveys, herbaria records, and other investigators records

# Field Sampling

- Sampled 196 plots during 2007-2008 in 11 states (NY, PA, OH, WV, VA, TN, KY, NC, SC, IN, MO)
- 30-m sampling plots
  - Searched for, and counted ginseng plants by size class
  - Measured elevation, aspect, slope, slope position, canopy cover
  - Recorded associated species and disturbance
  - Collected site photos and GPS coordinates
- Sampled DNA from up to 8 plants/plot
  - 802 genetics samples from 11 states
  - Measured height, # prongs, reproductive status

# Field Sampling



Plot size = 30x30 m (0.09ha)

20 plants = 222 plants/ha

○ Plants sampled for genetics





*Symptom Monitoring Questionnaire*

Site Preference	Soil Type	Aspect (S)	Aspect (W)
1) Shrub	2) Tree	3) South	4) North
5) Groundcover	6) Grass	7) East	8) West
9) Hedgerow	10) Rock outcrop	11) Slope	12) Watercourse
13) Gully	14) Barren	15) Ridge	16) Flat
17) Forest	18) Pasture	19) Riverbank	20) Roadside
21) Low	22) Medium	23) High	24) Very High

Diagnosing herbaceous species		
1) Asteraceae	2) Rosaceae	3) Gramineae
4) Leguminosae	5) Poaceae	6) Fabaceae
7) Compositae	8) Malvaceae	9) Rubiaceae
10) Solanaceae	11) Rosaceae	12) Rosaceae
13) Malvaceae	14) Asteraceae	15) Asteraceae
16) Poaceae	17) Malvaceae	18) Malvaceae
19) Rosaceae	20) Malvaceae	21) Malvaceae
22) Asteraceae	23) Malvaceae	24) Malvaceae
25) Rosaceae	26) Malvaceae	27) Malvaceae
28) Malvaceae	29) Malvaceae	30) Malvaceae
31) Asteraceae	32) Malvaceae	33) Malvaceae
34) Malvaceae	35) Malvaceae	36) Malvaceae
37) Malvaceae	38) Malvaceae	39) Malvaceae

TUCK COVER HERE

**FTA®**  
**Plant Card**

Whatman®

NY-0074 4/13/2007 VV	NY-0076 4/13/2007 JY

4 pt. Edge  
10 pt. Edge



7/11/12

FTA®  
Plant Card  
1000000000

Sample ID: 1000000000

Date: 7/11/12

Location:

Species:

Description:

Notes:

7/11/12

USGS 2007-2009 American Ginseng

New Survey

Date: 11/19/08 (MM/DD/YY)

Land Unit/Area

Ownership

State

WY, ME  
Dakota

Presumed Wild  
Unknown  
Cultivated  
Resumed  
Tilled

238.3 + 0.7 m

(Example: WV-001)

12

11.5

Lot No. FT6802308



plants

Ginseng genetics sample: (max of 8 per plot; divide total # plants by 8 to get # plants)

Plant: spread sampling equally among plot quadrants if possible; write plot number and sample #.

Plot #: 3 Sample #: 3 Height: 21cm Bud scan: —

Sample #: b Plot #: 3 Height: 31cm Bud scan: —

Sample #: c Plot #: — Height: — Bud scan: —

Sample #: d Plot #: — Height: — Bud scan: —

Co-occurring herbaceous species:

- Ebony Spleenwort
- Mayapple
- Sharp-toothed hepatic
- Blue cohosh
- Trillium spp.
- Spice bush
- Black cohosh
- Paw paw
- Bloodroot
- Jack-in-the-pulpit
- Wild ginger
- Goldenseal

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+ ginseng  
area  
(clone)

Site Photos:

1)

2)

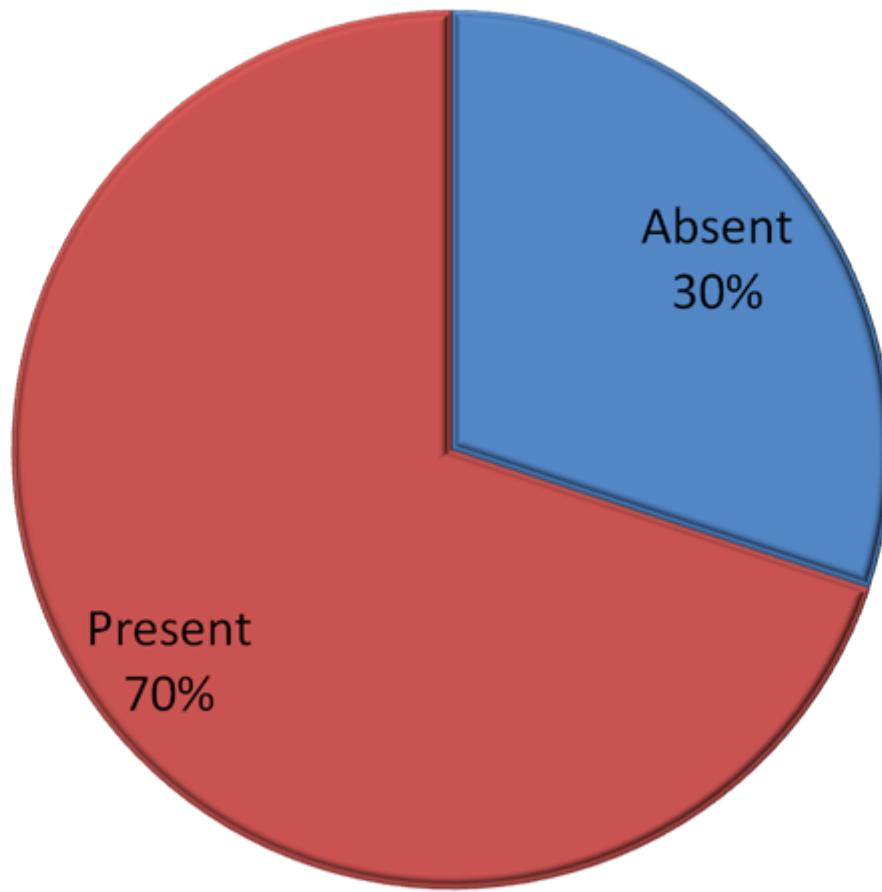
3)

4)

Notes:

