Intermountain Forest Tree Nutrition Co-op and the USDA Forest Service, RMRS *Armillaria* root disease study

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Review of *Armillaria* species in North America

Armillaria can be divided into 3 classifications

"Bad" *Armillaria* primary pathogens	NABS I and NABS VI
"Good" *Armillaria* secondary saprophytes	NABS III, V, VII, X, and XI
Unknown pathogenicity	NABS II and NABS IX

(NABS = North American Biological Species)

Armillaria species found on Nutrition Co-op plots throughout the Inland Northwestern United States.

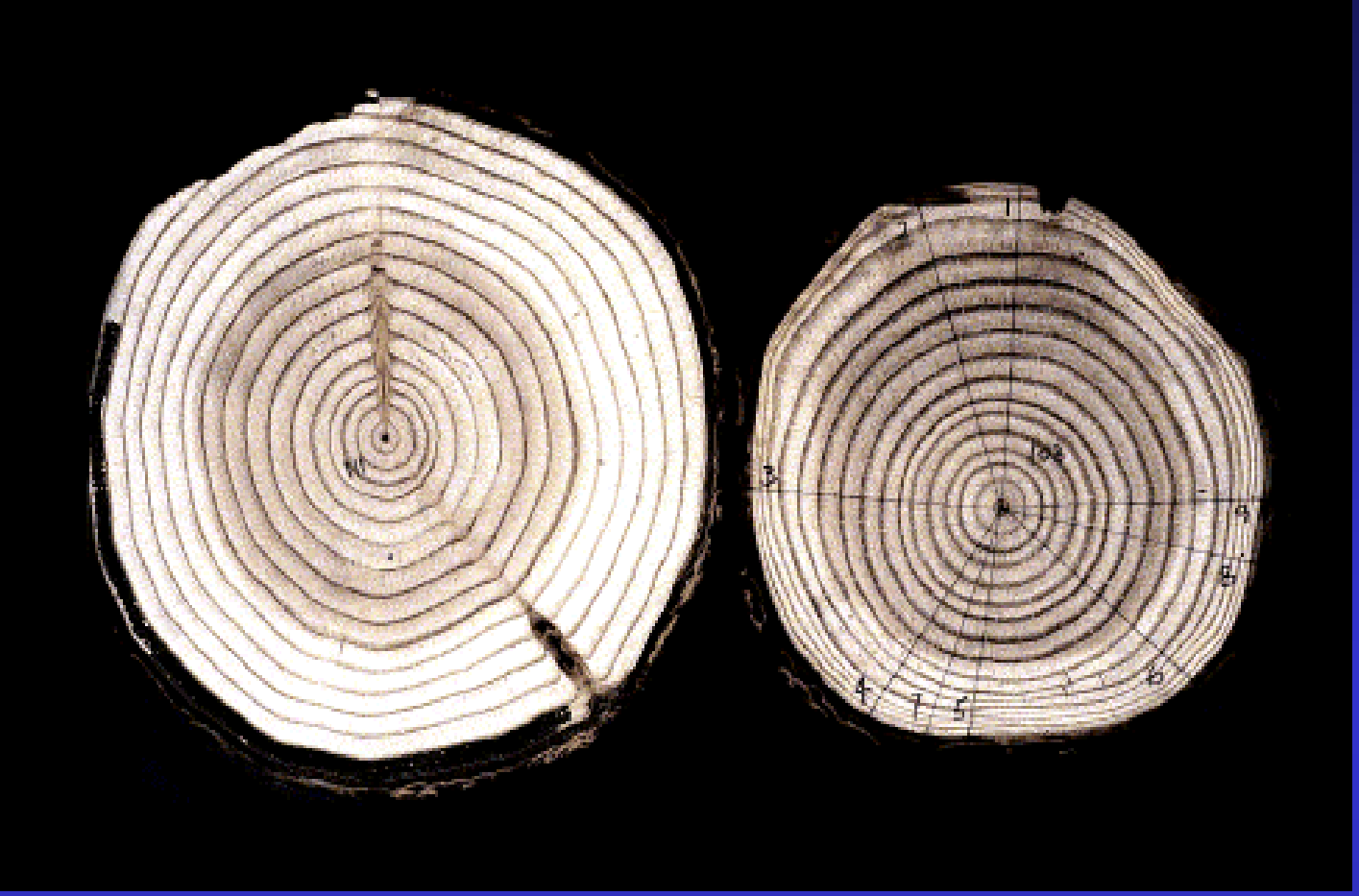
"Bad" *Armillaria* - primary pathogen
NABS I – *Armillaria ostoyae*

- Common
- Known as a highly virulent pathogen of conifers
- Causes tree mortality
- Causes tree growth loss (often with no apparent visible symptoms)

Example of *Armillaria ostoyae* mortality centers

Average mortality rates for sites covering Northern Idaho, northeastern Oregon, and Eastern Washington vary from 8.8% for Douglas-fir, 4.2% for grand fir, and 1.5% for redcedar habitat types based on 8 sites (Moore et al. 2000).

Growth loss and mortality due to *Armillaria* in British Columbia have been cited as between 1.4 and 3.8 million m^3 annually (White and Morrison, 1999).



The effects of root disease on annual ring width. (Cruickshank et al. 2001)

Volume loss has been shown as high as 40% over 4-8 years in eighteen-year-old Douglas-fir. (Cruickshank, 2000)

Resinosus

a sign of

Armillaria ostoyae

infection

Unknown pathogenicity
NABS IX – *Armillaria nabsnona*

- Rare
- Found on wet sites
- Unknown ecological effects

Armillaria species found on nutrition co-op plots throughout the Inland Northwestern United States.

"Good" *Armillaria* – primary saprophytes

NABS III – *Armillaria calvescens*

NABS V – *Armillaria sinapina*

NABS VII – *Armillaria gallica*

- Closely related species
- Common
- Low pathogenicity; 5-10% found as bark fans
- Most individuals show no sign of pathogenicity and may be beneficial for tree growth and or protection.
- Difficult to identify

