Preserving and Resurrecting Rare Plant Genomes

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BGSU

OUTLINE

1. Preservation of biological materials – tissues and biomolecules

2. Ice as an environmental preservation matrix

3. Preserving and resurrecting a species

4. Sequencing and resurrecting plants – which tissues should be used, and where can you find them?

1. Preservation of biological materials- tissues and biomolecules

Methods of Preservation

Propagation
Tissue Cultures
Drying – e.g Herbaria, salt
Mummifications – Human, Natural
Fossilization
Fixation
Nucleic Acid Extraction (stored frozen or dried)
Freezing - tissue

Methods of Preservation

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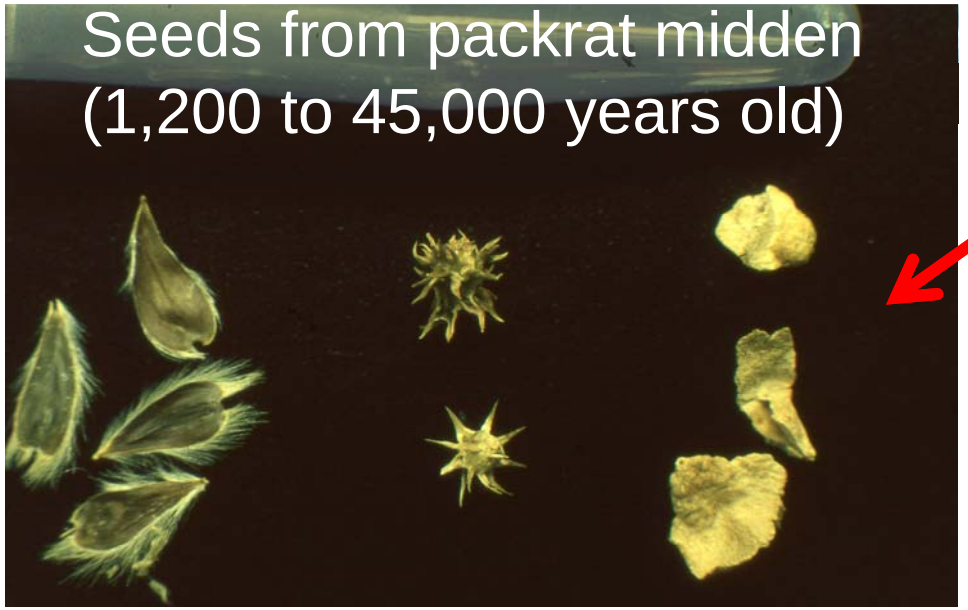
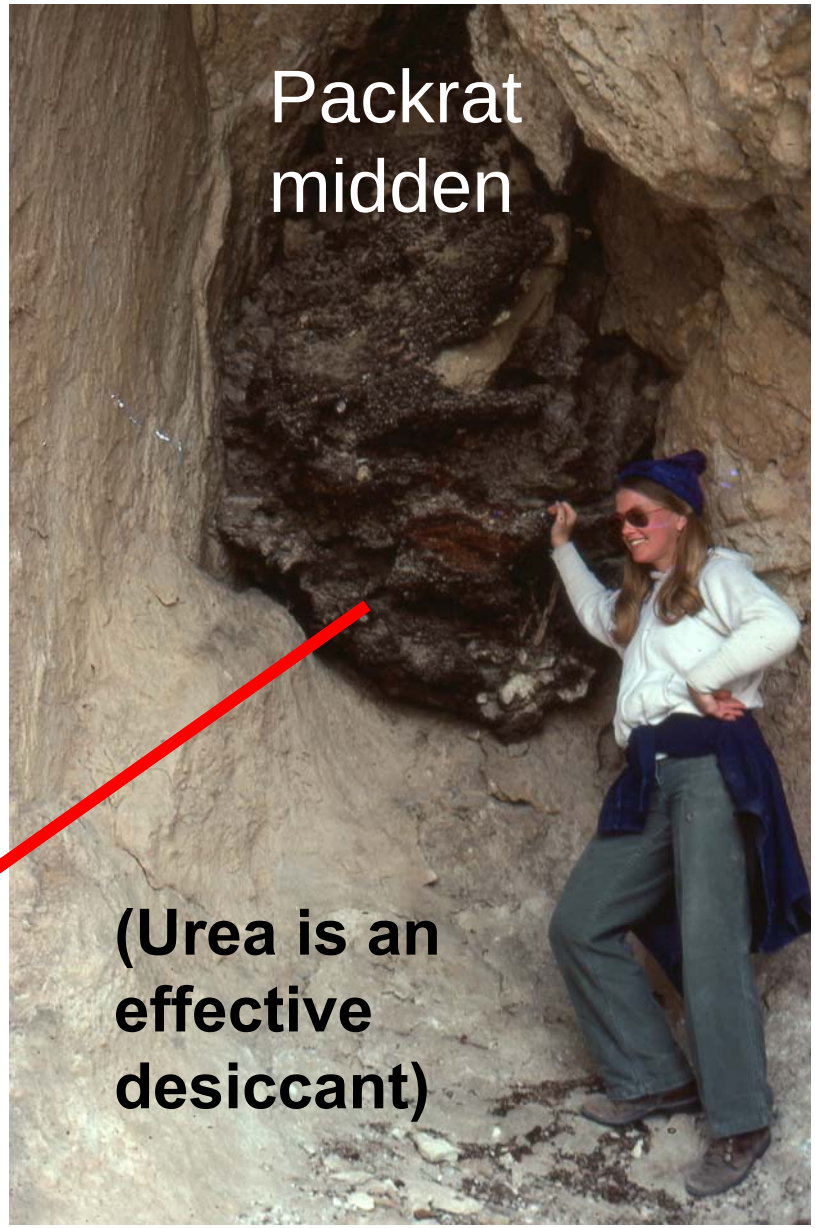
Freezing - tissue