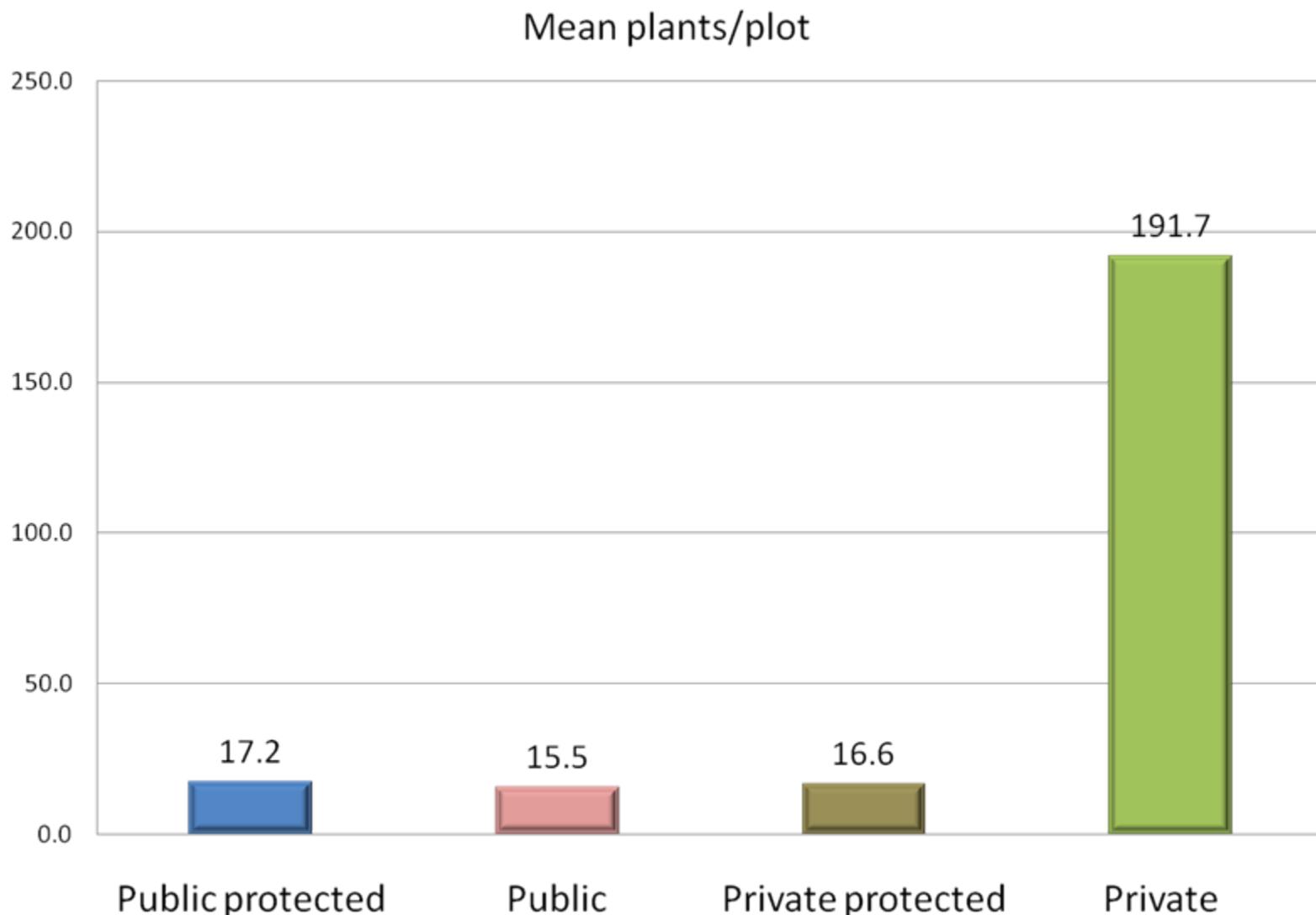
Wiley hill slope, near country road  
4 no time to do full plot, just ge-

# Ginseng occupancy in surveyed plots



Plots sampled 2007-2008, N=196

# Population status at field plots



\* 33.8 plants/plot across all sites sampled 2007-2008, N=196

# Genetic Analysis in Plants



- Long lived, out-crossing, late successional species retain most of their genetic variability within populations<sup>1</sup>
- Annual, selfing, and/or early successional species allocate relatively more genetic variability among populations<sup>1</sup>
- American ginseng ??
  - *Mixed mating system, self compatible*
  - long-lived
  - late successional forest species

<sup>1</sup>Nybom (2004) review of 307 studies using DNA markers

# Ginseng - Genetic Analysis

## Previous studies



- Allozymes:
  - More variation found among populations than within for wild populations, more within than among pop variation for cultivated (Grubbs and Case 2004)
  - Wild populations differ from cultivated pops in amount and distribution of genetic diversity, but differences in total genetic diversity not large (Grubbs and Case 2004)
  - Evidence of isolation by distance (Cruse-Sanders and Hamrick 2004)
- DNA markers [RAPD]:
  - High degree of genetic diversity within wild and cultivated ginseng (Schluter and Punja 2002, Boehm et al. 1999, Bai et al. 1997)
  - More variation detected within populations rather than among for both cultivated and wild populations (Schluter and Punja 2002)
  - Distinct populations observed (Lim et al. 1997), natural populations genetically distinct from cultivated (Schluter and Punja 2002, Boehm et al. 1999)
  - Evidence of mixing of wild populations with cultivated populations in PA (Boehm et al. 1999)

# Genetic Markers



- Allozyme markers (enzymes)
  - Advantages:
    - Good for range wide analysis (broad scale variation)
    - Inexpensive
  - Disadvantages:
    - Limited to protein encoding loci
    - Underestimate genetic diversity (fewer polymorphic loci)
    - Require fresh plant material, sample preservation
- DNA markers (e.g. RAPD, Microsatellites, AFLP)
  - Advantages:
    - More resolution for detecting population size, structure, gene flow, bottlenecks (more polymorphic loci)
    - Discrimination of individuals and source populations possible
    - Minimal sample preservation needed (FTA cards, silica gel)
  - Disadvantages:
    - Reproducibility (RAPDs only)
    - Anonymous regions of DNA, dominant markers (RAPD, AFLP)
    - High level of expertise required for marker development (microsatellites)

# Genetic Markers - Microsatellites



- Short sections of repeated sequences of DNA
- Mutations in non-coding (neutral) DNA region
  - Some mutations common to genus, species, populations
  - Some mutations unique to individual
- Variation in length of repeats = marker
- Uses sequence information

Flanking region

CTAGCTAG **GATAGATAGATA**

Flanking region

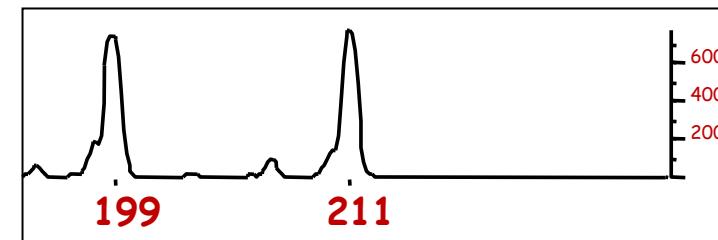
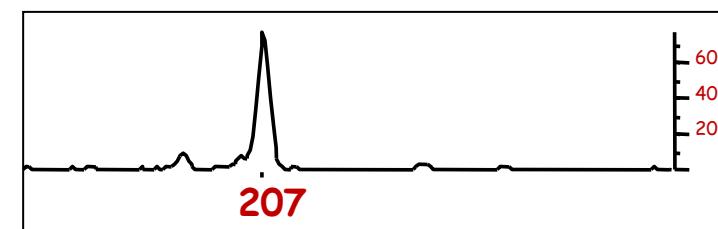
TTGTCA