"Good" *Armillaria* – primary saprophyte
NABS X – currently unnamed

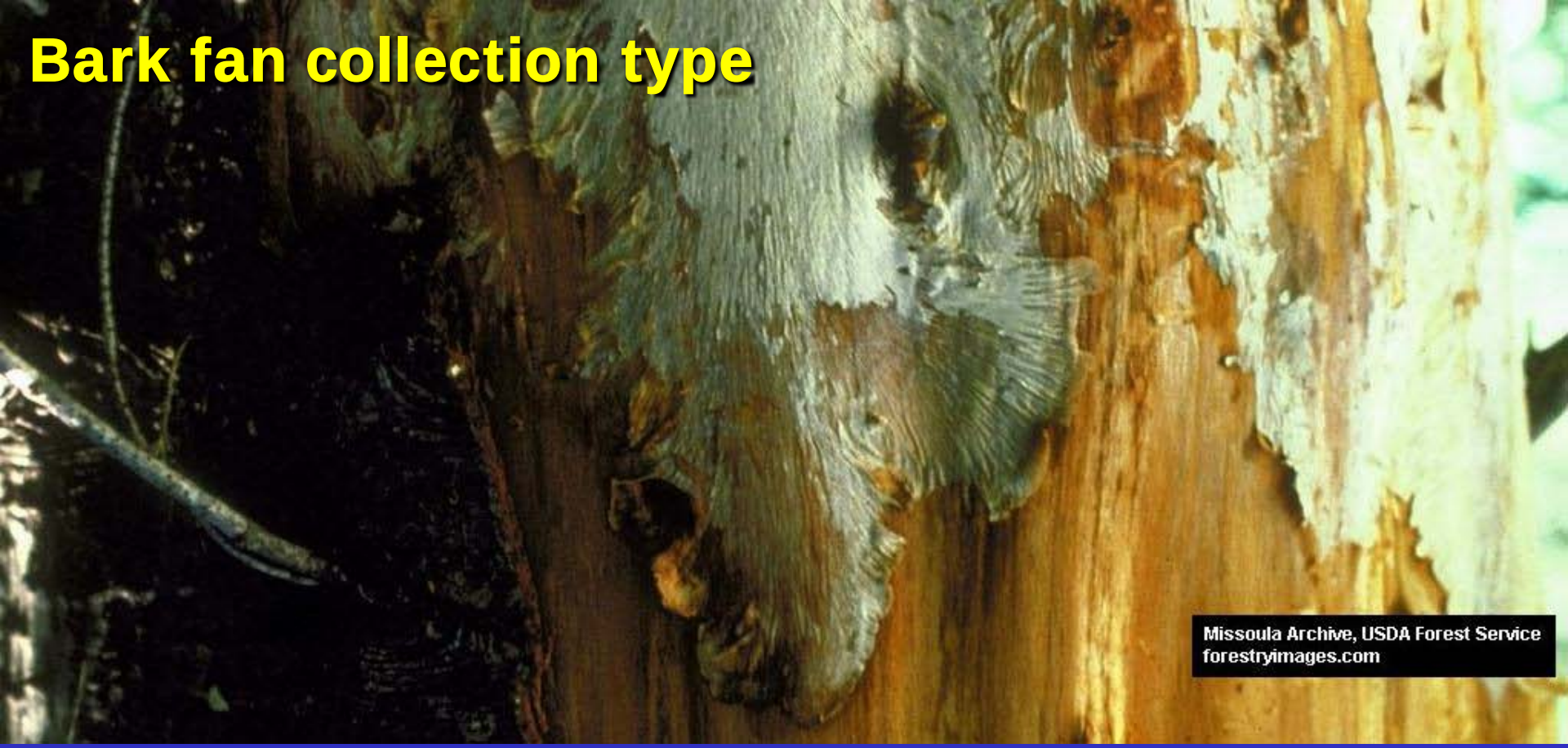
- Common
- Low pathogenicity; 5-10% found as bark fans
- Most individuals show no sign of pathogenicity and may be beneficial for tree growth and or protection.

1,310 *Armillaria* samples were collected from Nutrition Co-op plots during the summers of 1999 and 2000.

Three collection types were taken

- Bark fan
- Rhizomorph
- Wood

Bark fan collection type



Bark fans from live trees indicate high pathogenicity of an *Armillaria* individual.

Dead trees with bark fans do not necessarily indicate a pathogenic *Armillaria* individual.

Rhizomorph collection type

All species of *Armillaria* form rhizomorphs.

Most rhizomorphs are found on the surface of a root, some grow freely through the soil, and others can be found under the bark of a highly infected tree.

Presence of rhizomorphs does not necessarily indicate pathogenicity of an *Armillaria* individual.

Wood collection type

Wood samples are usually taken from trees that have recently been killed or are infected with a pathogenic *Armillaria* individual.

Often dark lines known as zone lines can be seen in infected wood.

Other collection types include caps and spores.

How do we identify *Armillaria* to species?

- From our collections we obtain pure fungal cultures known as isolates.

- Each isolate is paired against one another in a somatic incompatibility test to determine if it is from the same individual (genet).

- Isolates of the same genet will fuse together, while a distinct zone is formed between isolates of different genets.

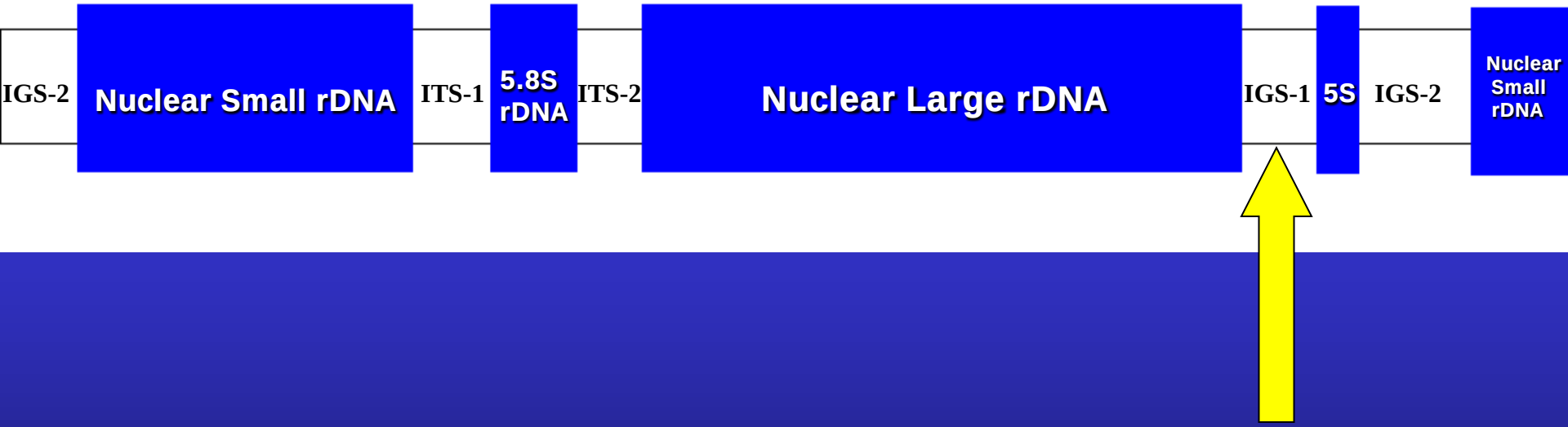
- Some genets have been known to cover an area as large as 900 ha, one having an estimated age of 2,400 years old. (Ferguson et al. 2000)

- The fungal isolates grow slowly, and it usually requires several months to run all the somatic incompatibility tests to reduce isolates to individual genets.

- Once the genets are obtained we use Polymerase Chain Reaction (PCR) to obtain multiple copies of a specific region of DNA that we use for genetic identification.

- The region of choice for our studies was the IGS-1 region of rDNA. This region repeats many times throughout an individual's DNA. PCR is used to amplify all the repeats of this region. The IGS-1 has a significant amount of variation between species for identification and it also displays variation within species.