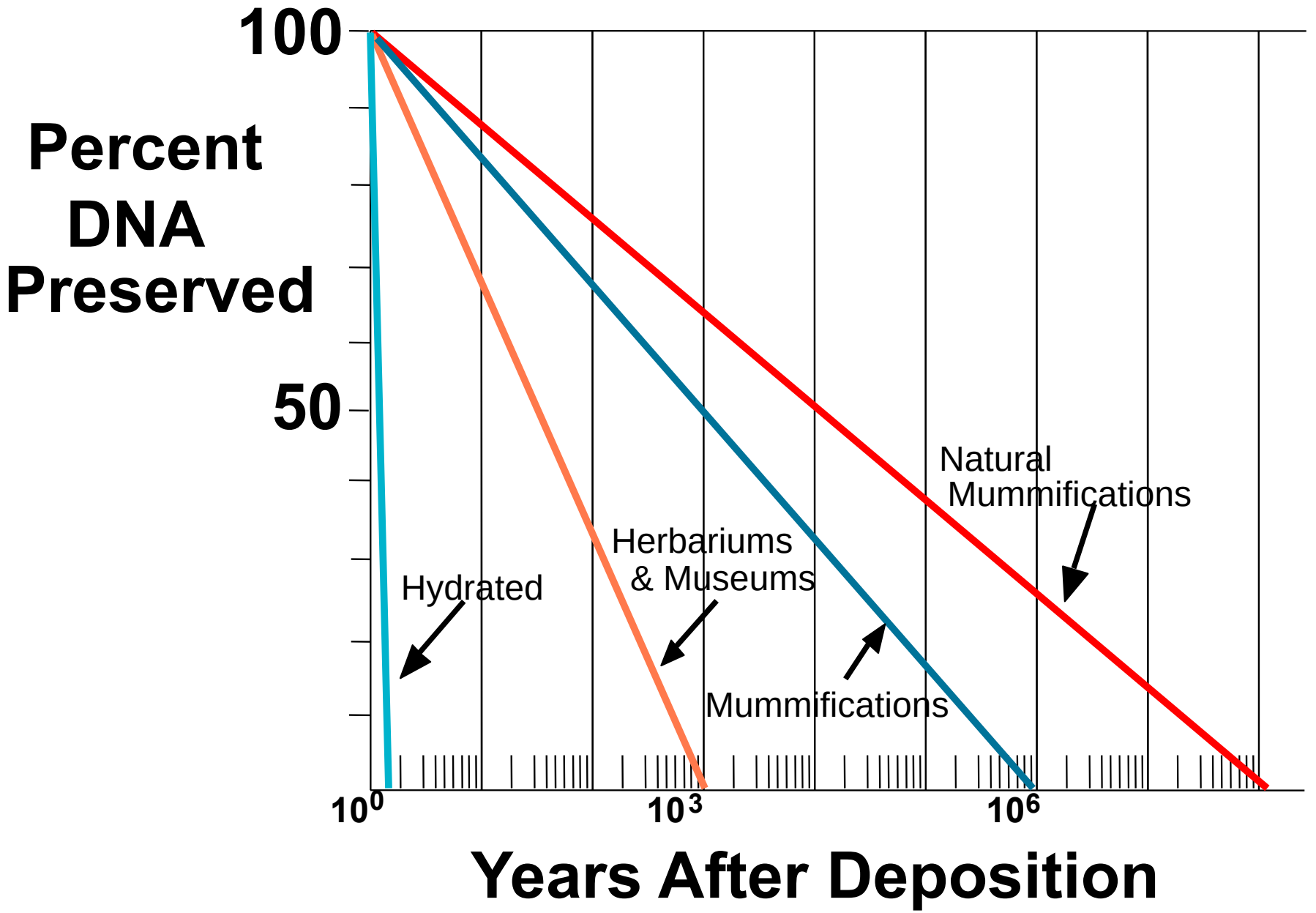
Herbaria and Preservation

Contamination/
Drying

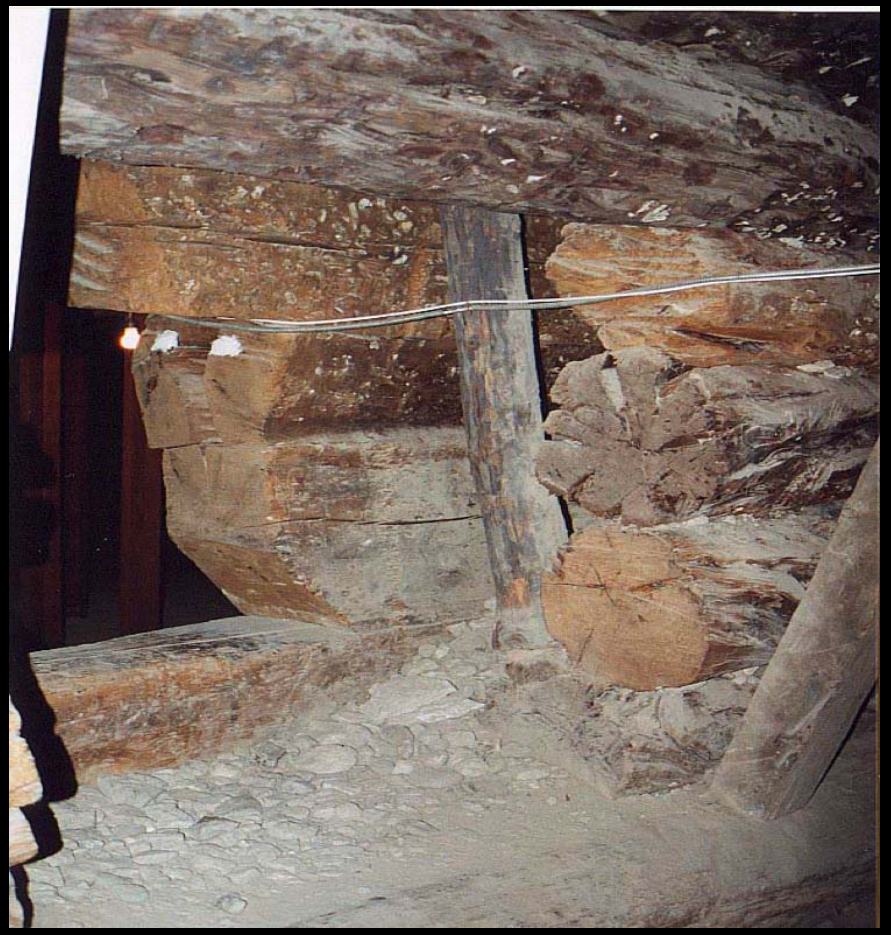
Natural Mummifications

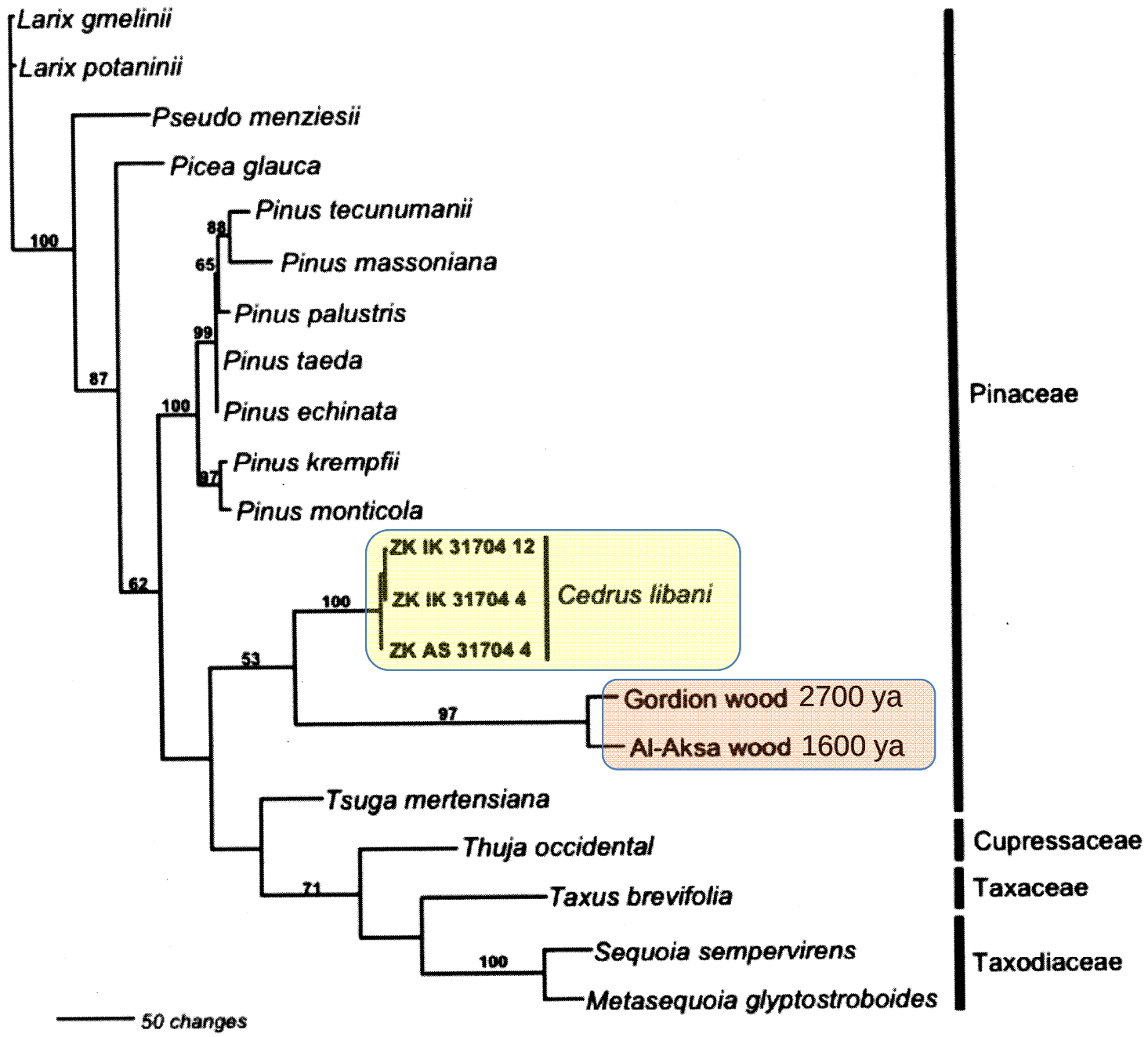





100
50
Percent
DNA
Preserved
Natural
Mummifications
Herbariums
& Museums
Hydrated
Mummifications
10^0
10^3
10^6
Years After Deposition

Gordion (King Midas' tumulus)
Cedar (*C. libani*)



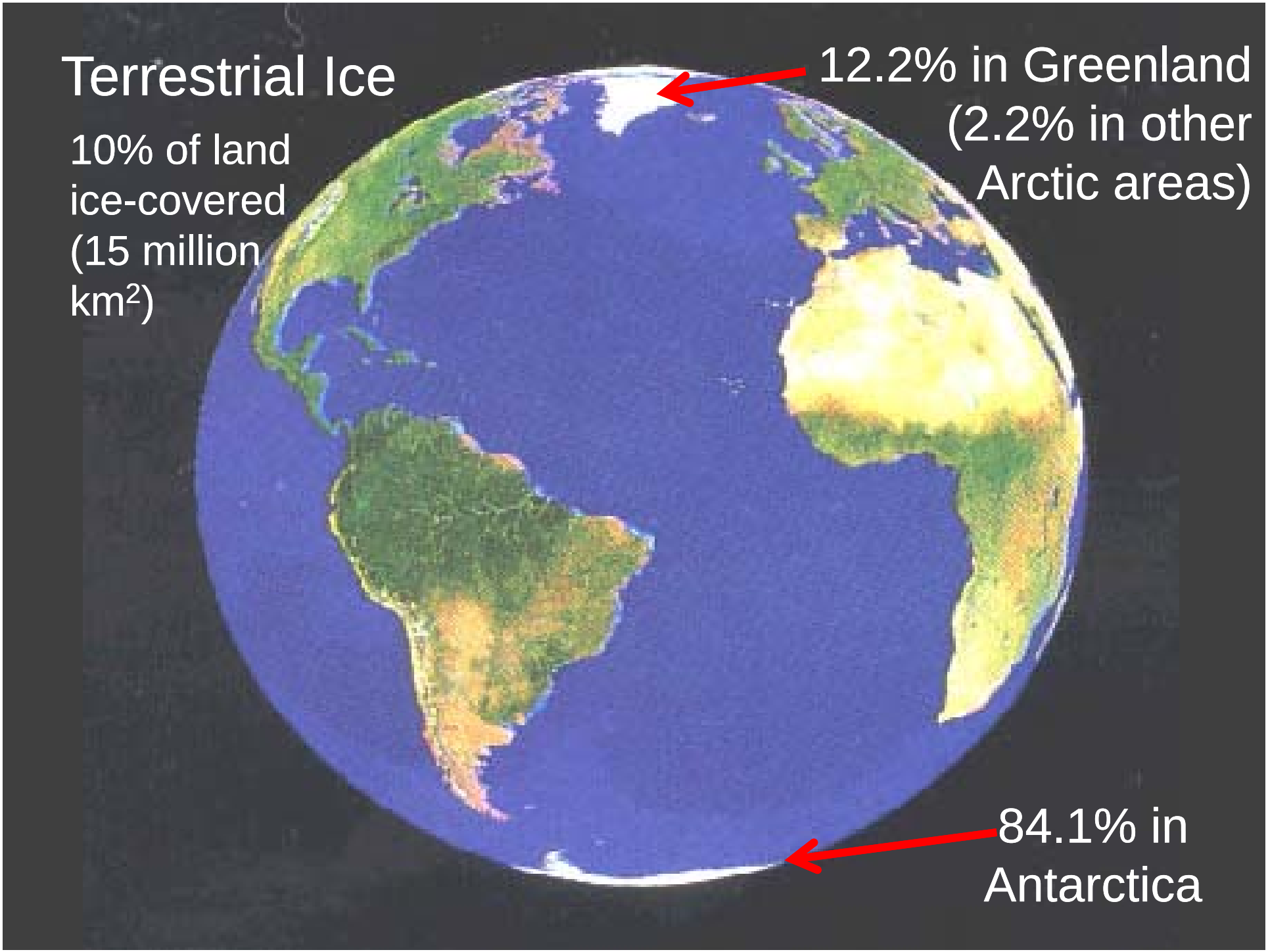

Larix gmelinii
Larix potaninii
Pseudo menziesii
Picea glauca
Pinus tecunumanii
Pinus massoniana
Pinus palustris
Pinus taeda
Pinus echinata
Pinus krempfii
Pinus monticola
ZK IK 31704 12
ZK IK 31704 4
ZK AS 31704 4
Cedrus libani
Gordion wood 2700 ya
Al-Aksa wood 1600 ya
Tsuga mertensiana
Thuja occidental
Taxus brevifolia
Sequoia sempervirens
Metasequoia glyptostroboides
100
88
65
99
87
100
97
62
100
53
97
71
100
50 changes
Pinaceae
Cupressaceae
Taxaceae
Taxodiaceae

2. Ice as an environmental preservation matrix

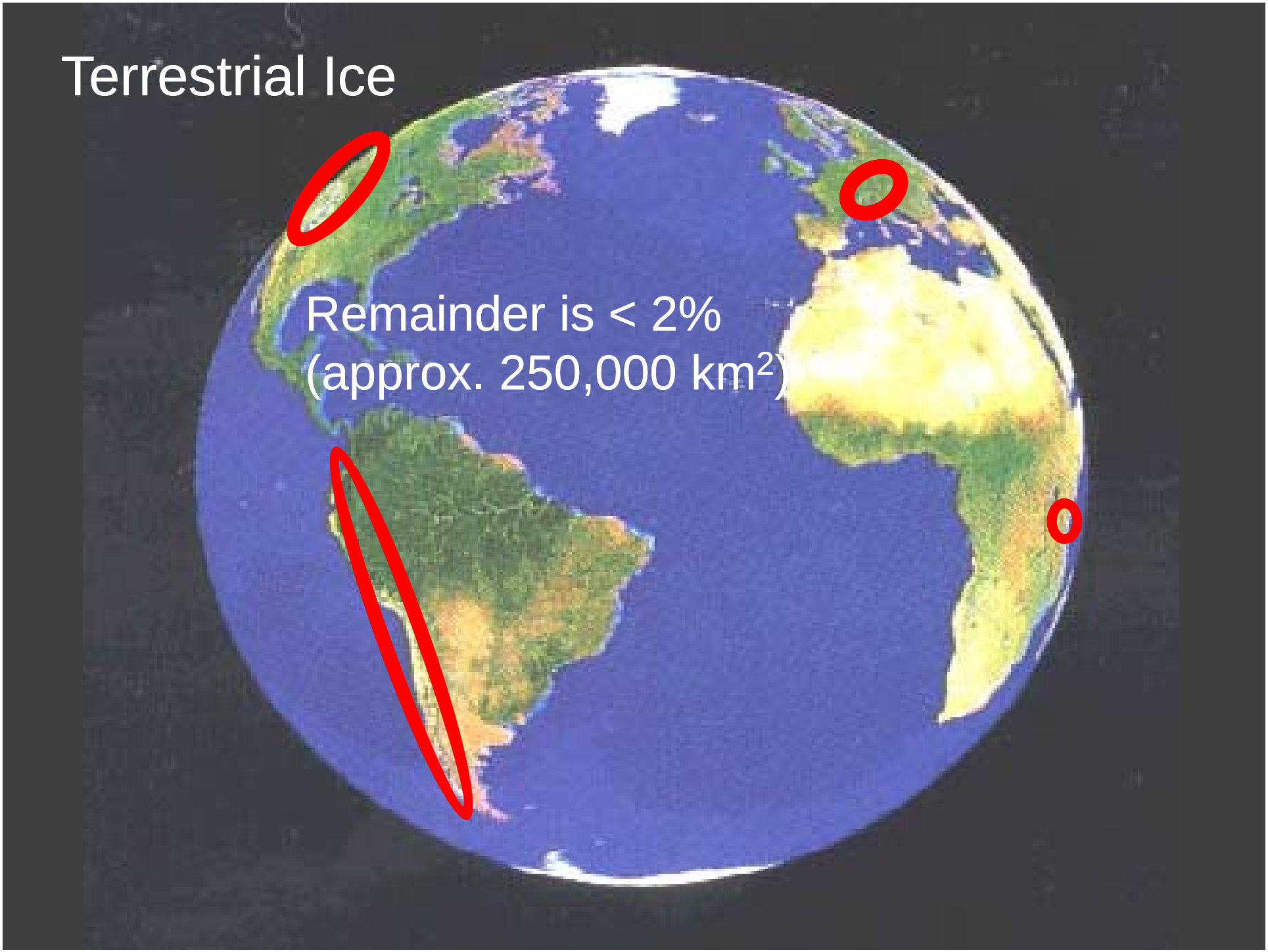
Primarily, the data are from studies of microbes (fungi, bacteria, and viruses), but DNA in microbes is chemically the same as DNA in plants.

Therefore, the conclusions are the same.