CTAGCTAG **GATAGATAGATA**

TTGTCA

CTAGCTAG **GATA** TTGTCA

CTAGCTAG **GATAGATAGATA** TTGTCA



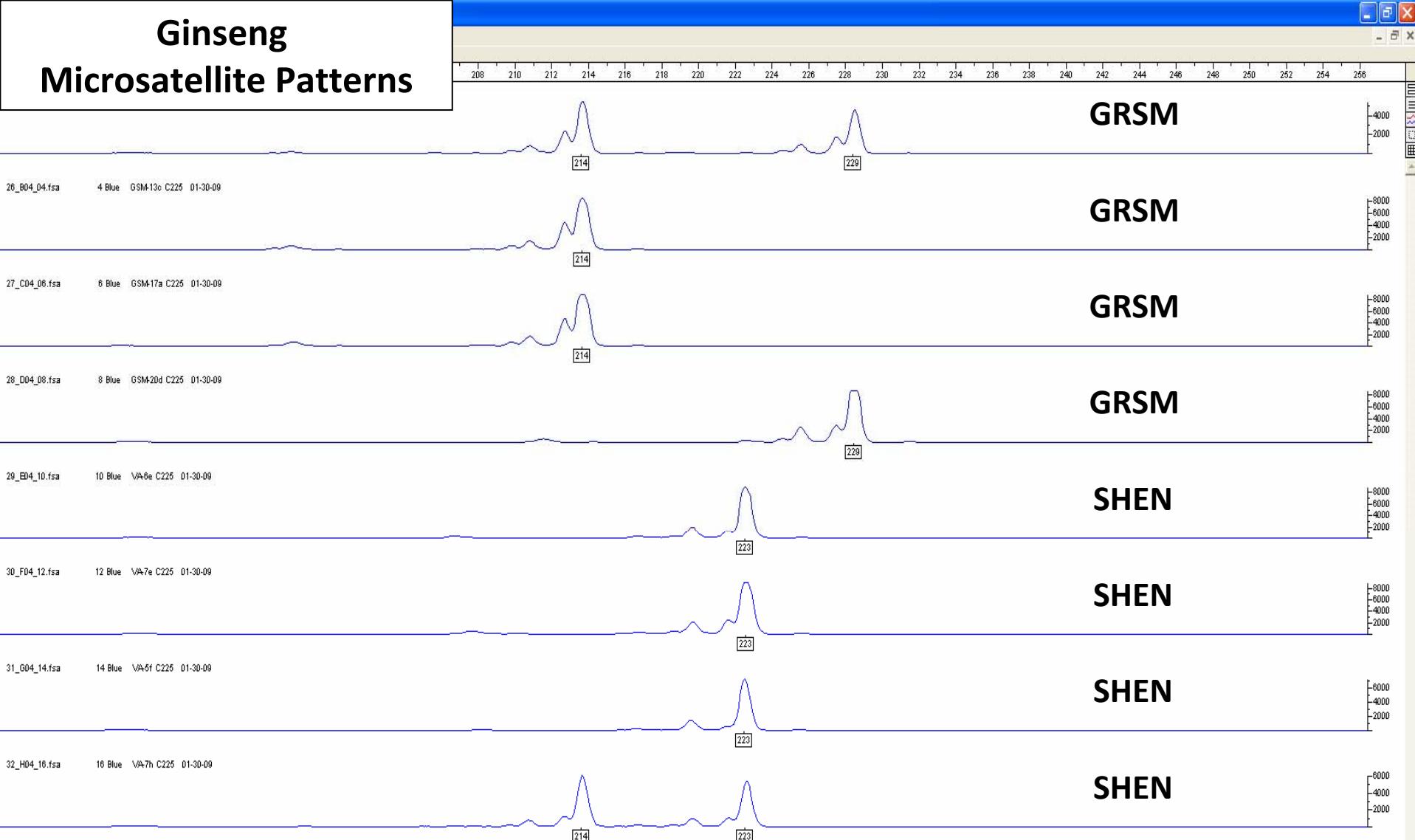
# Genetic Markers – Microsatellites

King Lab



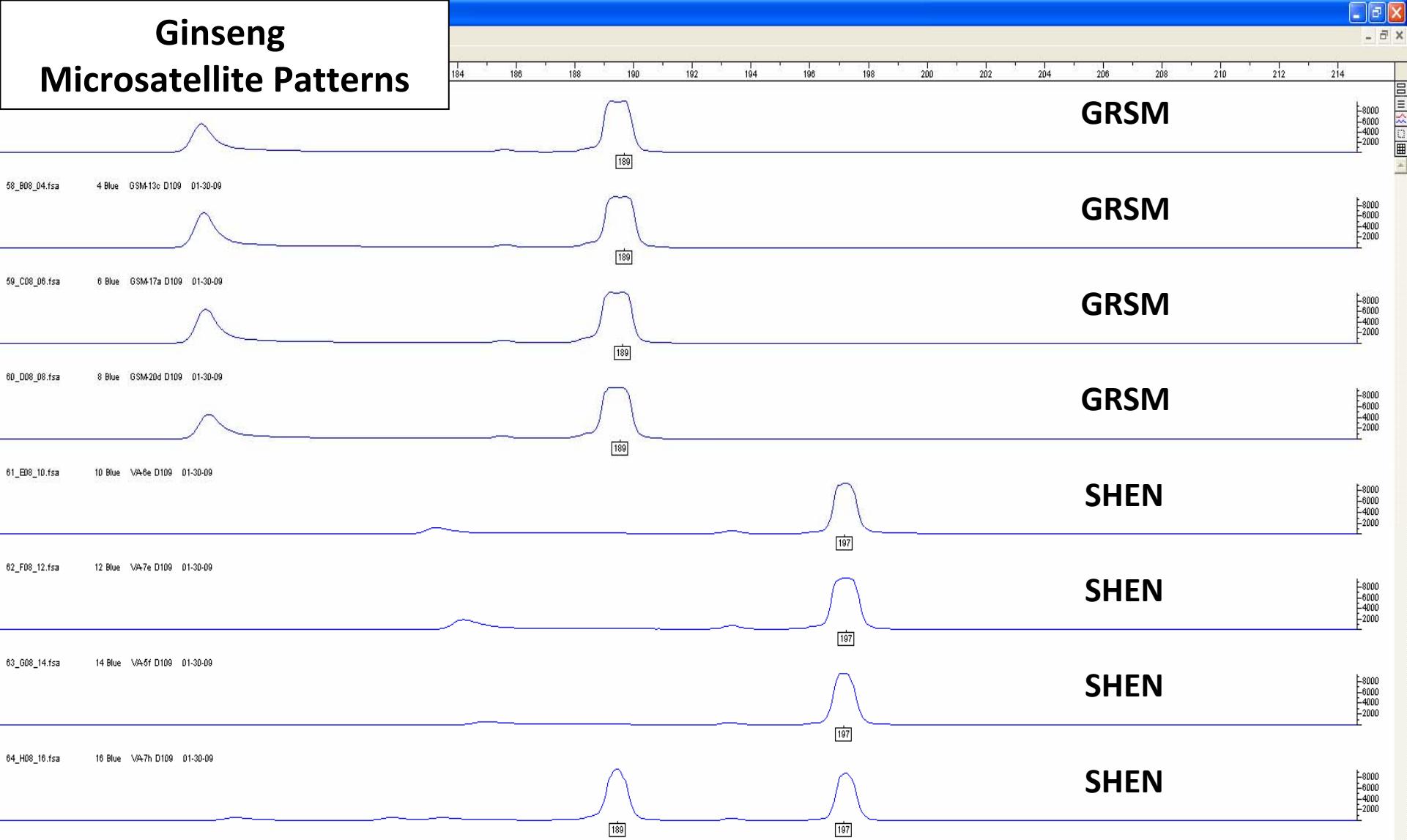
- Need 12-15 markers for population and individual discrimination
- Tested published markers for Korean ginseng (*Panax ginseng*)
  - Kim et al. 2007 [10/14], Ma et al. 2007 [14/22]
    - Amplification problems in *Panax quinquefolius*
    - Lack of polymorphism
- Developed our own set of 25 microsatellite markers for American ginseng, 14 look most promising
  - Enriched microsatellite DNA libraries created
  - Markers screened and tested in our laboratory