These regions of rDNA repeat many times



We amplify all the repeats of the IGS-1 region for a given isolate, then we obtain an IGS-1 region sequence

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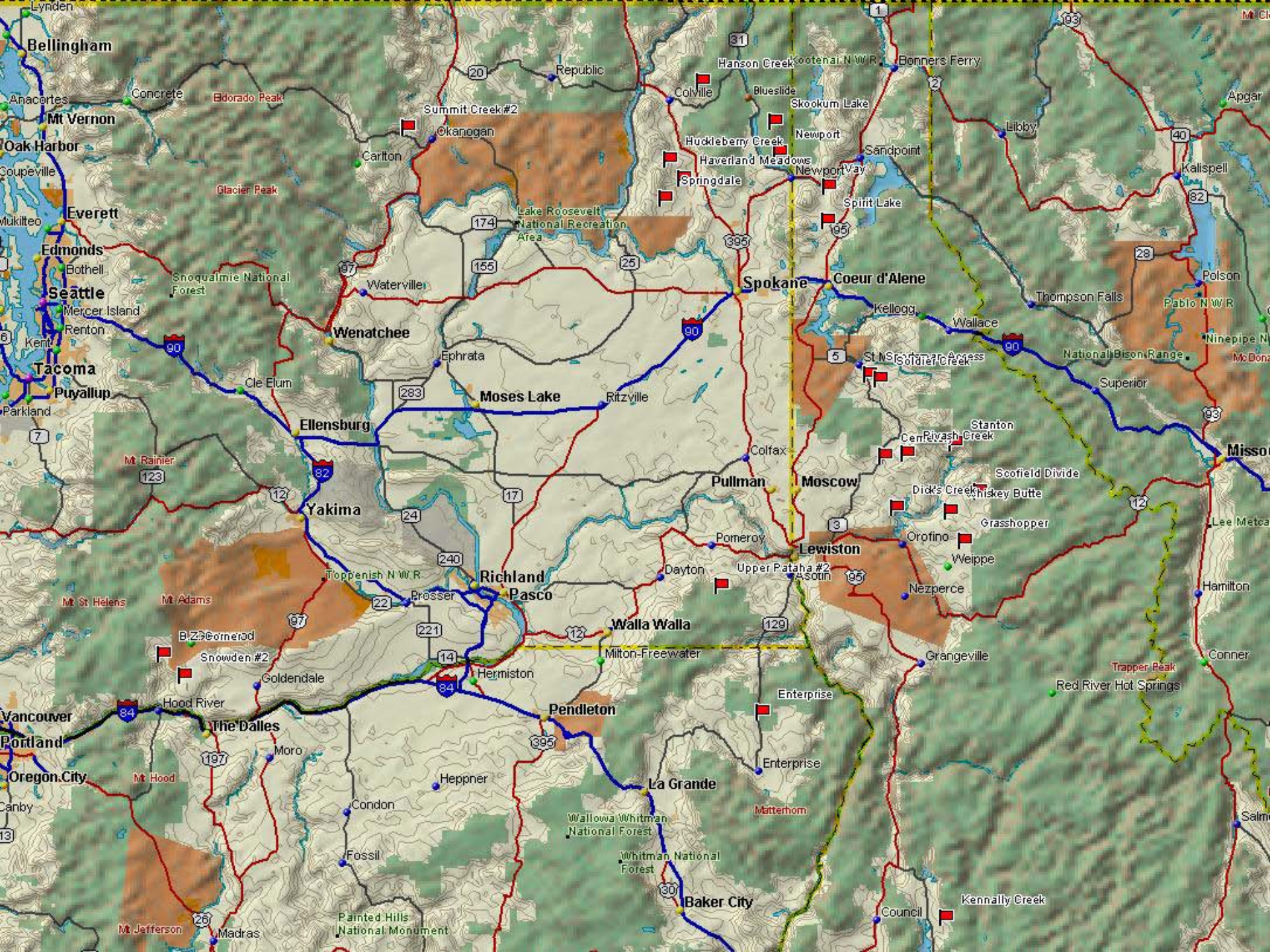
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Anacortes
Concrete
Eldorado Peak
Mt Vernon
Oak Harbor
Coupeville
Everett
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Seattle
Mercer Island
Renton
Kent
Tacoma
Puyallup
Parkland
Snoqualmie National Forest
Glacier Peak
Mt Rainier
Republic
Summit Creek #2
Okanogan
Carlton
Waterville
Wenatchee
Ephrata
Cle Elum
Moses Lake
Ritzville
Ellensburg
Yakima
Toppenish N W R
Prosser
Richland
Pasco
Mt St Helens
Mt Adams
B Z Corner
Snowden #2
Goldendale
Hood River
The Dalles
Vancouver
Portland
Oregon City
Mt Hood
Canby
Moro
Heppner
Condon
Fossil
Mt Jefferson
Madras
Painted Hills National Monument
Walla Walla
Milton-Freewater
Hermiston
Pendleton
La Grande
Wallowa Whitman National Forest
Whitman National Forest
Baker City
Colfax
Pullman
Moscow
Pomeroy
Dayton
Lewiston
Asotin
Upper Pataha #2
Nezperce
Grangeville
Enterprise
Council
Kennally Creek
Matterhorn
Trapper Peak
Red River Hot Springs
Hamilton
Conner
Lee Metca
Salm
Colville
Hanson Creek
Blueslide
Skookum Lake
Kootenai N W R
Bonners Ferry
Huckleberry Creek
Newport
Haverland Meadows
Springdale
Sandpoint
Spirit Lake
Lake Roosevelt National Recreation Area
Spokane
Coeur d'Alene
Kellogg
Wallace
Thompson Falls
Libby
Apgar
Kalispell
Polson
Pablo N W R
Ninepipe N
National Bison Range
McDona
Superior
Missoo
Stanton
Rivash Creek
Scofield Divide
Dick's Creek
Whiskey Butte
Grasshopper
Orofino
Weippe
Soldier Creek