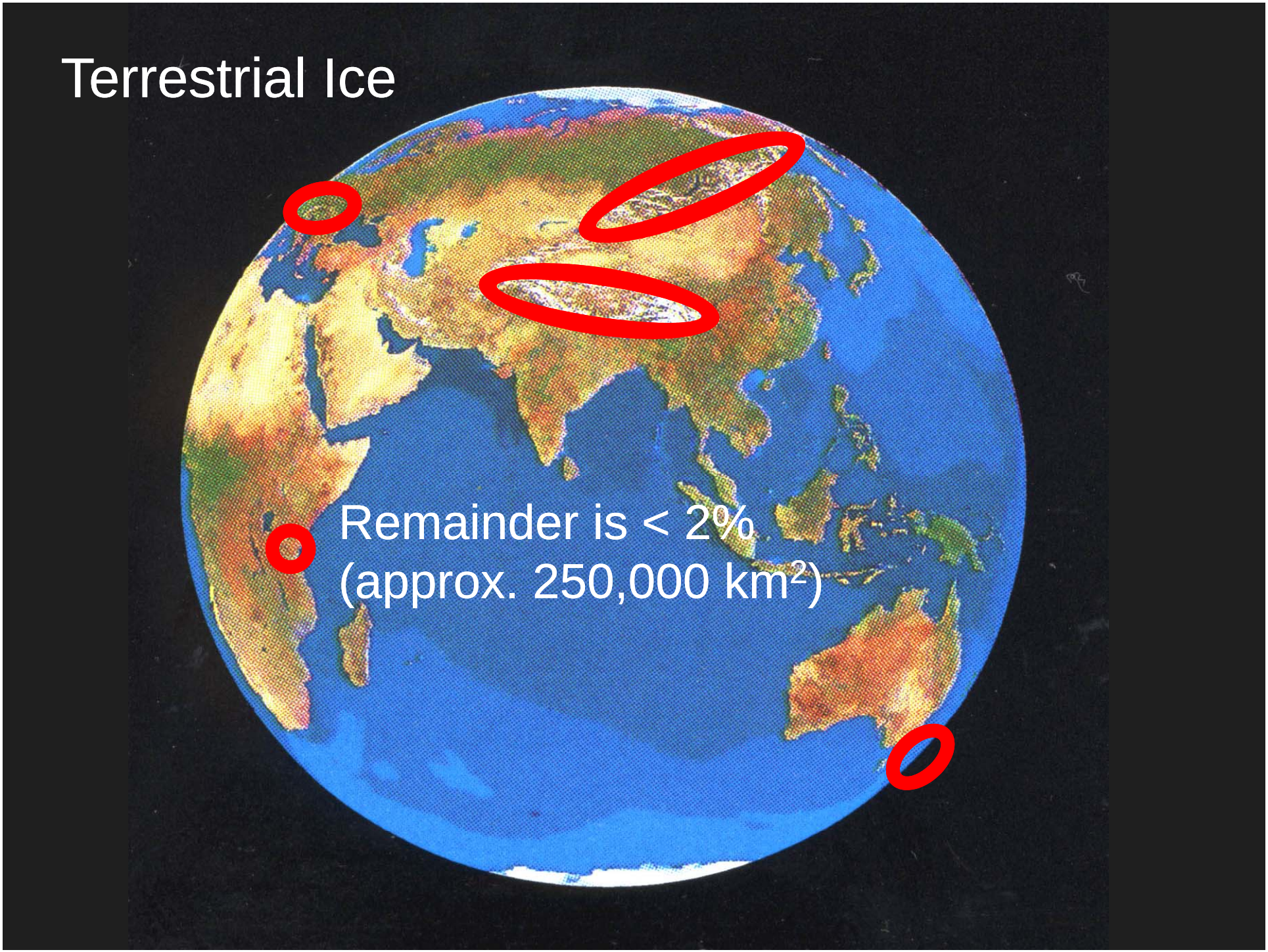
Glacial and Lake Ice


Terrestrial Ice
10% of land ice-covered (15 million km²)
12.2% in Greenland (2.2% in other Arctic areas)
84.1% in Antarctica

Terrestrial Ice



Terrestrial Ice



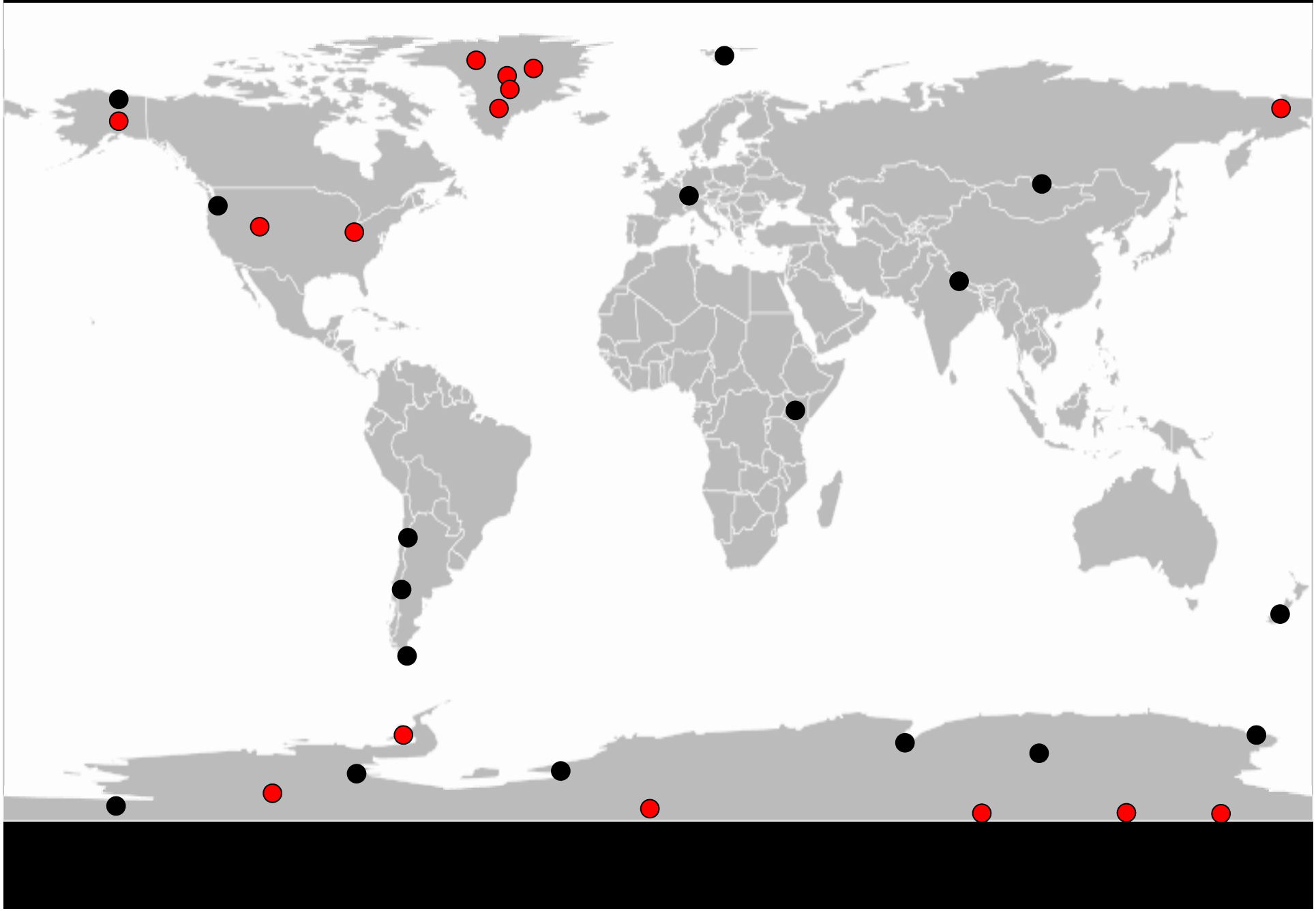
Freezing Preserves Most Organisms

Cultured from
tap water

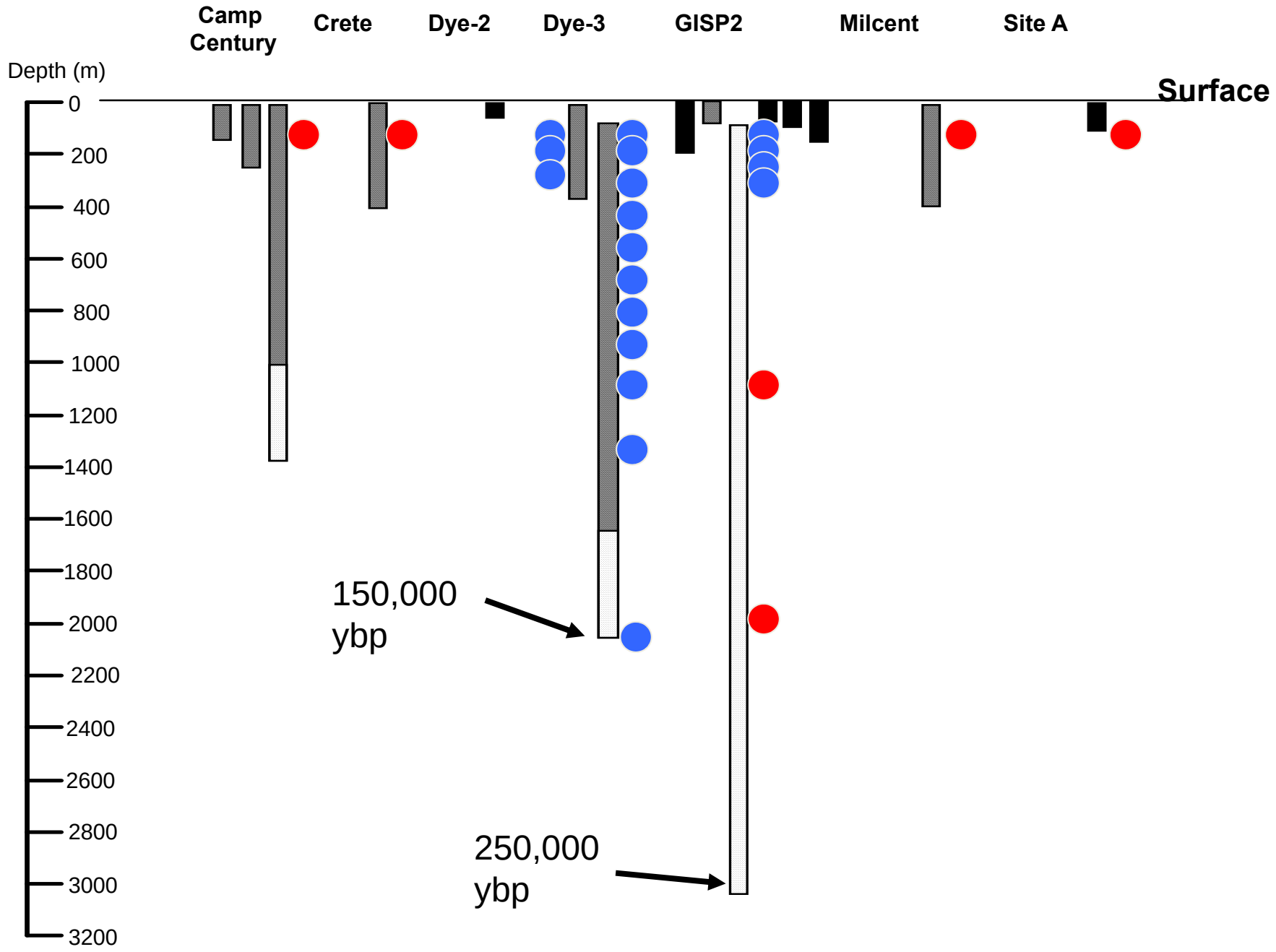
Cultured from
the same tap
water after freezing

Number of colonies is about equal.
Diversity sometimes drops.