# Ginseng Microsatellite Patterns



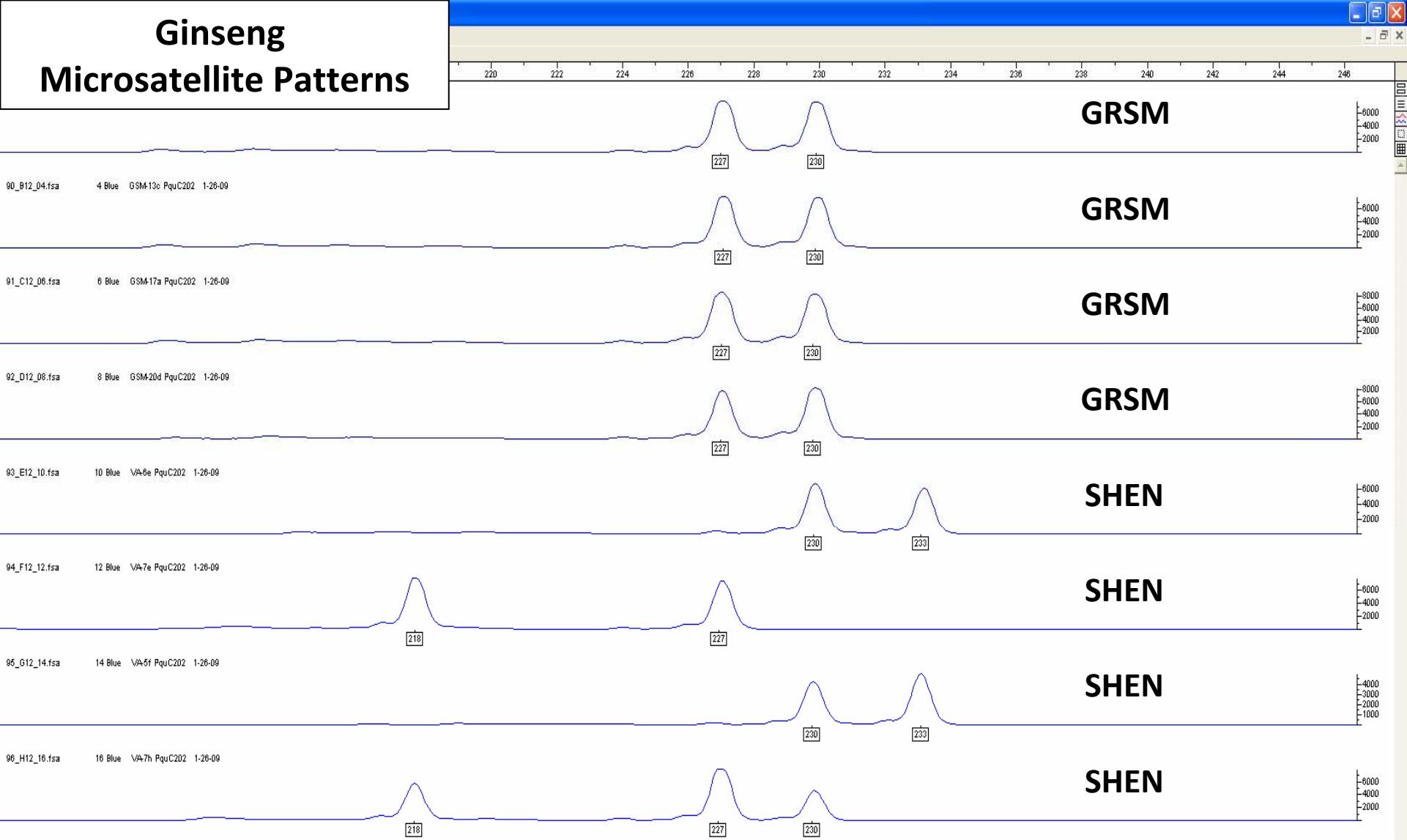
*PquC225*

# Ginseng Microsatellite Patterns



*PquD109*

# Ginseng Microsatellite Patterns



***PquC202***

# Test of microsatellite markers



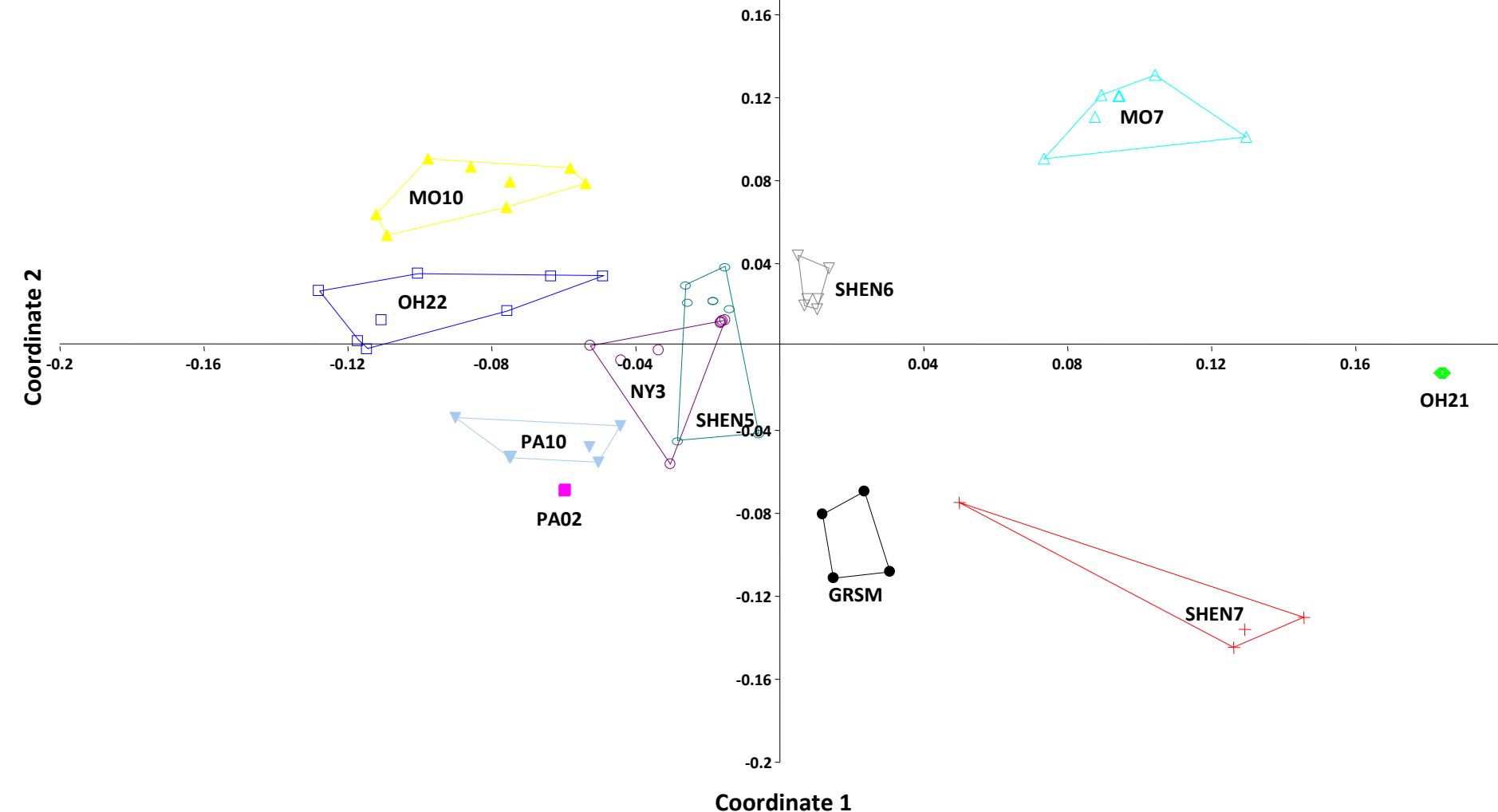
- Subset of sites across geographic range, and land ownerships (78 individuals)
- Test of allele frequency differences across 12 of 14 loci, 3-9 alleles each, (66 markers)
- Sites:
  - GRSM = Great Smoky National Park (4 plants)
  - SHEN5, SHEN6, SHEN7 = Shenandoah National Park, VA (3 sites, 18 plants)
  - MO10 = Roaring River State Park, MO (8 plants)
  - MO7 = Caney Mountain Conservation Area, MO (8 plants)
  - PA02 = Allegheny National Forest, PA (8 plants)
  - PA10 = Private land, western PA (8 plants)
  - NY3 = Private land, Cairo, NY (8 plants)
  - OH22 = Cultivated ginseng farm, OH (8 plants)
  - OH21 = Edge of Appalachia Preserve, OH (8 plants)