1999 and 2000 Nutrition Co-op genets by Species

221 Genets

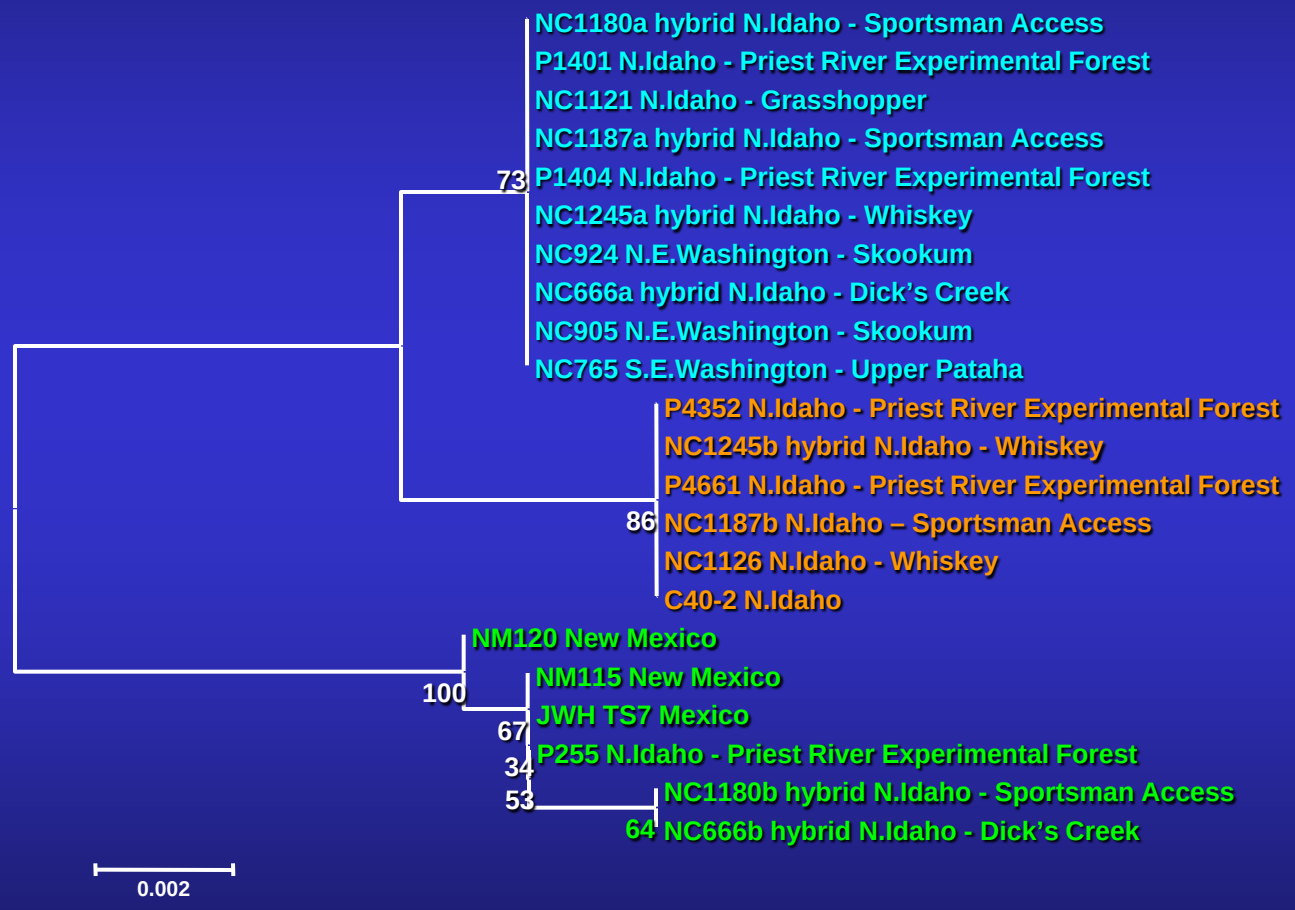
99 NABS X

62 NABS I

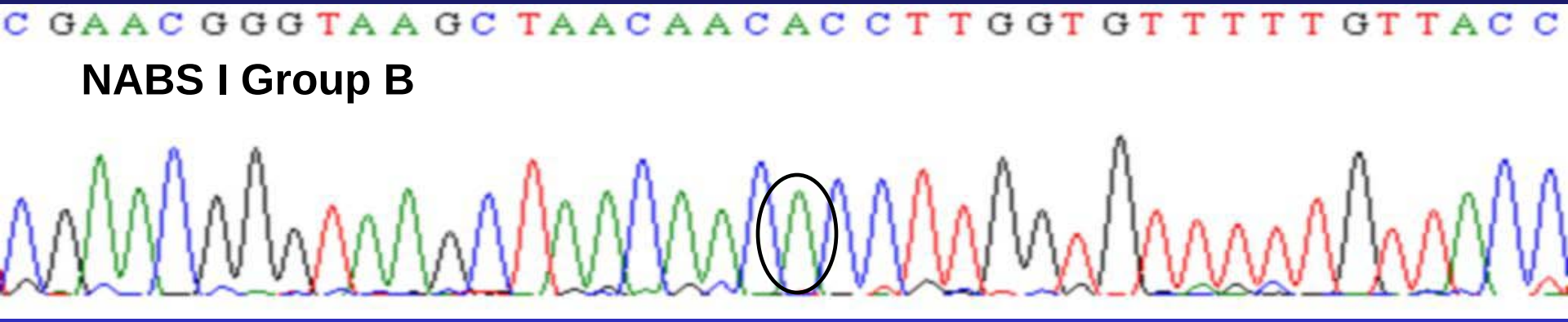
59 NABS III, V, or VII

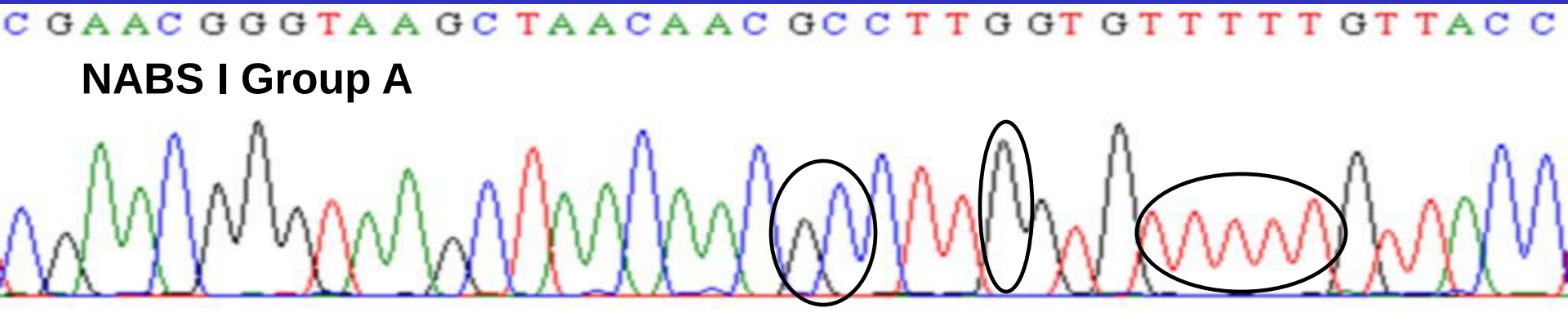
1 NABS IX

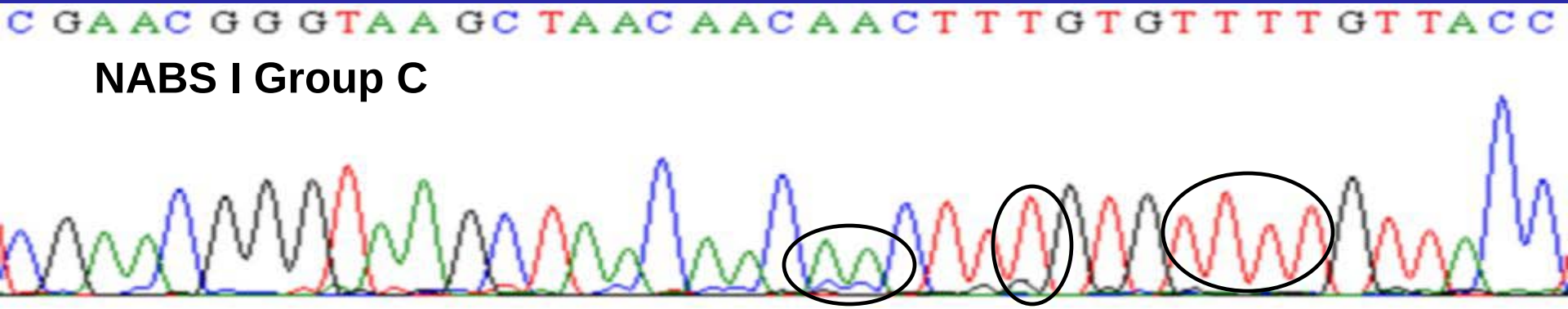
Phylogenetic tree showing genetic diversity of *Armillaria ostoyae*