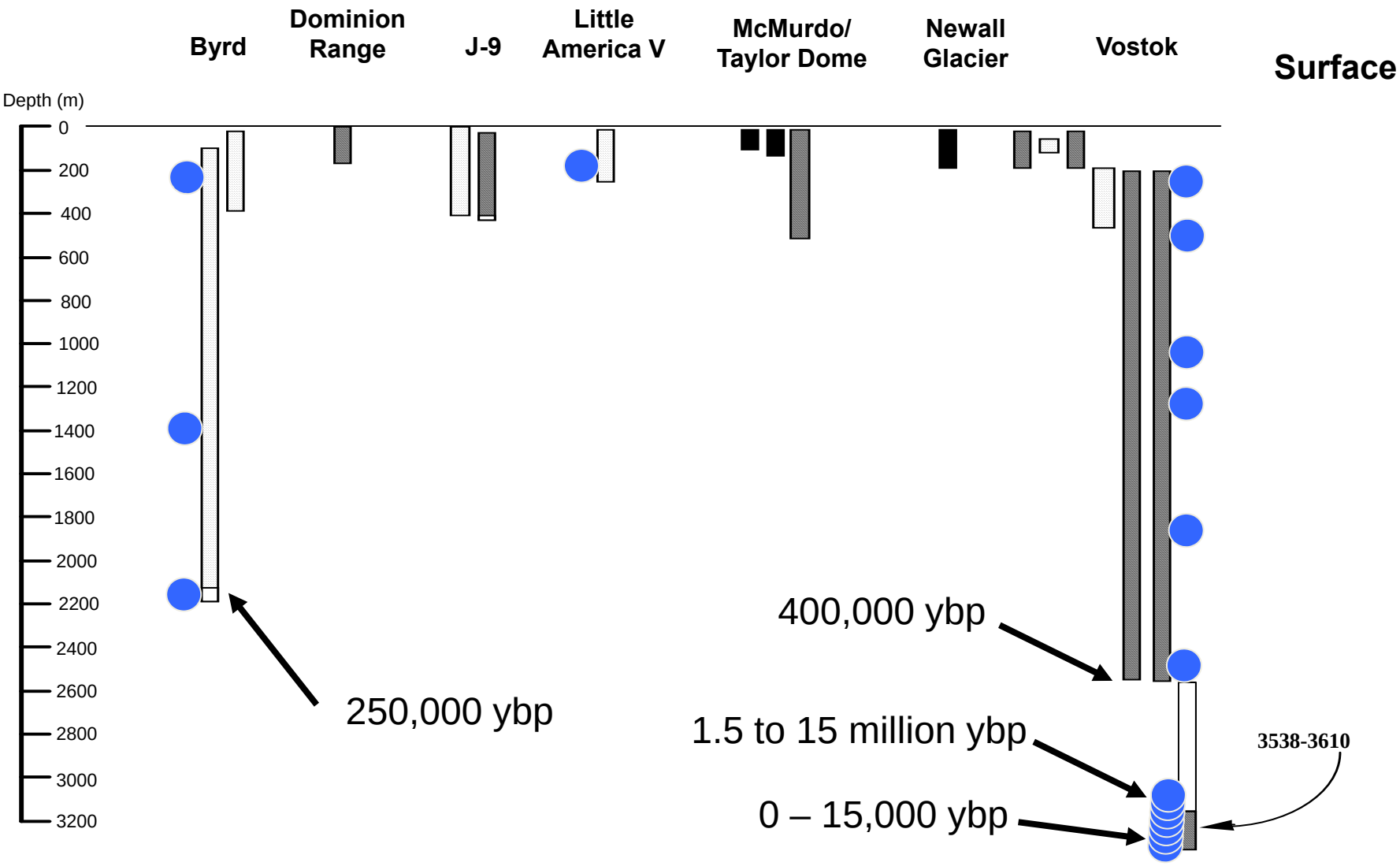
Ice Samples Collected Worldwide

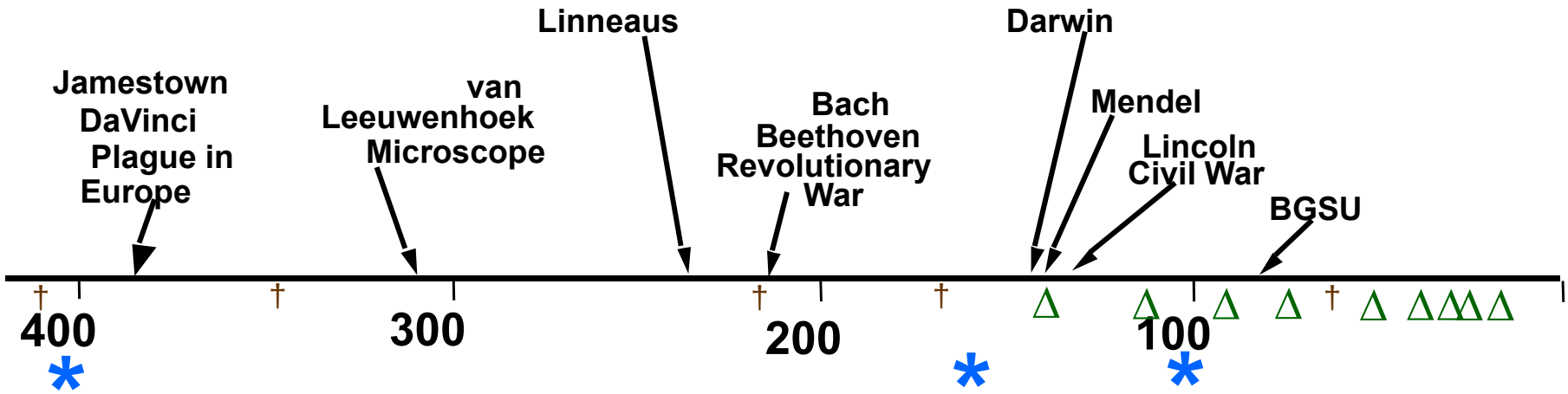


GREENLAND ICE CORES



ANTARCTICA ICE CORES



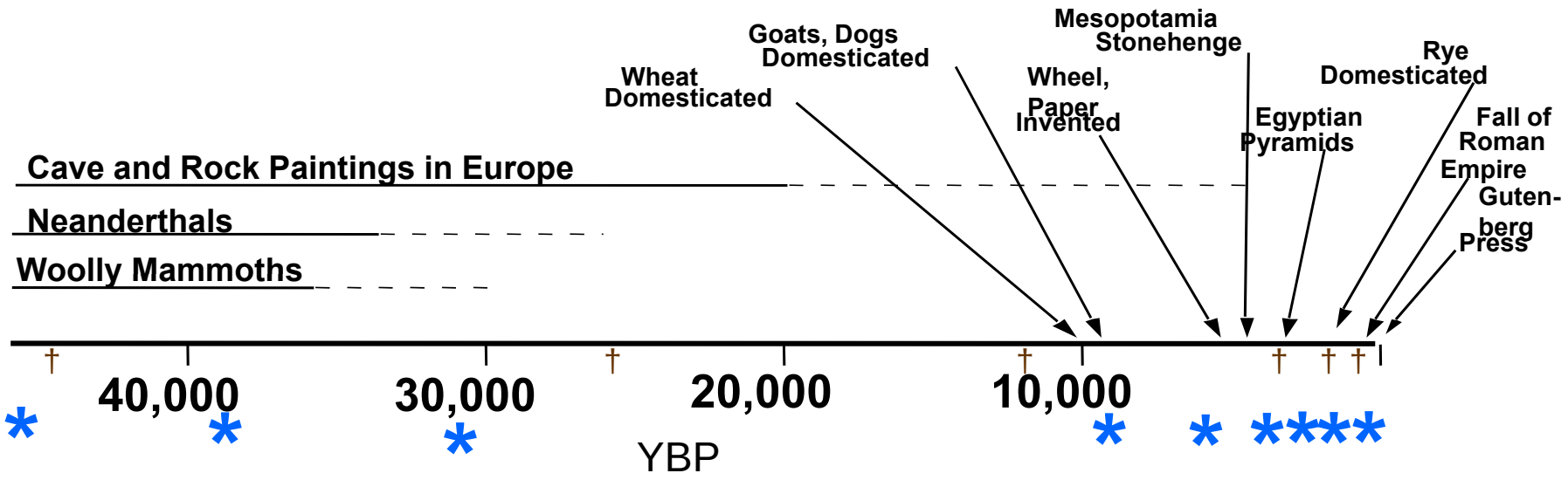


OUR SAMPLES

Δ = Herbarium

† = Mummified

* = Ice Cores



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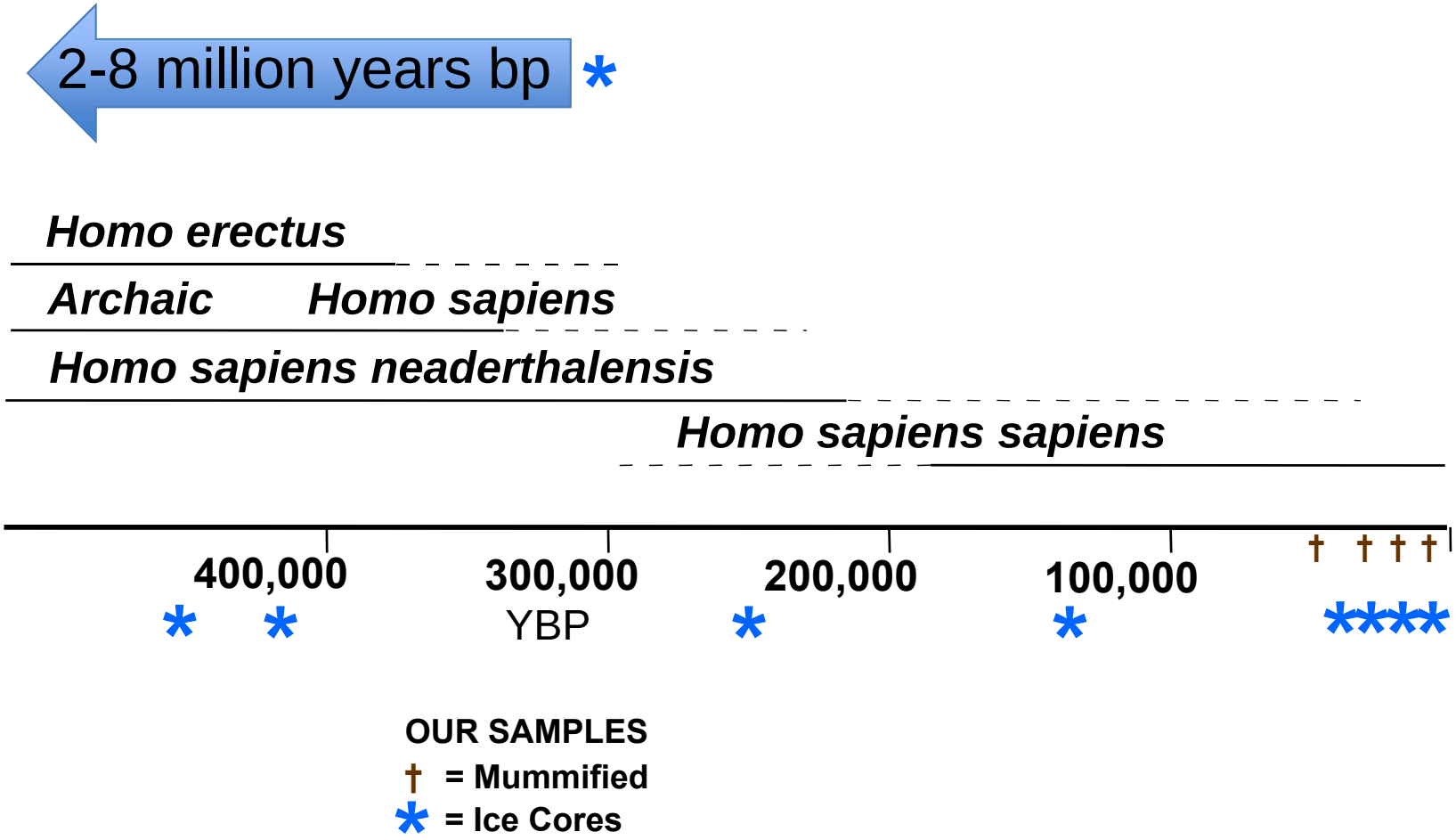
2-8 million years bp *
Homo erectus
Archaic
Homo sapiens
Homo sapiens neaderthalensis
Homo sapiens sapiens
400,000
300,000
200,000
100,000
YBP
† † † †
* * * * * ****
OUR SAMPLES
† = Mummified
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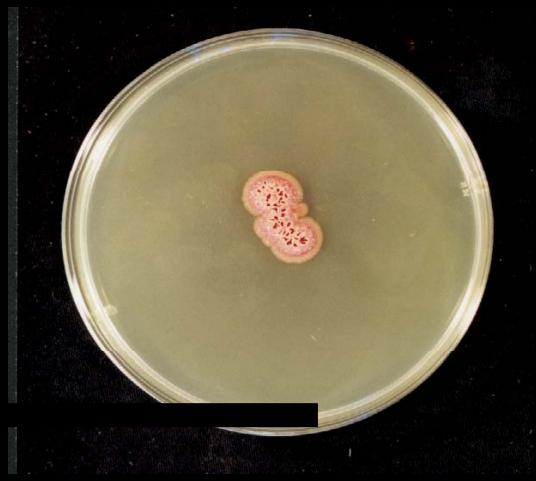
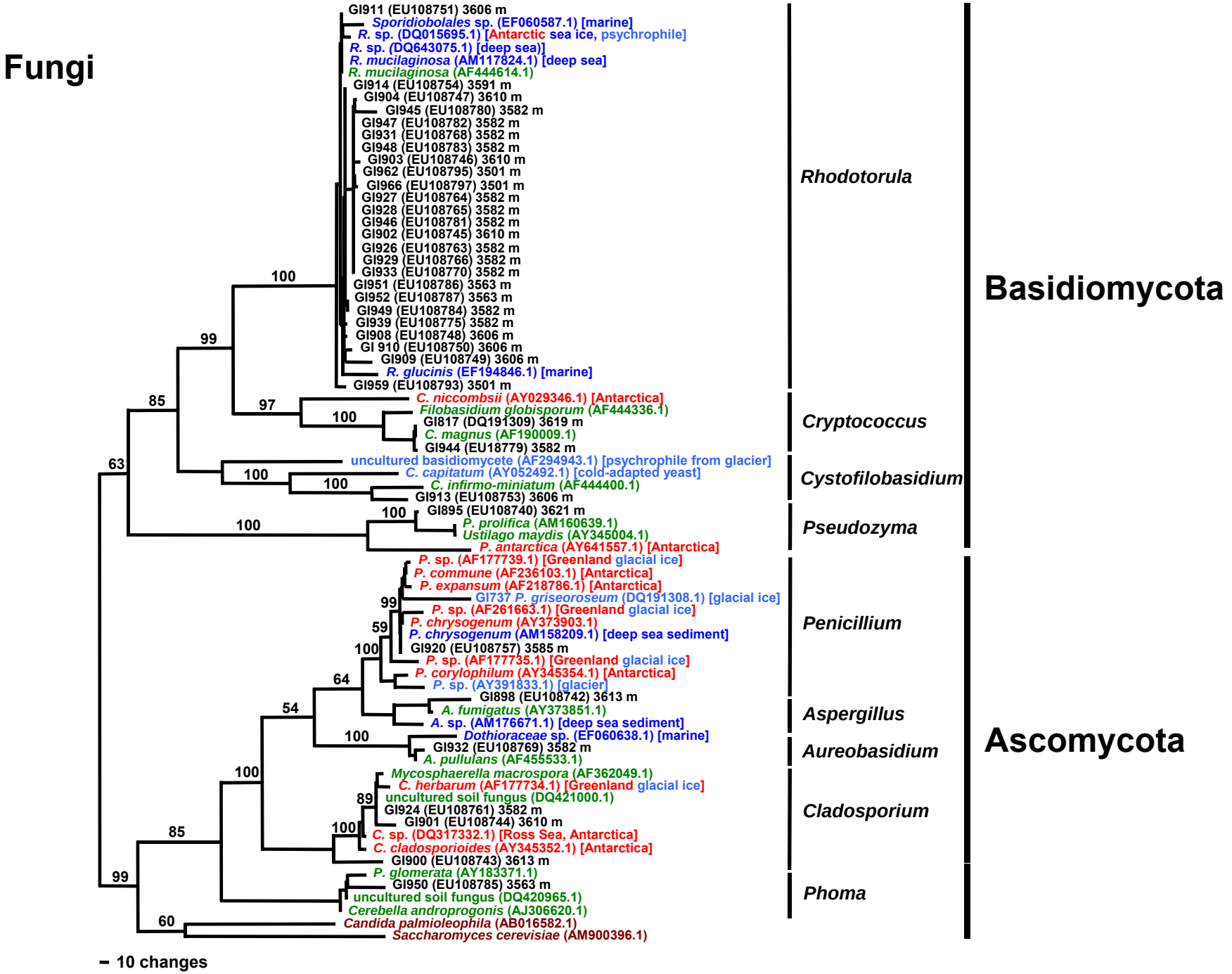
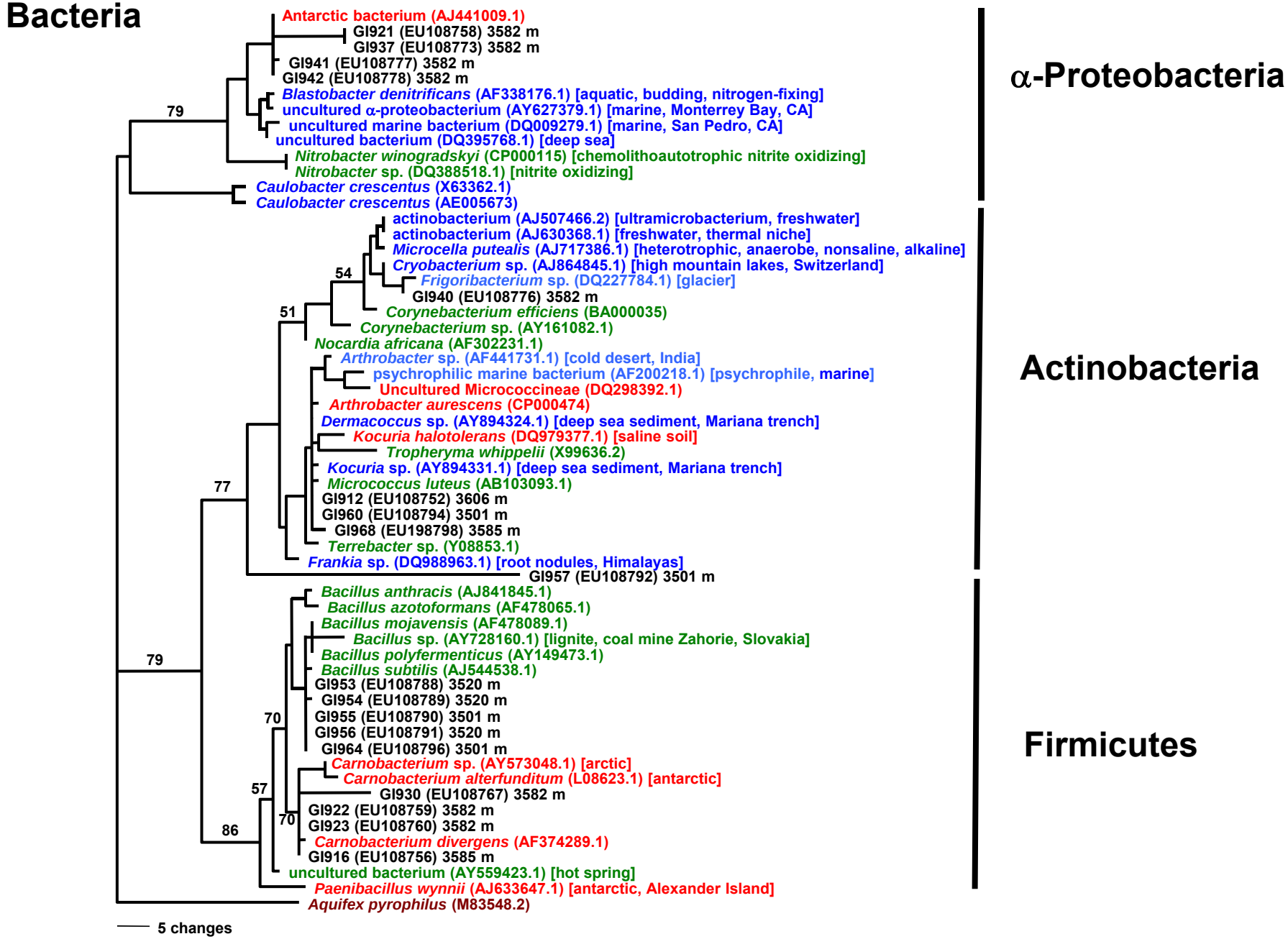
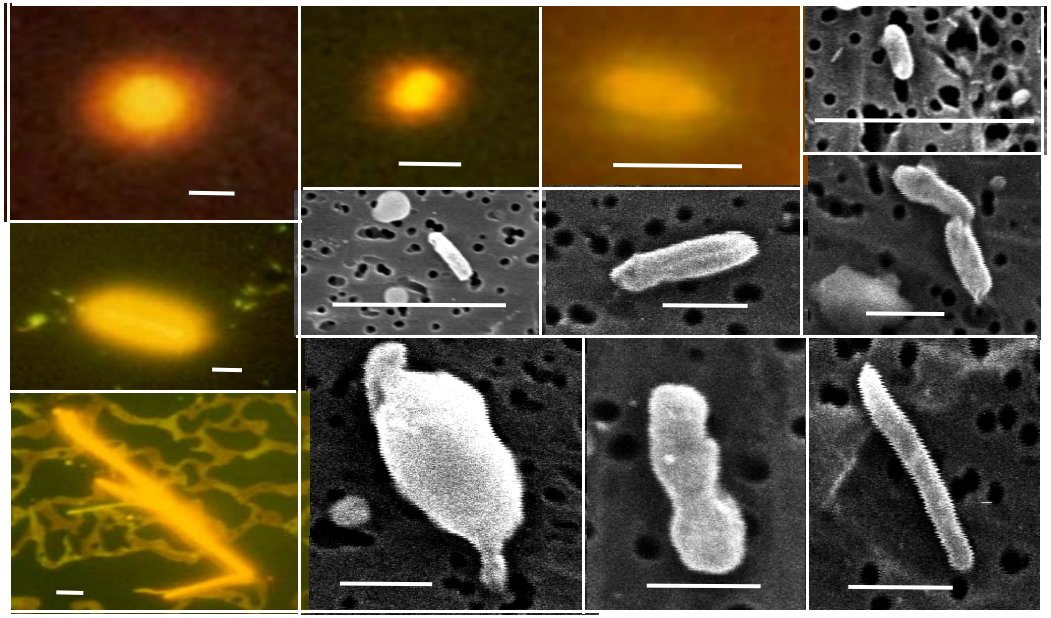
Fungi
(from 1,200
to 140,000
Year-old ice)