# Microsatellite DNA Collection Differentiation



Non-metric Multidimensional Scaling (MDS)  
Plot of individual Jaccard's distance values

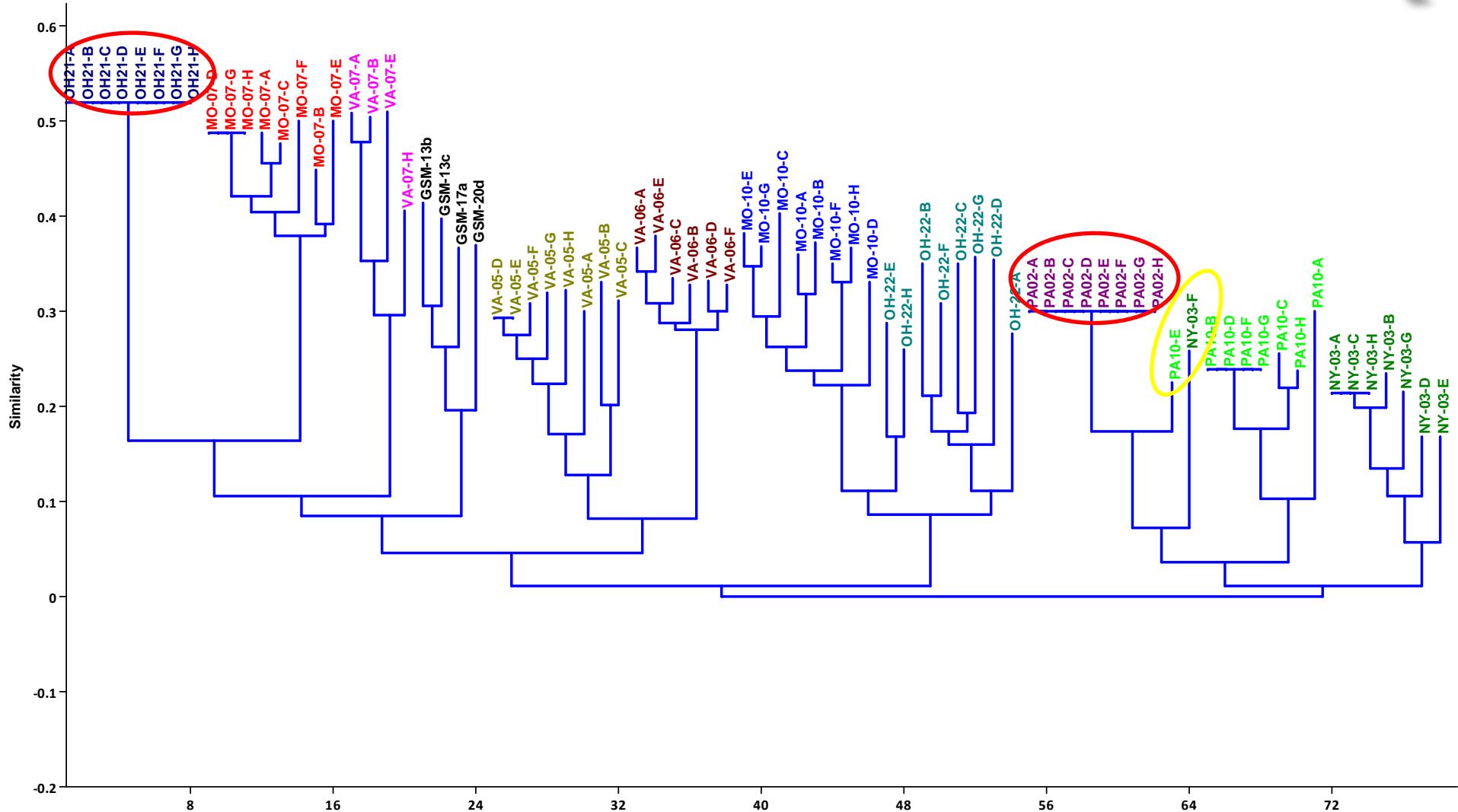


Polygons depict inter- and intra-collection variation in Jaccard's Distance

# Microsatellite DNA Collection Differentiation



Neighbor Joining Tree on Jaccard's distance values





# $\Phi_{PT}$ - Test of Genetic Differentiation

( $F_{ST}$  analog for binomial data)



Collection	GRSM	MO7	MO10	NY	OH22	OH21	PA02	PA10	SHEN5	SHEN6	SHEN7	Prob.
GRSM	0.000	0.002	0.002	0.002	0.003	0.007	0.004	0.001	0.005	0.007	0.023	GRSM
MO7	0.806	0.000	0.001	0.001	0.003	0.001	0.002	0.001	0.001	0.002	0.003	MO7
MO10	0.747	0.804	0.000	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.002	MO10
NY	0.651	0.760	0.680	0.000	0.001	0.001	0.001	0.001	0.002	0.001	0.006	NY
OH22	0.639	0.714	0.483	0.455	0.000	0.001	0.001	0.001	0.001	0.001	0.004	OH22
OH21	0.889	0.909	0.889	0.859	0.788	0.000	0.122	0.002	0.001	0.002	0.004	OH21
PA02	0.848	0.923	0.855	0.771	0.691	0.000	0.000	0.001	0.001	0.001	0.003	PA02
PA10	0.681	0.838	0.752	0.531	0.542	0.899	0.771	0.000	0.001	0.002	0.004	PA10
SHEN5	0.667	0.776	0.675	0.427	0.529	0.854	0.783	0.667	0.000	0.002	0.001	SHEN5
SHEN6	0.768	0.851	0.765	0.637	0.648	0.958	0.944	0.812	0.576	0.000	0.002	SHEN6
SHEN7	0.657	0.830	0.777	0.734	0.672	0.890	0.898	0.769	0.679	0.771	0.000	SHEN7
$\Phi_{PT}$	GRSM	MO7	MO10	NY	OH22	OH21	PA02	PA10	SHEN5	SHEN6	SHEN7	

Pair-wise  $\Phi_{PT}$  (below diagonal) and probability  $\Phi_{PT}$  value is > 0 (above).  
 Comparison in yellow is not statistically significant.