Genetic diversity within *Armillaria ostoyae*







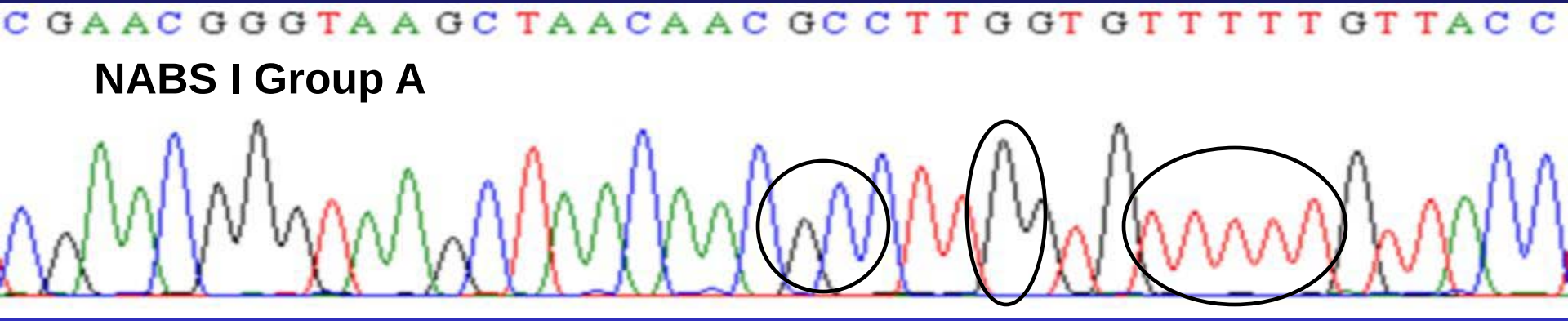
Hybridization also found within groups of *Armillaria ostoyae.*

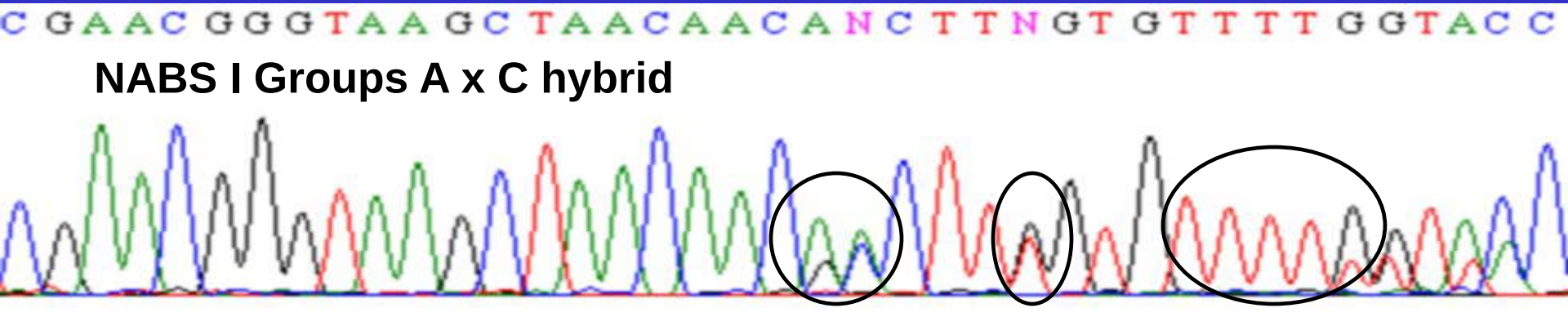
Hybridization within species is known as intraspecific hybridization.

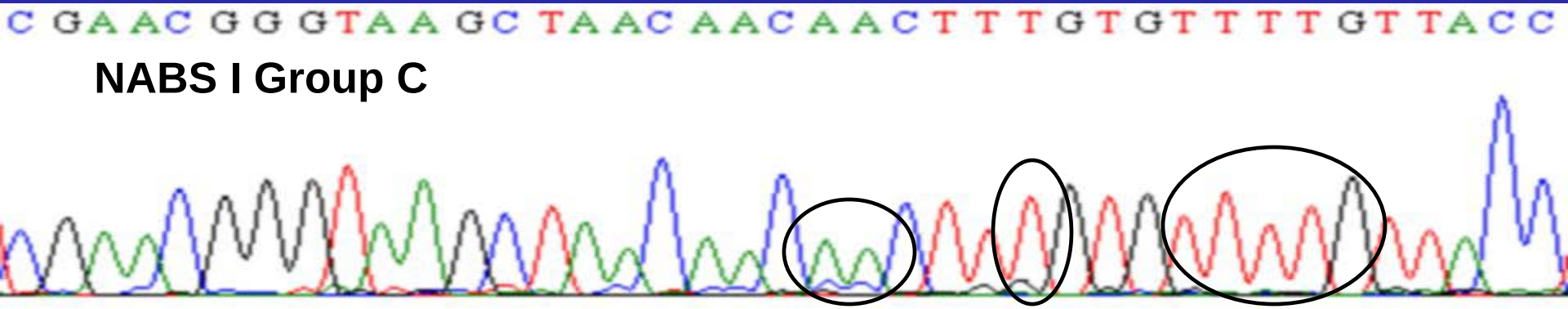
NABS I Groups A x B hybrid

NABS I Groups A x C hybrid

Armillaria ostoyae intraspecific hybridization







What does this hybridization mean?

- It is unlikely we are inducing the hybridization of *Armillaria* due to disturbance; many individuals have been around for hundreds of years.
- Possible “hybrid vigor”
- May adapt better to changing environment

Hybridization between NABS X and NABS VII

- After DNA analysis, 32 of the 99 NABS X genets are thought to be hybrids between NABS X and NABS VII.

- Some bark fans are associated with this hybrid combination. This may explain why some studies found 5-10% of NABS III, V, VII, and X in pathogenic situations indicated by bark fans from live trees.