Bacteria
(from 10,000
to 140,000
Year-old ice)



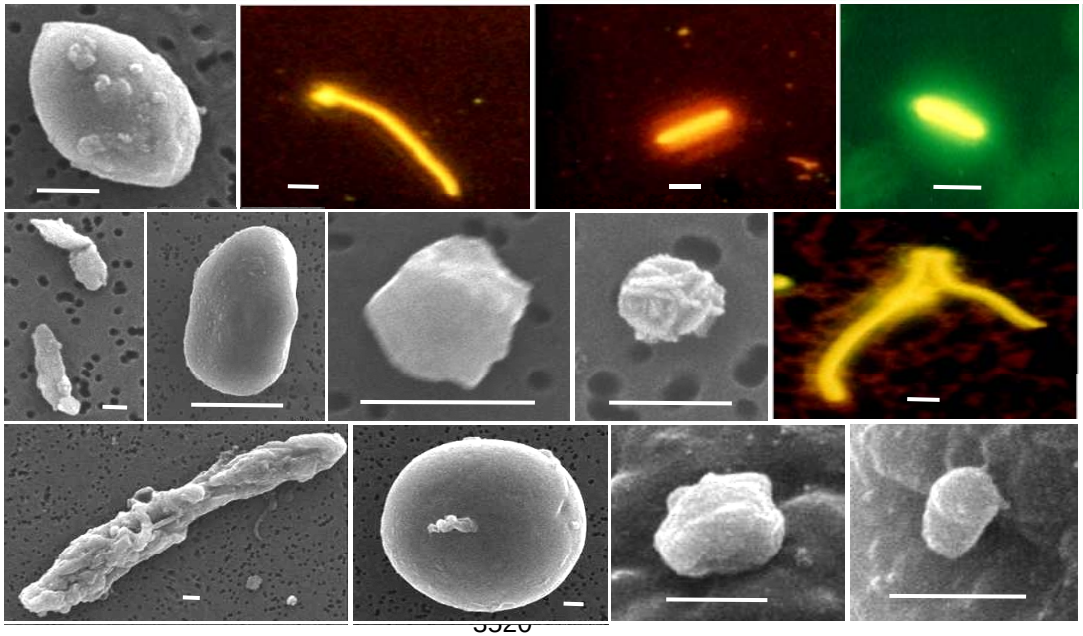

Fungi
GI911 (EU108751) 3606 m
Sporidiobolales sp. (EF060587.1) [marine]
R. sp. (DQ015695.1) [Antarctic sea ice, psychrophile]
R. sp. (DQ643075.1) [deep sea)]
R. mucilaginosa (AM117824.1) [deep sea]
R. mucilaginosa (AF444614.1)
GI914 (EU108754) 3591 m
GI904 (EU108747) 3610 m
GI945 (EU108780) 3582 m
GI947 (EU108782) 3582 m
GI931 (EU108768) 3582 m
GI948 (EU108783) 3582 m
GI903 (EU108746) 3610 m
GI962 (EU108795) 3501 m
GI966 (EU108797) 3501 m
GI927 (EU108764) 3582 m
GI928 (EU108765) 3582 m
GI946 (EU108781) 3582 m
GI902 (EU108745) 3610 m
GI926 (EU108763) 3582 m
GI929 (EU108766) 3582 m
GI933 (EU108770) 3582 m
GI951 (EU108786) 3563 m
GI952 (EU108787) 3563 m
GI949 (EU108784) 3582 m
GI939 (EU108775) 3582 m
GI908 (EU108748) 3606 m
GI 910 (EU108750) 3606 m
GI909 (EU108749) 3606 m
R. glucinis (EF194846.1) [marine]
GI959 (EU108793) 3501 m
C. niccombsii (AY029346.1) [Antarctica]
Filobasidium globisporum (AF444336.1)
GI817 (DQ191309) 3619 m
C. magnus (AF190009.1)
GI944 (EU18779) 3582 m
uncultured basidiomycete (AF294943.1) [psychrophile from glacier]
C. capitatum (AY052492.1) [cold-adapted yeast]
C. infirmo-miniatum (AF444400.1)
GI913 (EU108753) 3606 m
GI895 (EU108740) 3621 m
P. prolifica (AM160639.1)
Ustilago maydis (AY345004.1)
P. antarctica (AY641557.1) [Antarctica]
P. sp. (AF177739.1) [Greenland glacial ice]
P. commune (AF236103.1) [Antarctica]
P. expansum (AF218786.1) [Antarctica]
GI737 P. griseoroseum (DQ191308.1) [glacial ice]
P. sp. (AF261663.1) [Greenland glacial ice]
P. chrysogenum (AY373903.1)
P. chrysogenum (AM158209.1) [deep sea sediment]
GI920 (EU108757) 3585 m
P. sp. (AF177735.1) [Greenland glacial ice]
P. corylophilum (AY345354.1) [Antarctica]
P. sp. (AY391833.1) [glacier]
GI898 (EU108742) 3613 m
A. fumigatus (AY373851.1)
A. sp. (AM176671.1) [deep sea sediment]
Dothioraceae sp. (EF060638.1) [marine]
GI932 (EU108769) 3582 m
A. pullulans (AF455533.1)
Mycosphaerella macrospora (AF362049.1)
C. herbarum (AF177734.1) [Greenland glacial ice]
uncultured soil fungus (DQ421000.1)
GI924 (EU108761) 3582 m
GI901 (EU108744) 3610 m
C. sp. (DQ317332.1) [Ross Sea, Antarctica]
C. cladosporioides (AY345352.1) [Antarctica]
GI900 (EU108743) 3613 m
P. glomerata (AY183371.1)
GI950 (EU108785) 3563 m
uncultured soil fungus (DQ420965.1)
Cerebella andropogonis (AJ306620.1)
Candida palmioleophila (AB016582.1)
Saccharomyces cerevisiae (AM900396.1)
100
99
85
97
100
63
100
100
100
100
99
59
100
64
54
100
100
89
100
85
99
60
Rhodotorula
Cryptococcus
Cystofilobasidium
Pseudozyma
Penicillium
Aspergillus
Aureobasidium
Cladosporium
Phoma
Basidiomycota
Ascomycota
10 changes