# Allocation to Collection



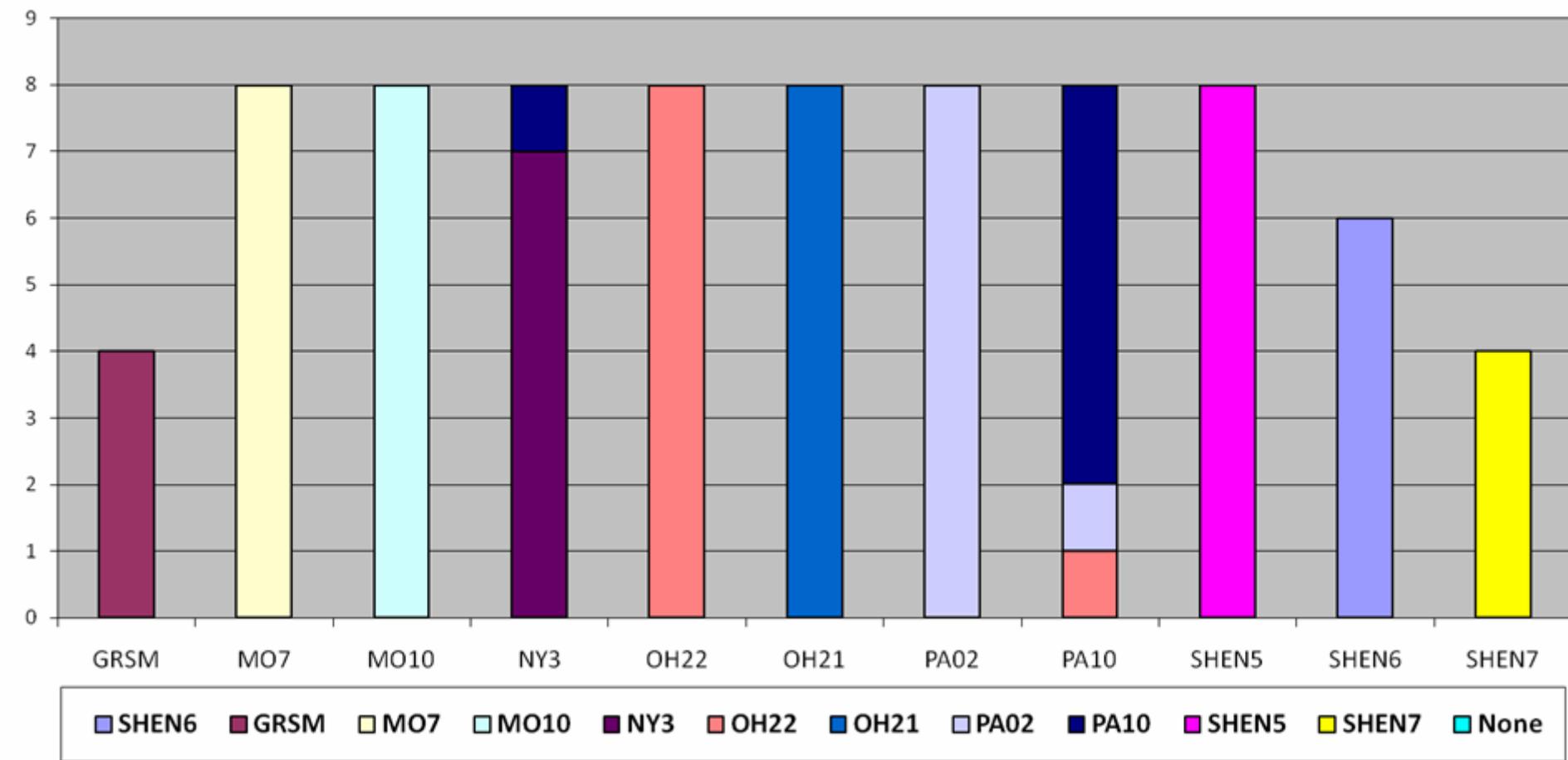
allocated to	GRSM	MO7	MO10	NY3	OH22	OH21	PA02	PA10	SHEN5	SHEN6	SHEN7
GRSM	4	0	0	0	0	0	0	0	0	0	0
MO7	0	8	0	0	0	0	0	0	0	0	0
MO10	0	0	8	0	0	0	0	0	0	0	0
NY3	0	0	0	7	0	0	0	0	0	0	0
OH22	0	0	0	0	8	0	0	1	0	0	0
OH21	0	0	0	0	0	8	0	0	0	0	0
PA02	0	0	0	0	0	0	8	1	0	0	0
PA10	0	0	0	1	0	0	0	6	0	0	0
SHEN5	0	0	0	0	0	0	0	0	8	0	0
SHEN6	0	0	0	0	0	0	0	0	0	6	0
SHEN7	0	0	0	0	0	0	0	0	0	0	4
Correct Assignment (%)	100.0%	100.0%	100.0%	87.5%	100.0%	100.0%	100.0%	75.0%	100.0%	100.0%	100.0%

Overall Assignment Success: 96.2%

Incorrect assignments are distributed vertically and are highlighted in yellow.