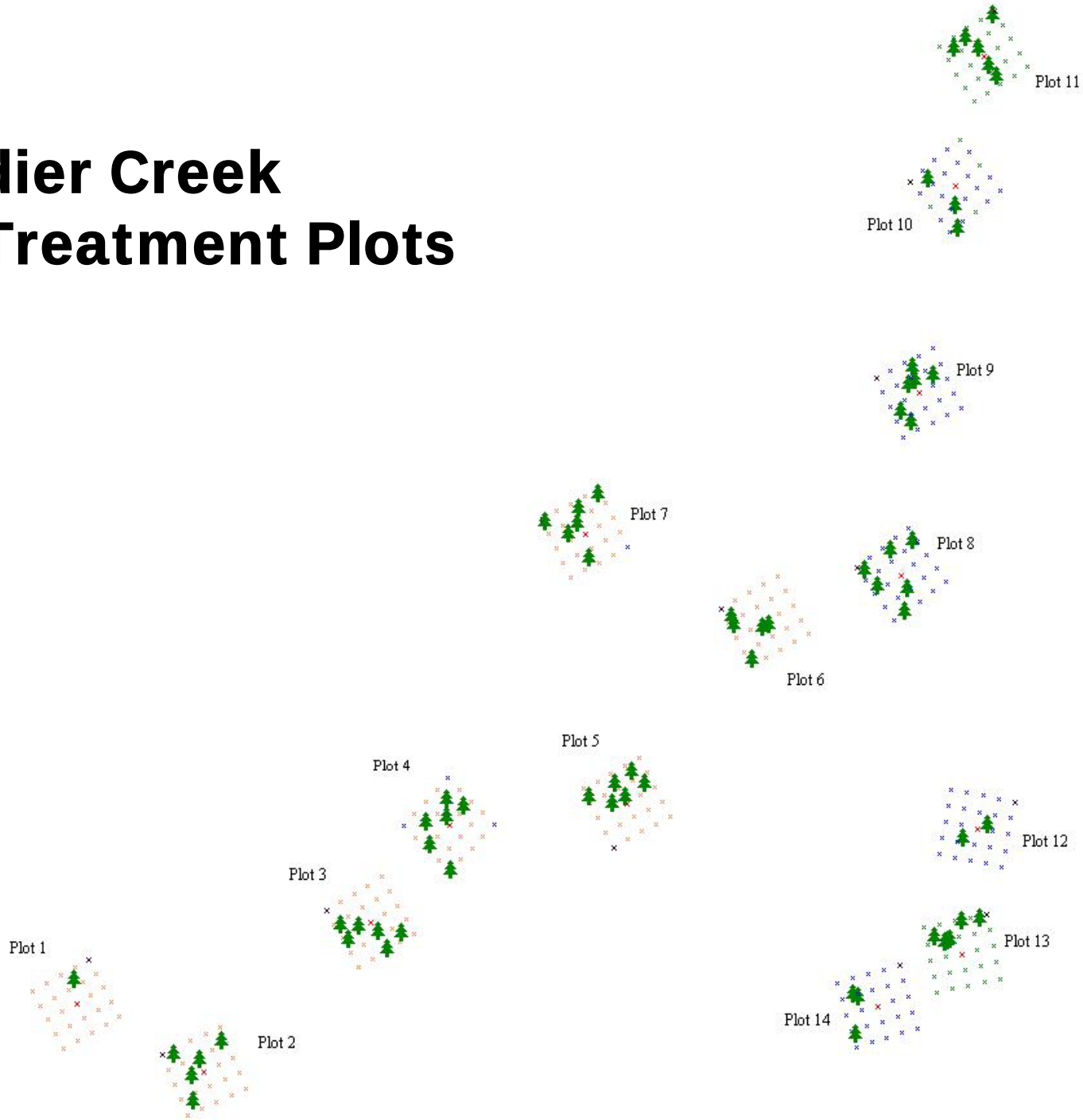
- A study from Ida Creek in northern Idaho showed 3% of 951 NABS X isolates to be from bark fans (McDonald et al. 1998). These isolates were collected before we had DNA analysis tools to distinguish NABS X from NABS X hybrids.

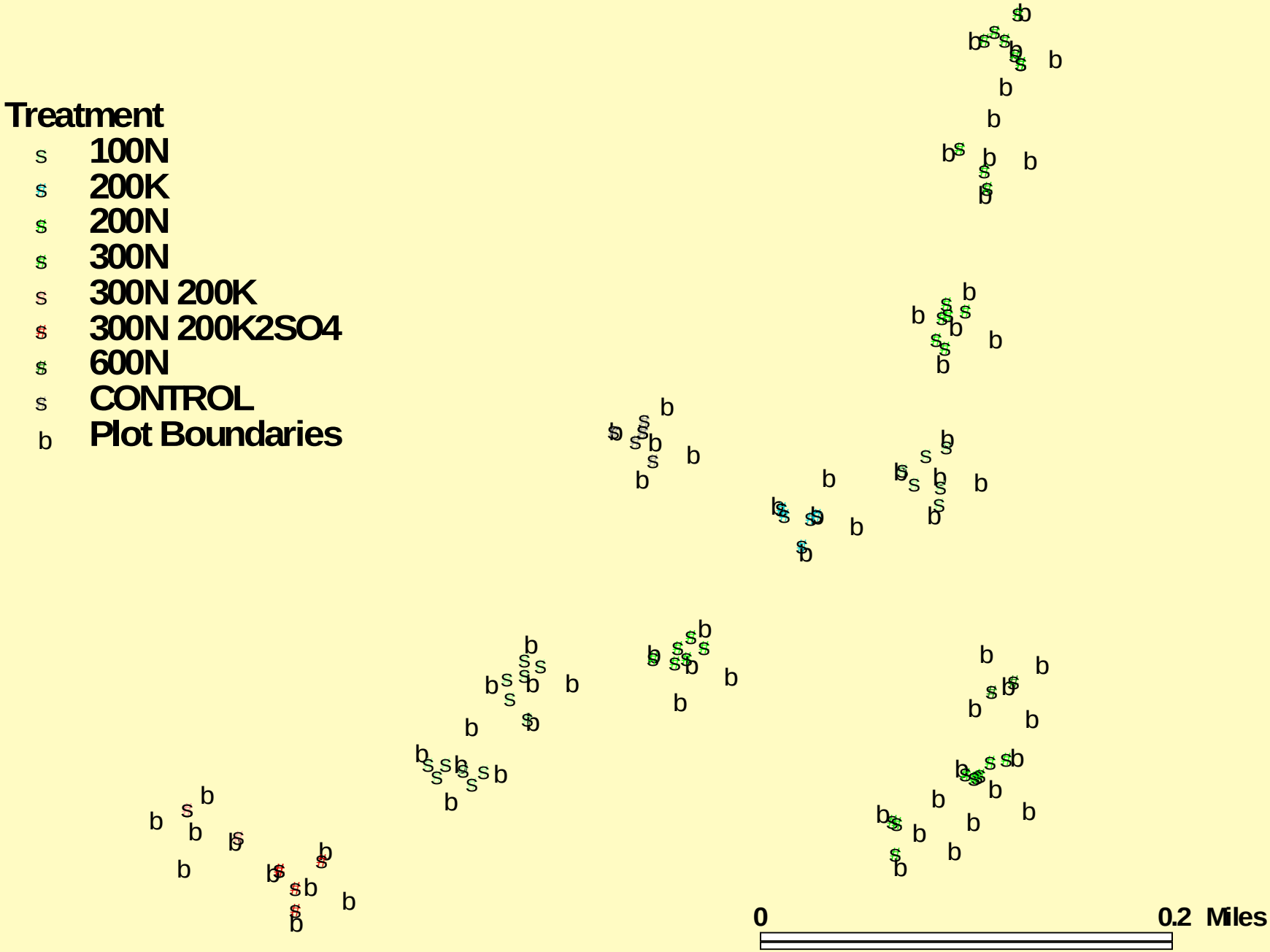
- More studies need to be done to determine if NABS X hybrids are solely responsible for low level pathogenicity of NABS X.