Bacteria
Antarctic bacterium (AJ441009.1)
GI921 (EU108758) 3582 m
GI937 (EU108773) 3582 m
GI941 (EU108777) 3582 m
GI942 (EU108778) 3582 m
Blastobacter denitrificans (AF338176.1) [aquatic, budding, nitrogen-fixing]
uncultured α-proteobacterium (AY627379.1) [marine, Monterrey Bay, CA]
uncultured marine bacterium (DQ009279.1) [marine, San Pedro, CA]
uncultured bacterium (DQ395768.1) [deep sea]
Nitrobacter winogradskyi (CP000115) [chemolithoautotrophic nitrite oxidizing]
Nitrobacter sp. (DQ388518.1) [nitrite oxidizing]
Caulobacter crescentus (X63362.1)
Caulobacter crescentus (AE005673)
actinobacterium (AJ507466.2) [ultramicrobacterium, freshwater]
actinobacterium (AJ630368.1) [freshwater, thermal niche]
Microcella putealis (AJ717386.1) [heterotrophic, anaerobe, nonsaline, alkaline]
Cryobacterium sp. (AJ864845.1) [high mountain lakes, Switzerland]
Frigoribacterium sp. (DQ227784.1) [glacier]
GI940 (EU108776) 3582 m
Corynebacterium efficiens (BA000035)
Corynebacterium sp. (AY161082.1)
Nocardia africana (AF302231.1)
Arthrobacter sp. (AF441731.1) [cold desert, India]
psychrophilic marine bacterium (AF200218.1) [psychrophile, marine]
Uncultured Micrococcineae (DQ298392.1)
Arthrobacter aurescens (CP000474)
Dermacoccus sp. (AY894324.1) [deep sea sediment, Mariana trench]
Kocuria halotolerans (DQ979377.1) [saline soil]
Tropheryma whippelii (X99636.2)
Kocuria sp. (AY894331.1) [deep sea sediment, Mariana trench]
Micrococcus luteus (AB103093.1)
GI912 (EU108752) 3606 m
GI960 (EU108794) 3501 m
GI968 (EU198798) 3585 m
Terrebacter sp. (Y08853.1)
Frankia sp. (DQ988963.1) [root nodules, Himalayas]
GI957 (EU108792) 3501 m
Bacillus anthracis (AJ841845.1)
Bacillus azotoformans (AF478065.1)
Bacillus mojavensis (AF478089.1)
Bacillus sp. (AY728160.1) [lignite, coal mine Zahorie, Slovakia]
Bacillus polyfermenticus (AY149473.1)
Bacillus subtilis (AJ544538.1)
GI953 (EU108788) 3520 m
GI954 (EU108789) 3520 m
GI955 (EU108790) 3501 m
GI956 (EU108791) 3520 m
GI964 (EU108796) 3501 m
Carnobacterium sp. (AY573048.1) [arctic]
Carnobacterium alterfunditum (L08623.1) [antarctic]
GI930 (EU108767) 3582 m
GI922 (EU108759) 3582 m
GI923 (EU108760) 3582 m
Carnobacterium divergens (AF374289.1)
GI916 (EU108756) 3585 m
uncultured bacterium (AY559423.1) [hot spring]
Paenibacillus wynnii (AJ633647.1) [antarctic, Alexander Island]
Aquifex pyrophilus (M83548.2)
79
54
51
77
79
70
57
70
86
5 changes
α-Proteobacteria
Actinobacteria
Firmicutes



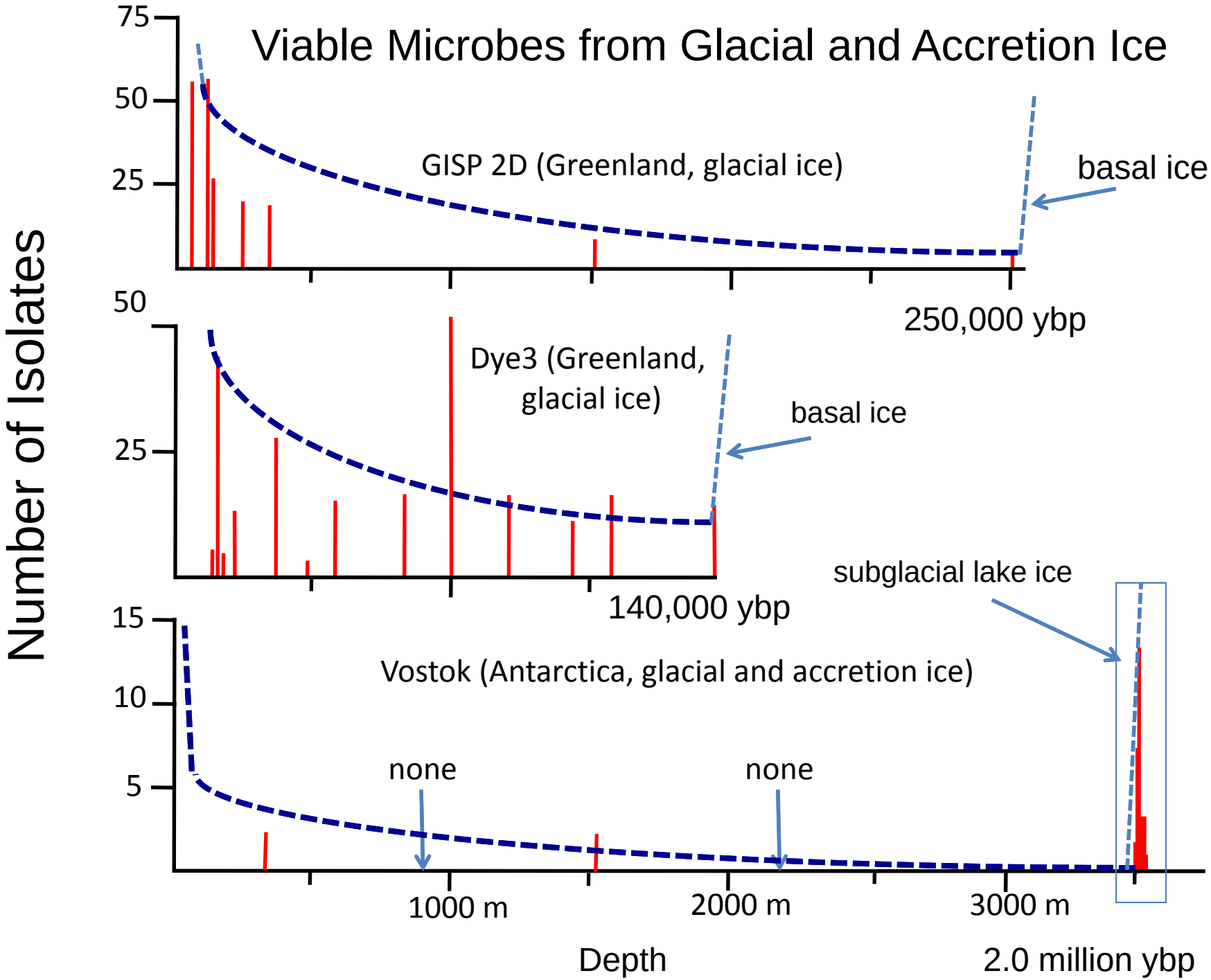
3501 (glacial)

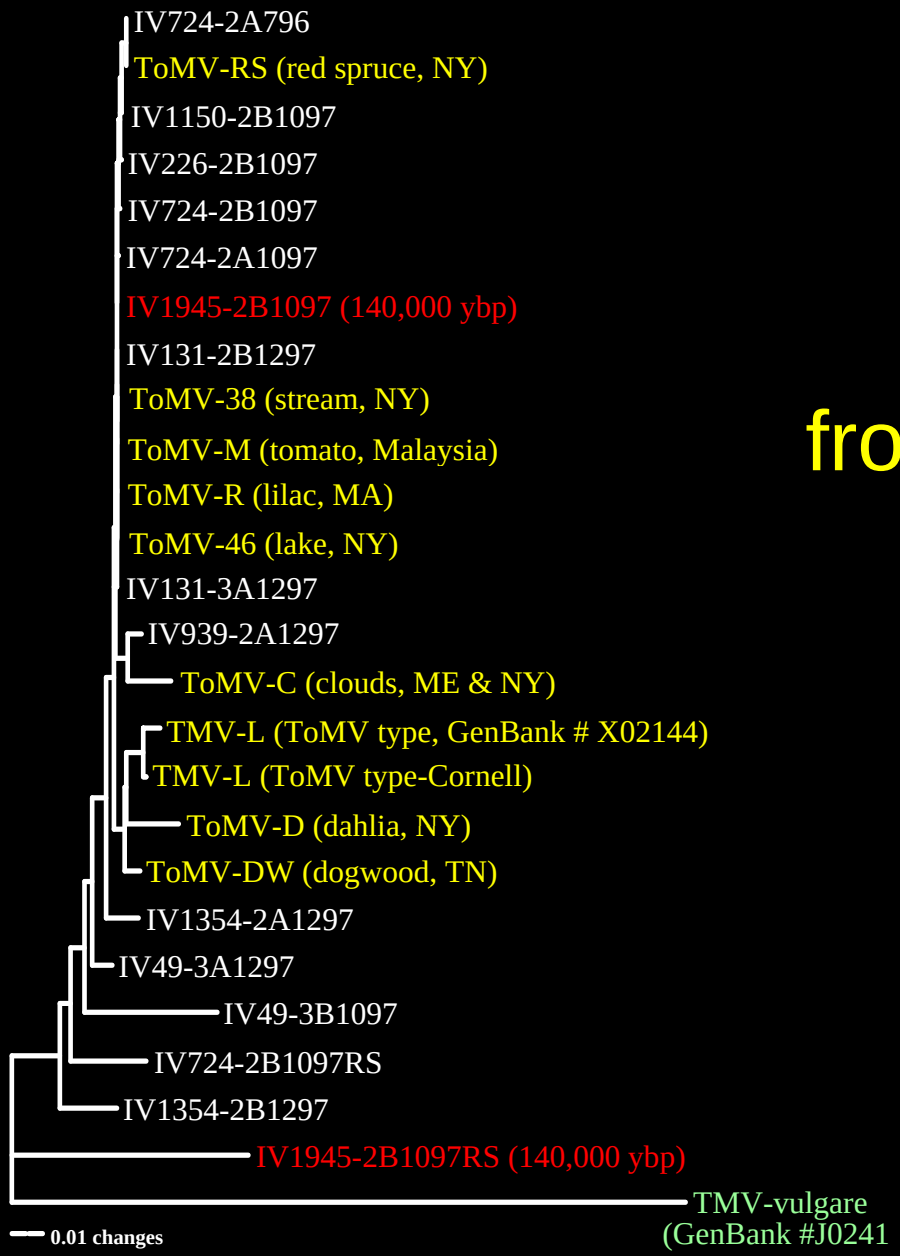
1.5 million year-old ice



3520 (glacial)

2.0 million year-old ice


Viable Microbes from Glacial and Accretion Ice
Number of Isolates
75
50
25
GISP 2D (Greenland, glacial ice)
basal ice
250,000 ybp
50
25
Dye3 (Greenland, glacial ice)
basal ice
140,000 ybp
15
10
5
subglacial lake ice
Vostok (Antarctica, glacial and accretion ice)
none
none
1000 m
2000 m
3000 m
Depth
2.0 million ybp



Tomato Mosaic Tobamovirus (an RNA virus) from 500 to 140,000 year-old Greenland ice

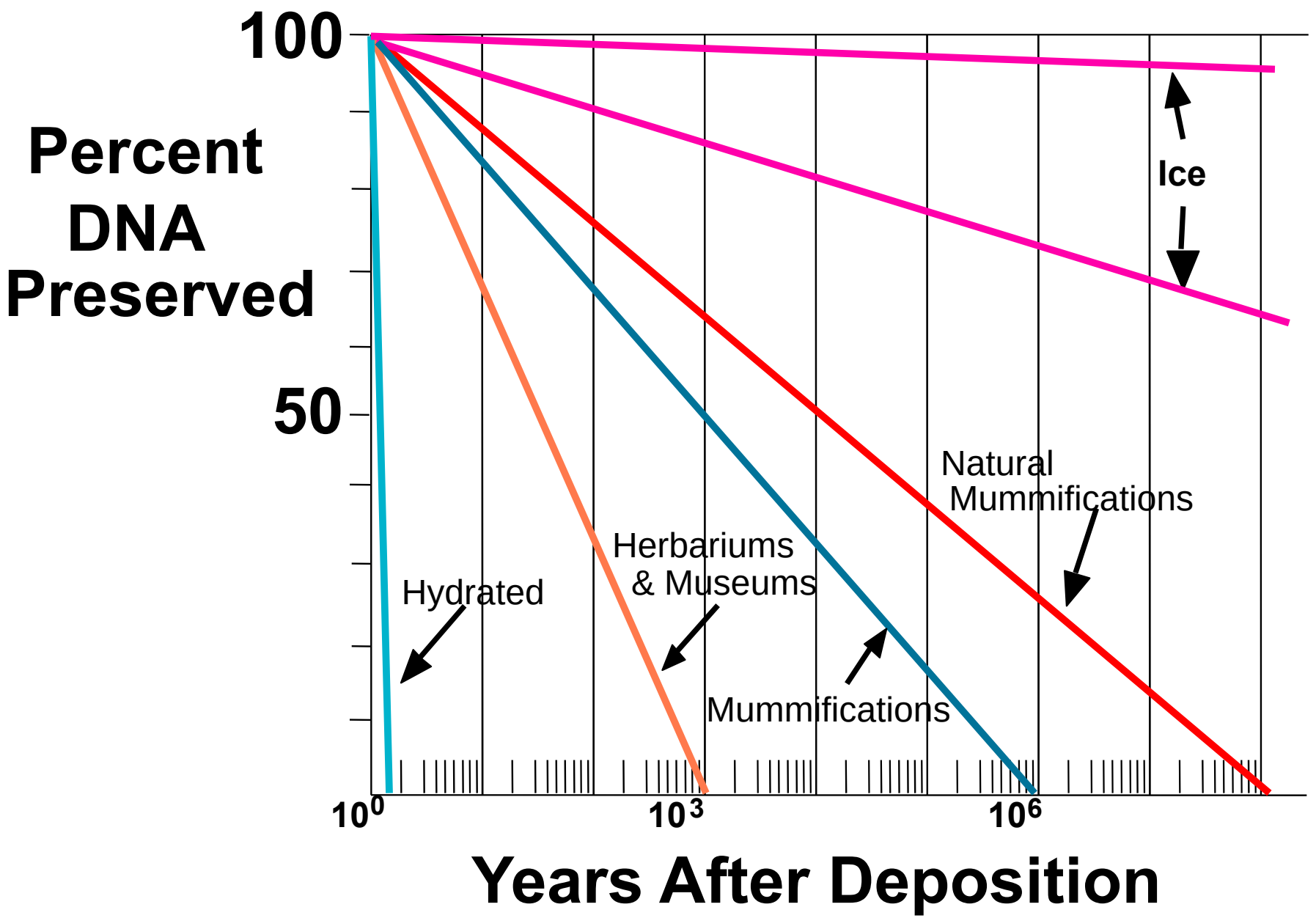
Willerslev et al 2007 – Greenland ice - Plants

Recent ice – micro and macrofossils, including pollen; sequences from arctic species

Older ice – 500 to 140,000 ybp; pollen grains; sequences from arctic/temperate taxa

Basal (silty) ice – 450,000 to 900,000 ybp; few pollen grains; sequences from taxa characteristic of a boreal forest.