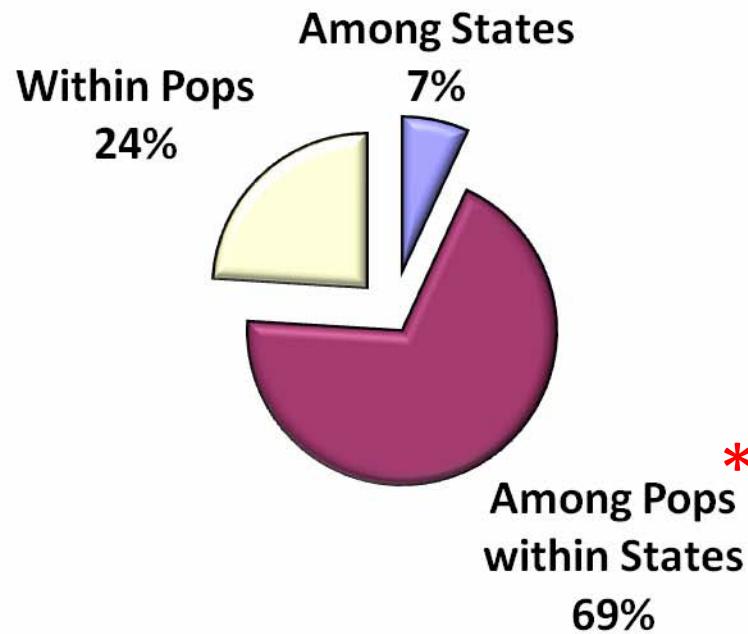
# Microsatellite DNA Allocation Graphic



# **AMOVA** (*A*nalysis of *M*olecular *V*Ariance)



## Partitioning of Genetic Variation



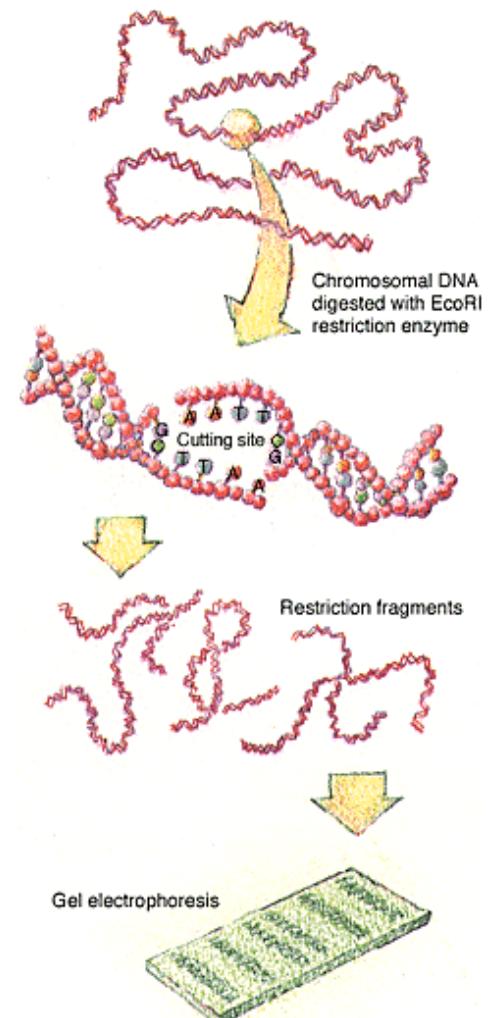
\*

Preliminary results (AMOVA, pair-wise  $\Phi_{PT}$ , and assignment tests),  
needs validation with larger dataset



# Genetic Markers - AFLP

- AFLP = “Amplified Fragment Length Polymorphism”
  - No sequence information required
  - Highly repeatable
- Two step process:
  1. Cut DNA with restriction enzymes (primers), bind adapters to end of fragments
  2. Use PCR to amplify fragments
- Polymorphisms from:
  - Sequence variation in and around restriction sites
  - Insertions or deletions in amplified fragments
- High numbers of dominant (presence/absence) allelic markers produced

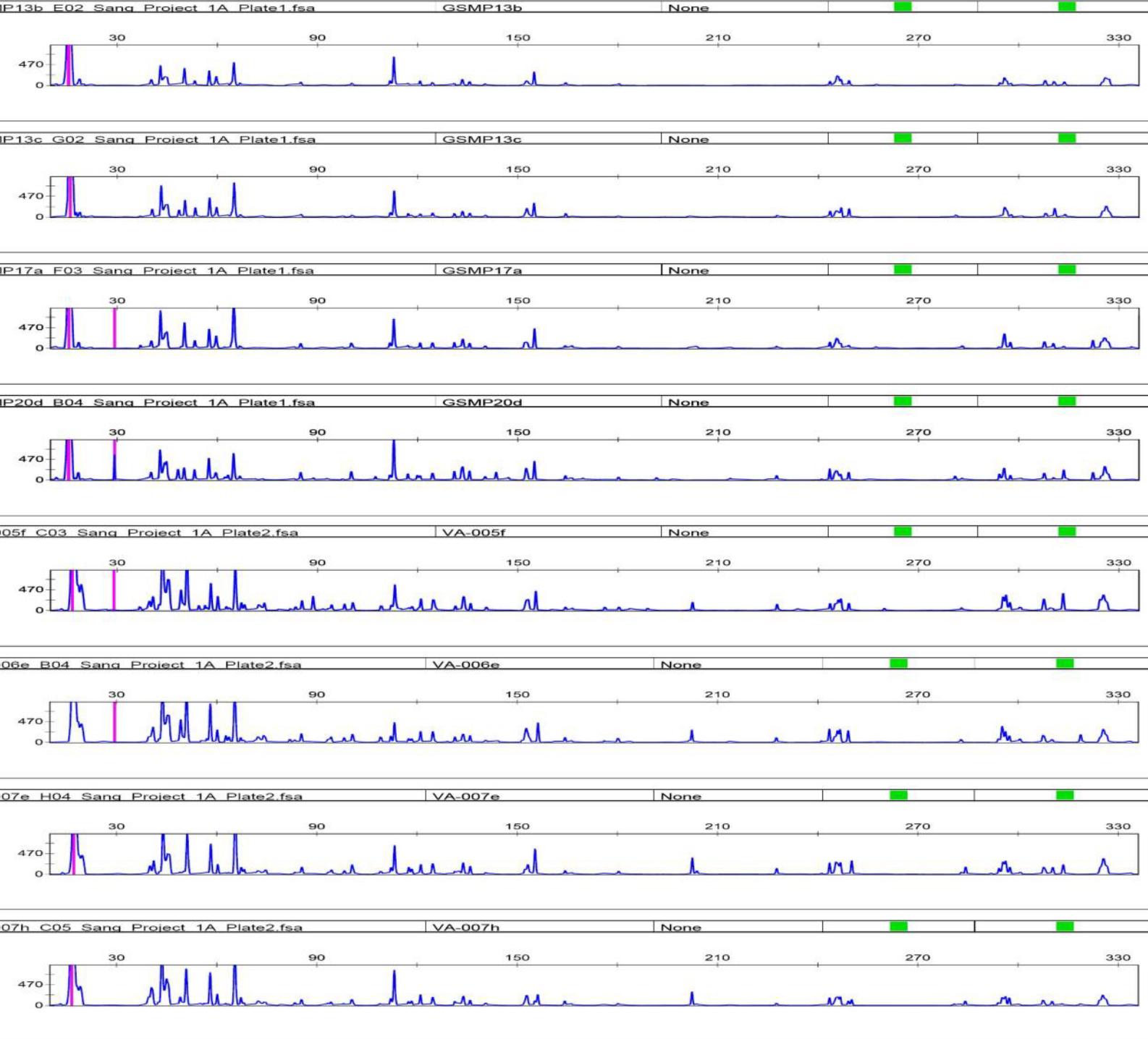


# Genetic Markers – AFLP

David DeViney, M.S. Student, ASU



- We tested 3 primer pairs for American ginseng:
  - 1) “Pqu1-A”: EcoRI-ACT, MseI-CAA
  - 2) “Pqu4-H”: EcoRI-ACC, MseI-CTT
  - 3) “Pqu7-A”: EcoRI-AGG, MseI-CAA
- Number of allelic markers generated for American ginseng:
  - 378 with 3 primers (1+2+3)
  - 240 with 2 primers (1+2)