Types of information collected from Nutrition Co-op sites

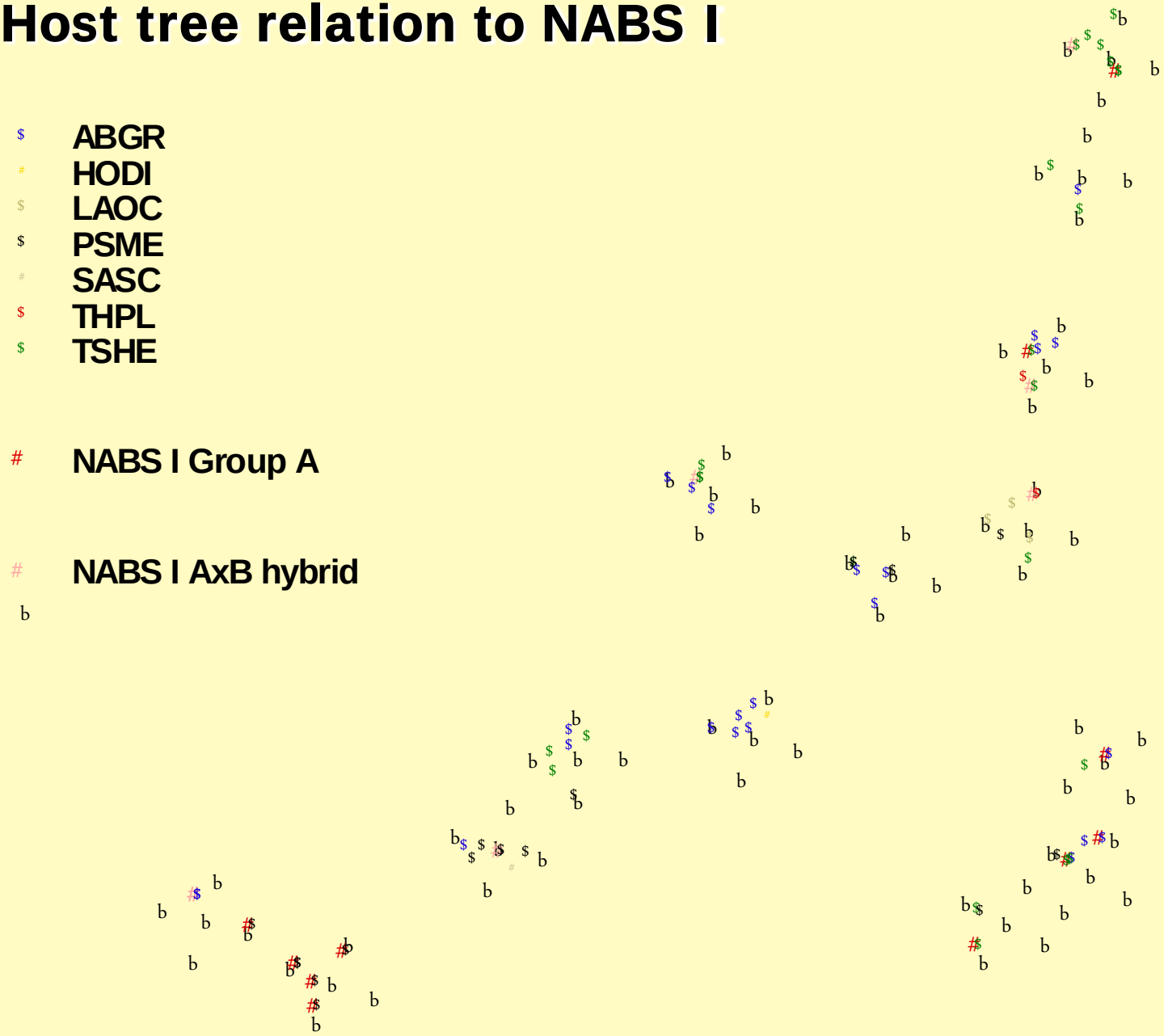
- Isolate ID
- Location
- Host Species
- Collection Type (Bark Fan, Rhizomorph, or Wood)
- Host Status (Dead or Alive)
- Habitat Type
- Fertilization Treatment
- Species ID
- Rock Type