Oldest ice – 2.4 million ybp; no sequences; temperate climate at that time.


100
50
Percent
DNA
Preserved
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Hydrated
Mummifications
10^0
10^3
10^6
Years After Deposition

Freezing and Thawing Influenza A Virus

- Influenza A = an RNA virus with a membrane (from host).

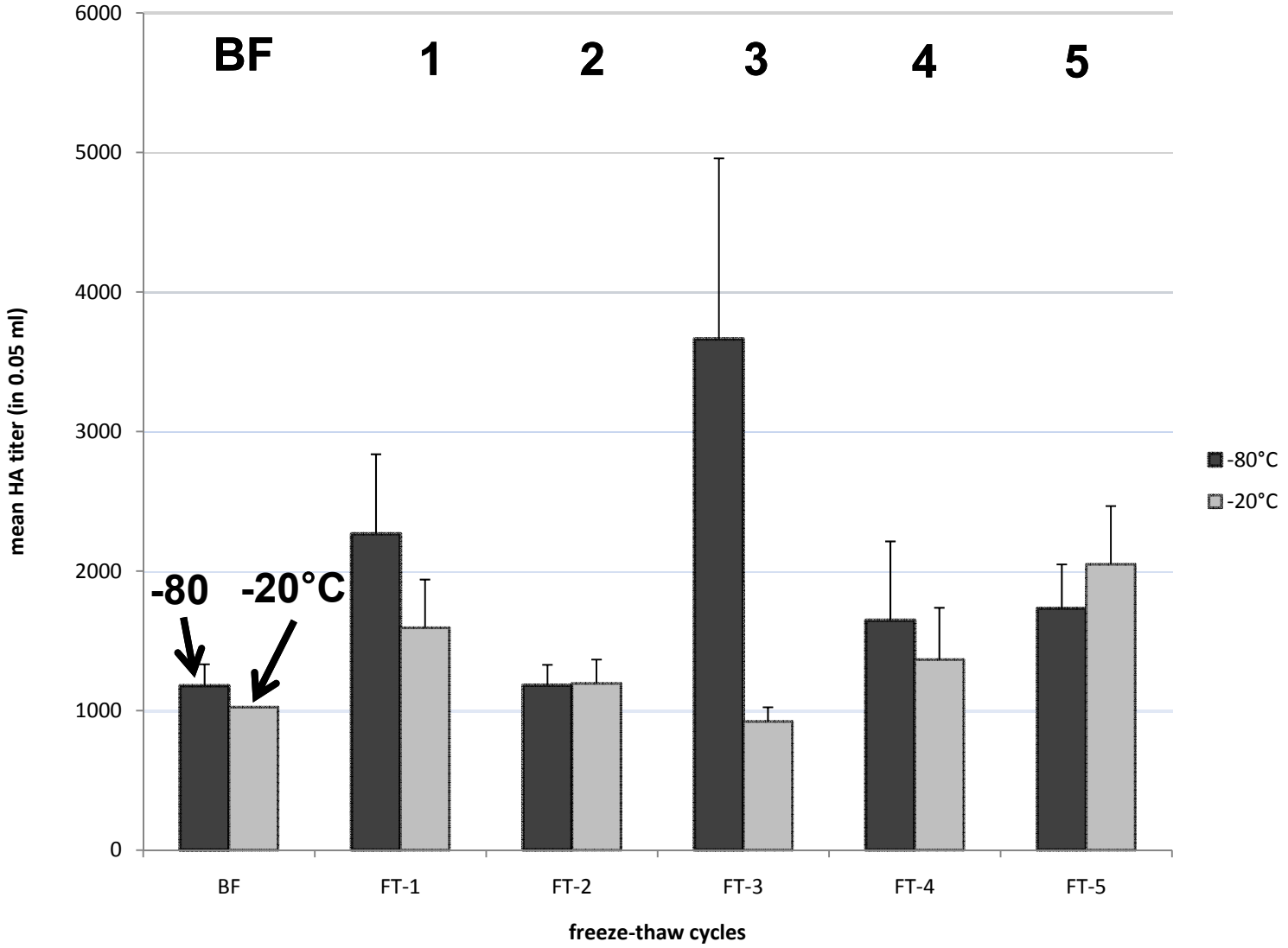
- Are there viable influenza A viruses in lake ice?
  YES.

- Do influenza A viruses survive multiple cycles of freezing and thawing at -20°C and -80°C?
  YES.

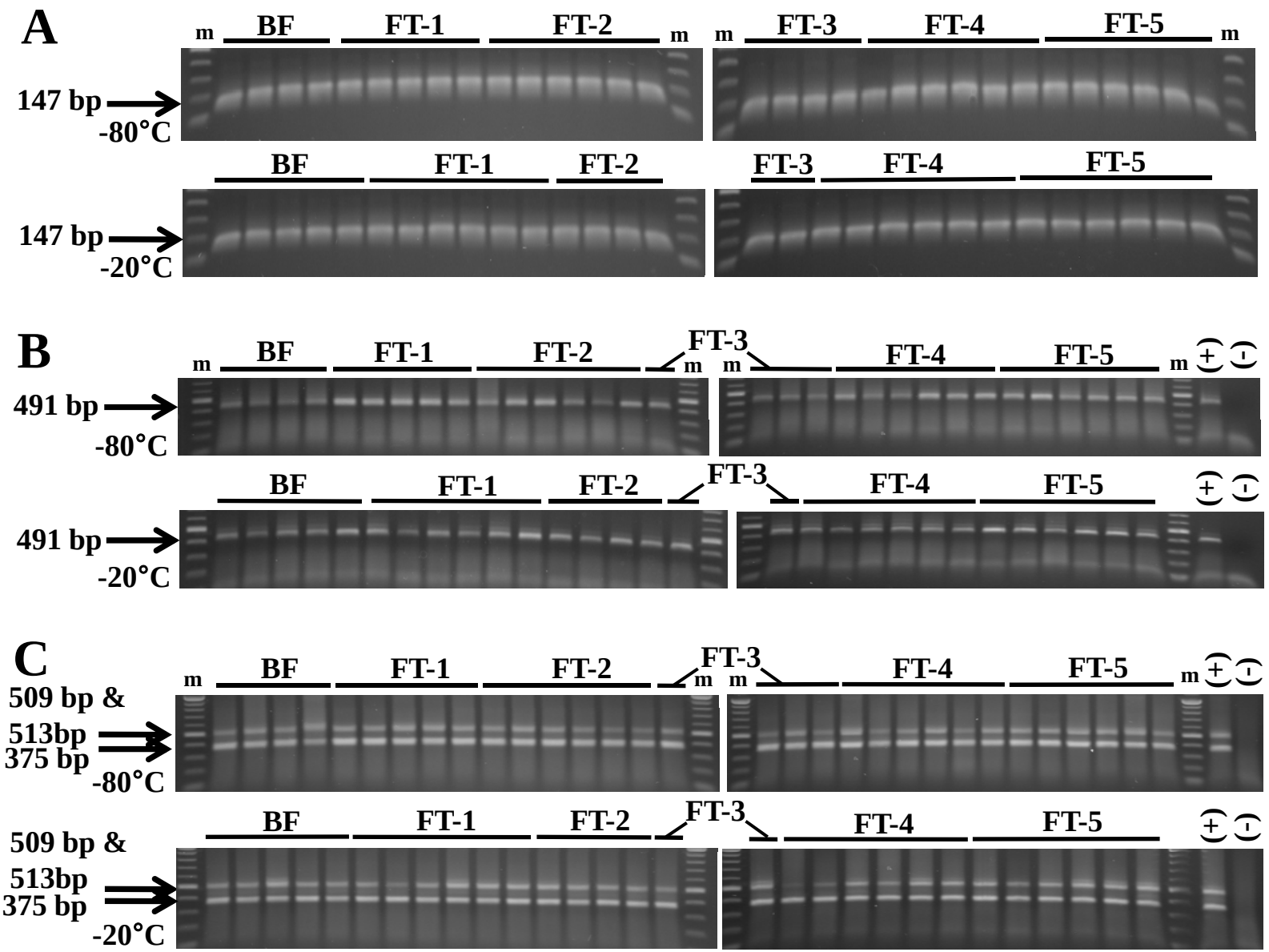
Influenza A Assays

SAMPLE		RNA	VIABILITY
[Others' studies]			
Water + birds	(WW)	+	+
Water + chicks	(JP)	++	++
Water, few birds (end of fall)	(AK)	+	+
Mud, ice-covered lake	(AK)	+	nt
[Our Lab]			
Water/Ice (winter – few birds; sp – 10 000s) (M, H2, H3, H4, H5, H6, H7, H11)		+	+
Ice (winter – no birds; sp – 10 000s) (M, H1, H2, H4, H7, H11, H13)		+	+

Viability of Infleunza A after freezing and thawing



RT-PCR confirmation of Influenza A after each freeze-thaw cycle



DNA Preservation

pH	6.0 and 11.0 (6.5-7.5 for RNA)
Temperature	Cold
Humidity	Low
Light conditions	Dark

Optimal Below freezing, dry, dark, pH 7.0

3. Preserving and resurrecting a plant species

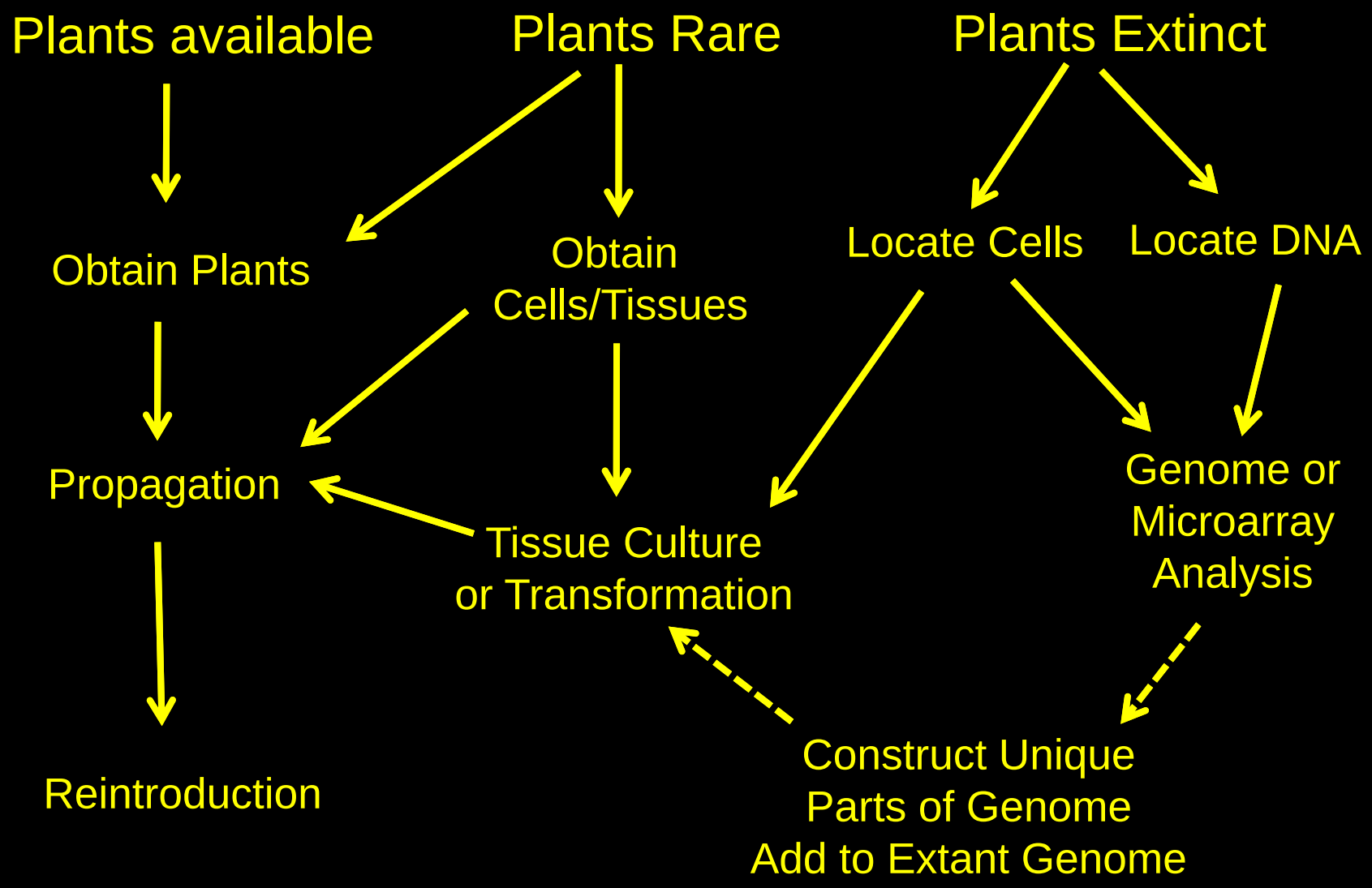
Status of Plant

Threatened – Plant and DNA specimens available

Endangered – Plant and DNA specimens may be available

Extinct – Locate preserved cells and/or DNA (often dilute and degraded)

Resurrecting a Species



4. Sequencing and resurrecting plants – which tissues should be used, and where can you find them?

Which Tissues?