GRSM

GRSM

GRSM

GRSM

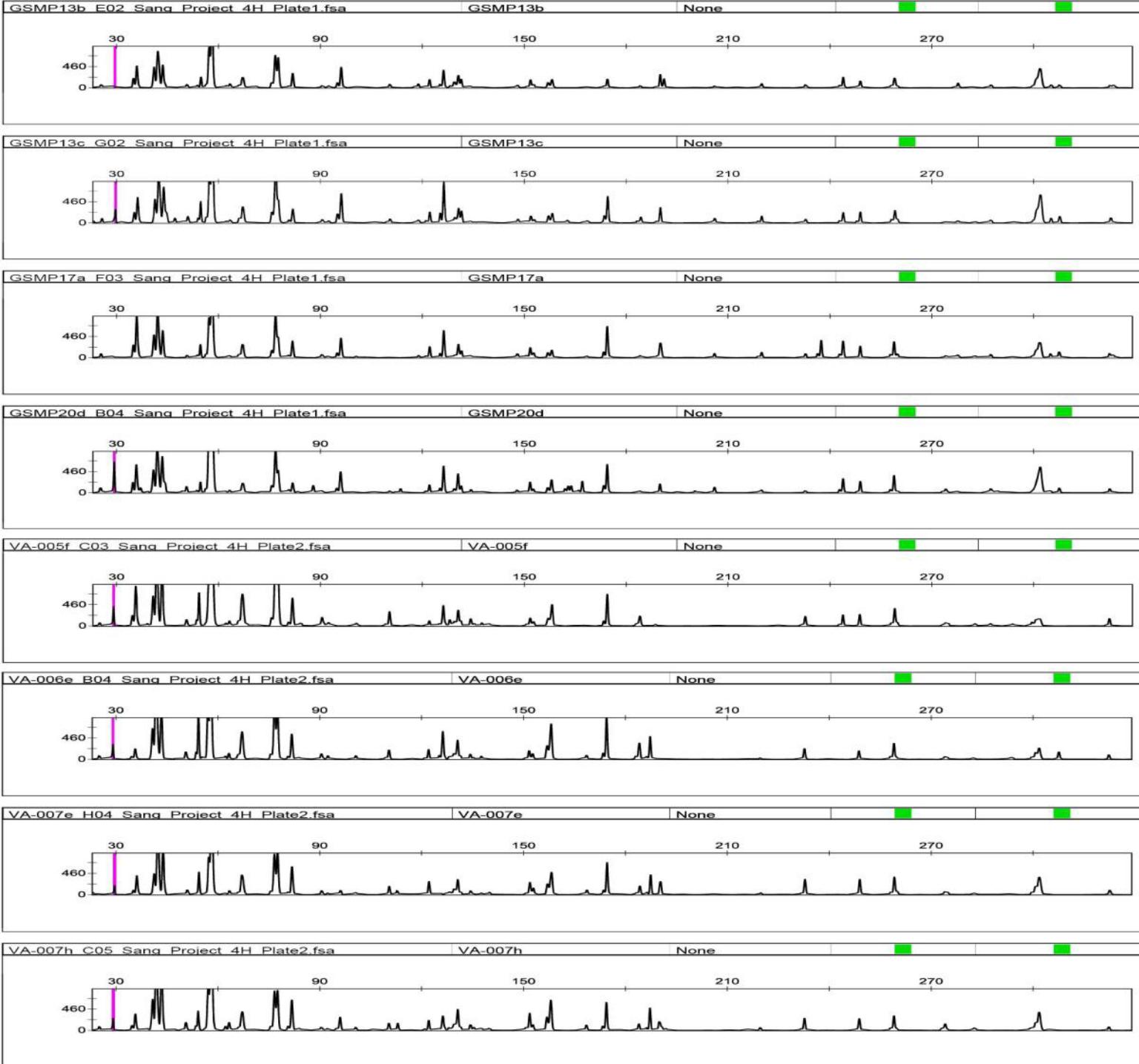
SHEN

SHEN

SHEN

SHEN

Paul-A



Pau4-H

GRSM

GRSM

GRSM

GRSM

SHEN

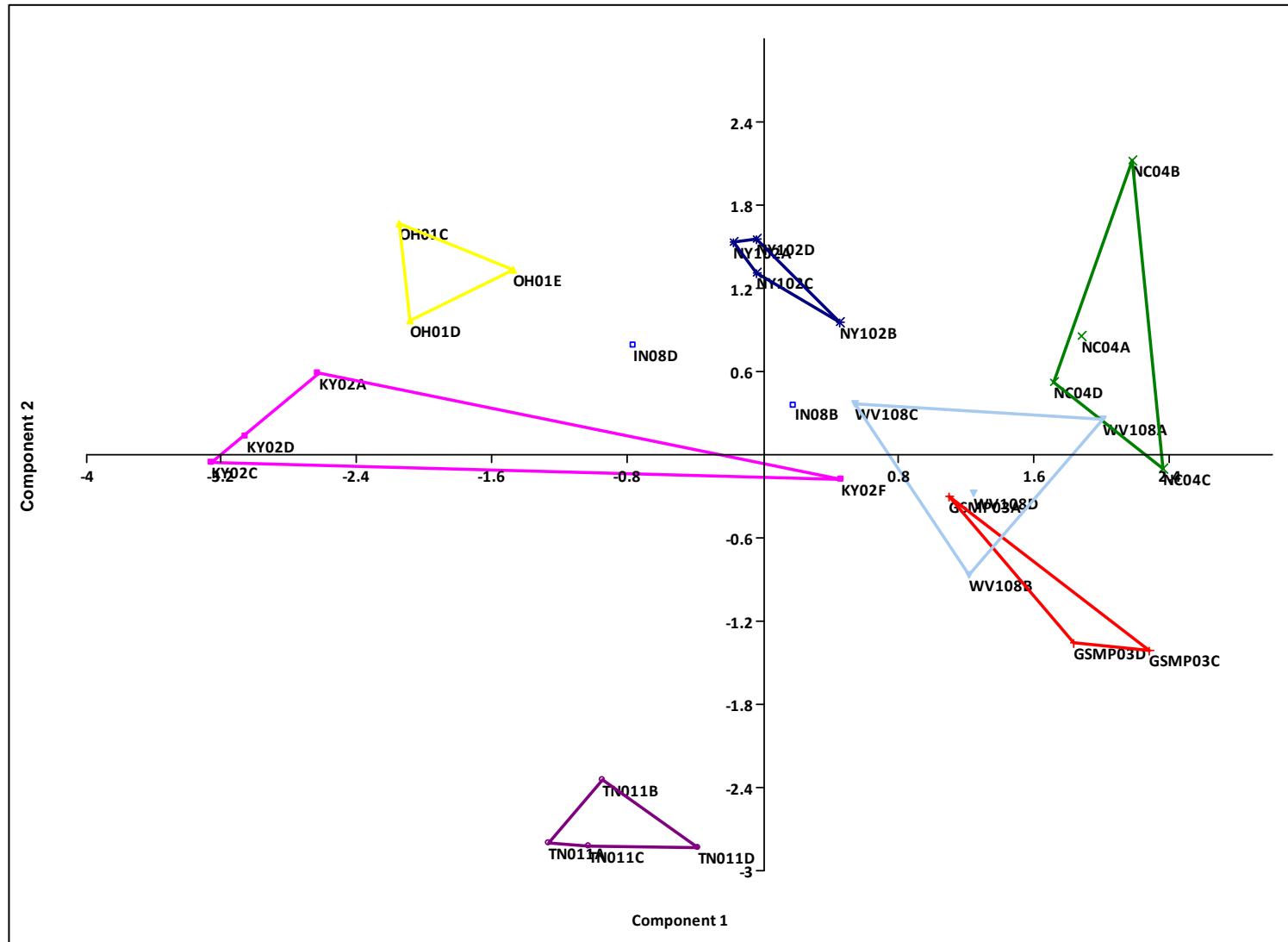
SHEN

SHEN

SHEN

# Population discrimination – AFLP

## Principal Components Analysis



# Remaining tasks:

- Winter-Spring 2009
  - Develop new ginseng distribution models
  - Survey all genetic samples for AFLP and microsatellite variation
- Early summer 2009
  - Collect additional genetic samples
- Late summer-early fall 2009
  - Data analysis
    - Assess phylogeographic and population structure
    - Diversity within/among sites by land ownership
    - Genetic diversity compared to habitat distribution/quality

# Acknowledgements

- Marcus Springmann (USGS) – field data collection, genetic laboratory assistance
- Chris Walter (USGS) – field data collection, assistance with GIS data compilation
- Pat Ford (FWS), state ginseng coordinators – contacts with land owners
- Bob Beyfuss, Eric Burkhart – contacts with private and state landowners
- Numerous landowners and land managers who provided access to their property for sampling