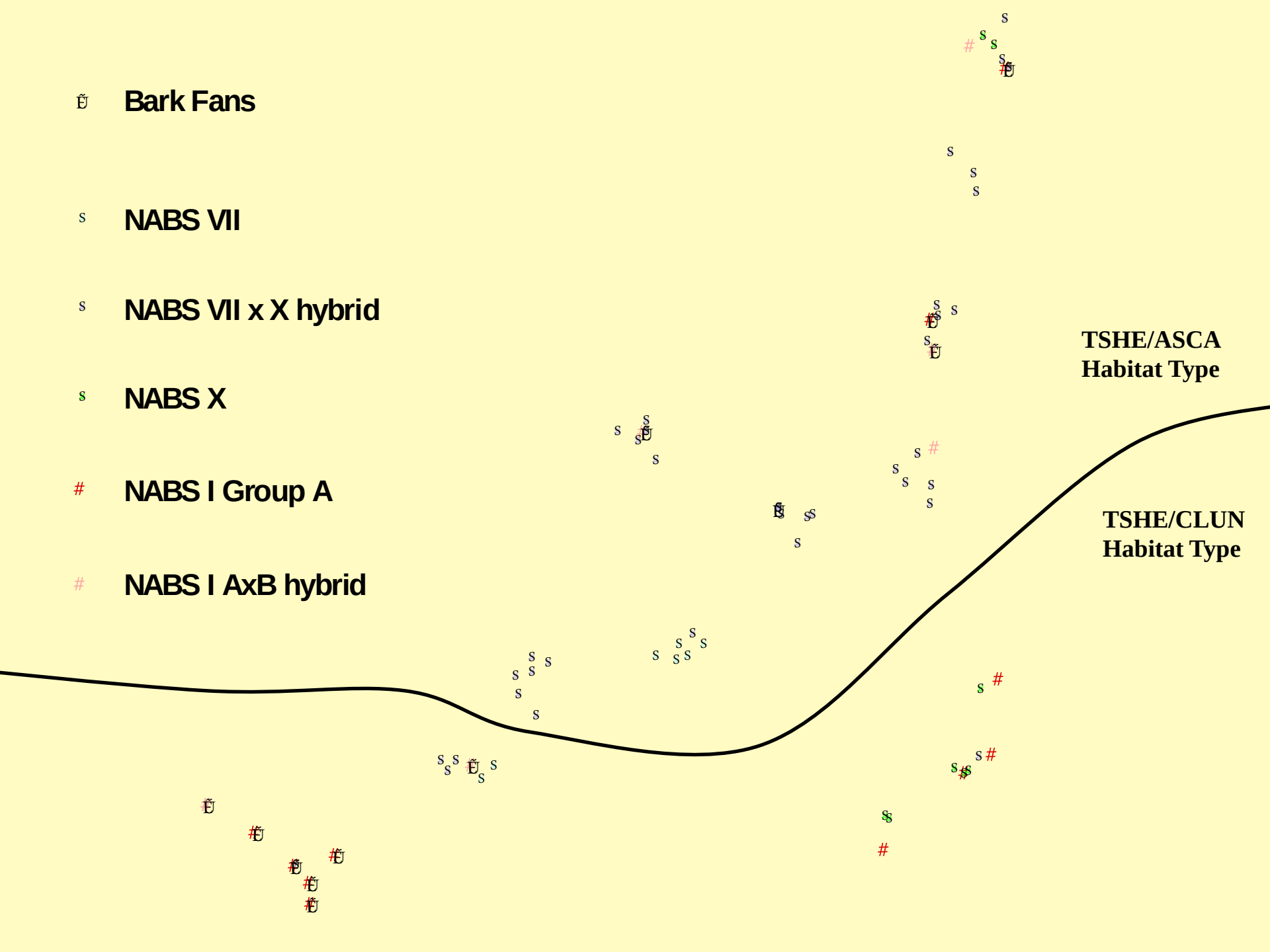
Soldier Creek
14 Treatment Plots




Treatment
100N
200K
200N
300N
300N 200K
300N 200K2SO4
600N
CONTROL
Plot Boundaries
0
0.2 Miles

Host tree relation to NABS I




Bark Fans
NABS VII
NABS VII x X hybrid
NABS X
NABS I Group A
NABS I AxB hybrid
TSHE/ASCA
Habitat Type
TSHE/CLUN
Habitat Type

Each species, hybrid, or grouping of *Armillaria* may occupy different preferred habitat type ranges.

Habitat Type is based on

- **Moisture**
- **Light**
- **Temperature**
- **Soil Characteristics**
- **Rock Types**

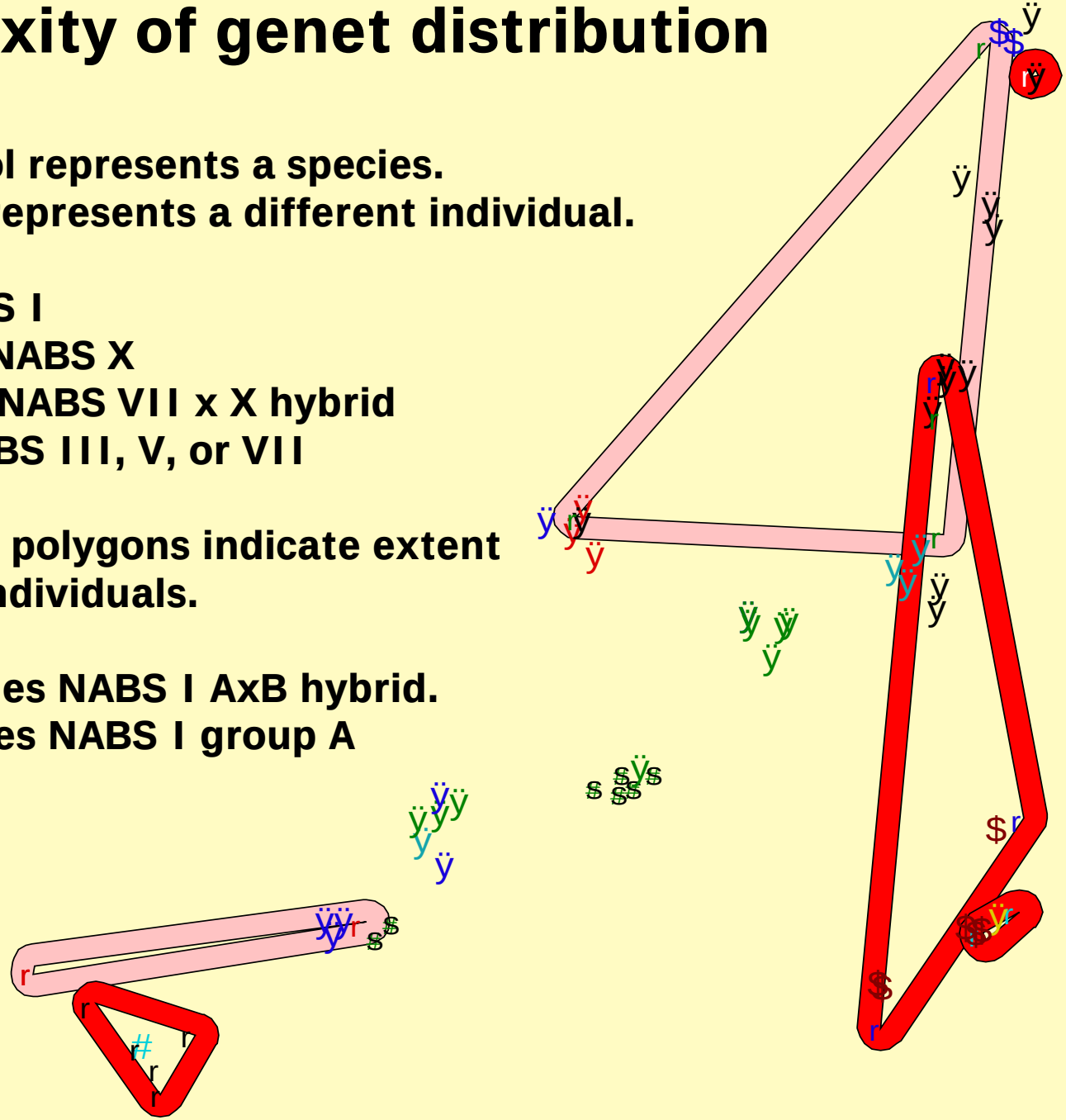
Complexity of genet distribution

Each symbol represents a species.
Each color represents a different individual.

Star = NABS I
Triangle = NABS X
Squiggle = NABS VII x X hybrid
Circle = NABS III, V, or VII

Highlighted polygons indicate extent of NABS I individuals.

Pink indicates NABS I AxB hybrid.
Red indicates NABS I group A



Armillaria interactions with the environment are extremely complex, however with new tools such as molecular genetic techniques and geographic information systems we are in a good position to find answers.

Understanding these interactions will help forest managers learn the conditions leading to mortality and growth loss and will help in management decisions to minimize loss of productivity on *Armillaria* infected sites.

Continued work on the isolates collected from Nutrition co-op plots will begin to yield statistically significant results showing how *Armillaria* interacts with the environment.

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