Intact Genomes Best

- Pollen (haploid)
- Embyros (axis is diploid)
- Young leaves, shoot apices, and primordia (diploid)

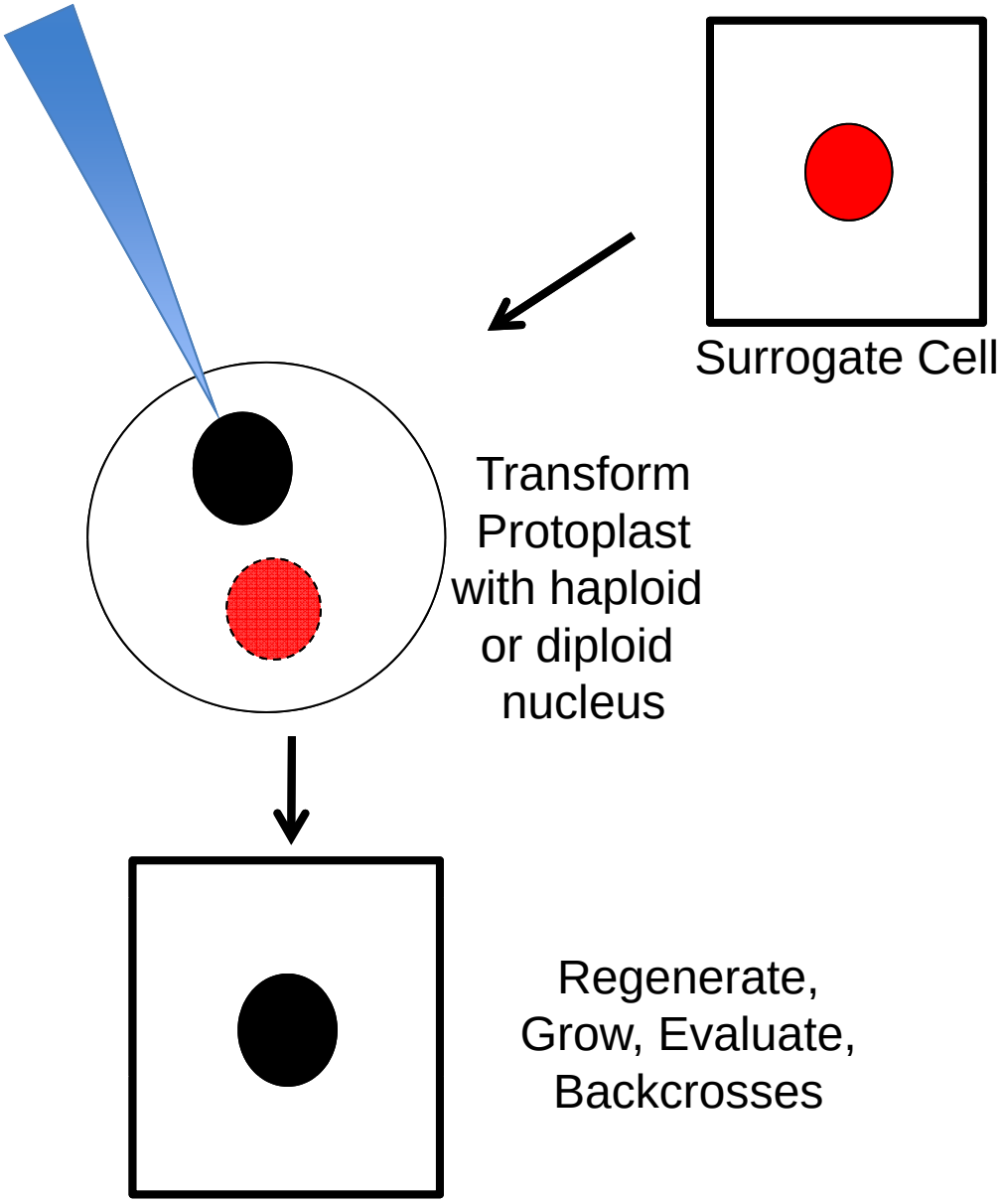
Degraded - Avoid

- Expanded/older leaves (especially with deciduous and annual plants – more DNA in evergreen species)
- Wood, Stems, Roots

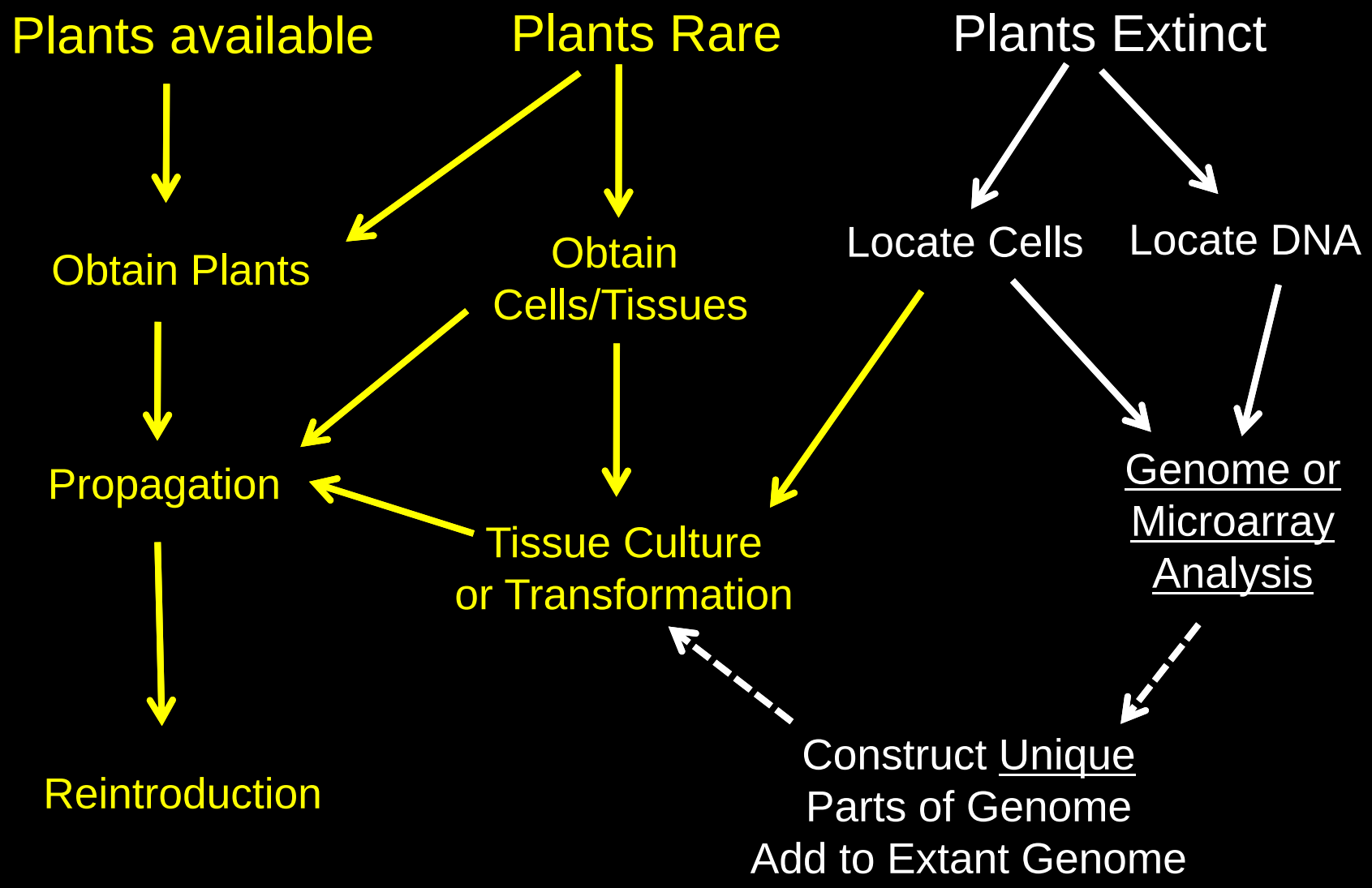
Polyploid - Avoid

- Cotyledons, endosperm, storage tissues

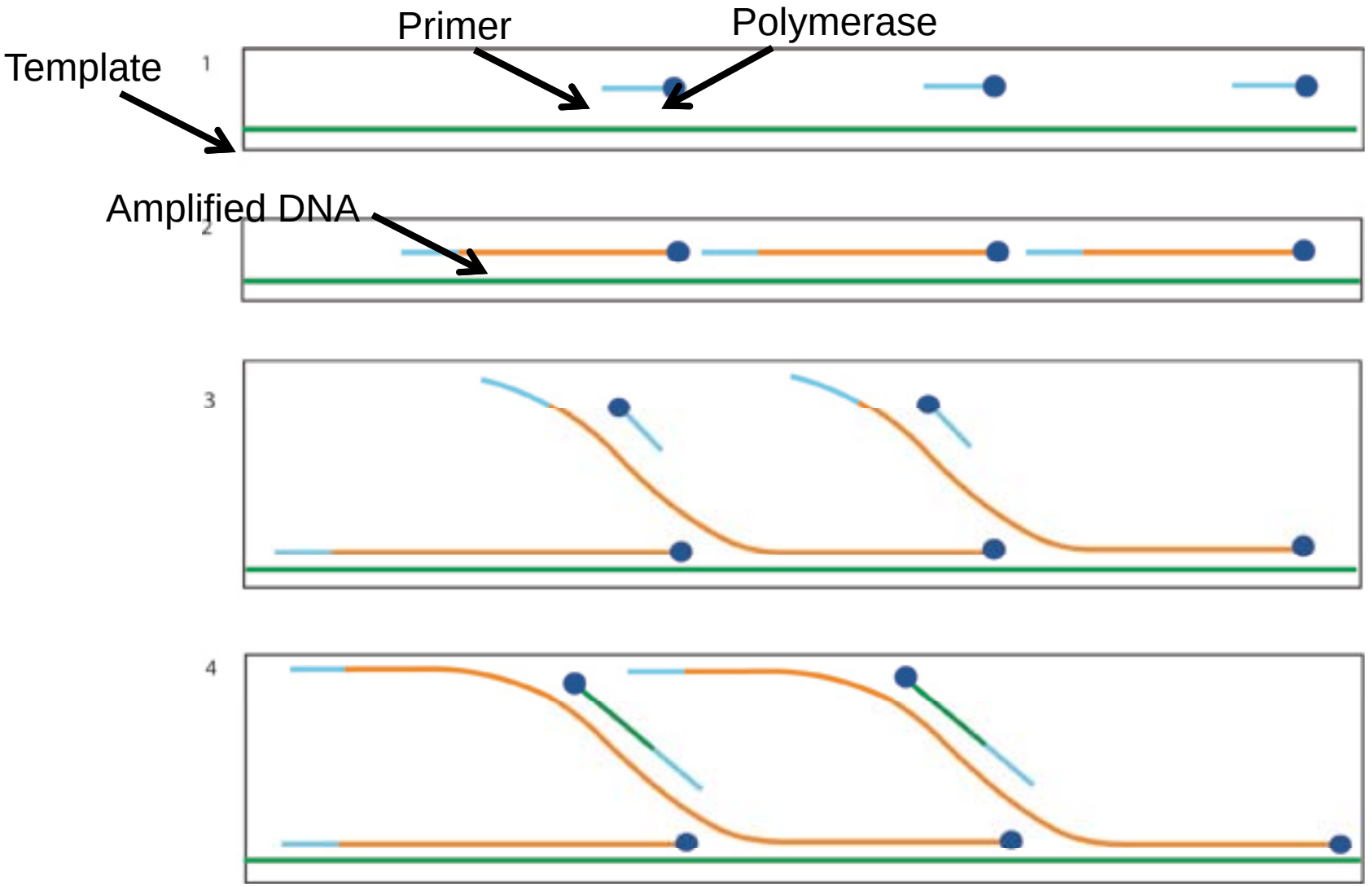
Resurrecting an Extinct Plant When Nuclei Are Available



Resurrecting a Species

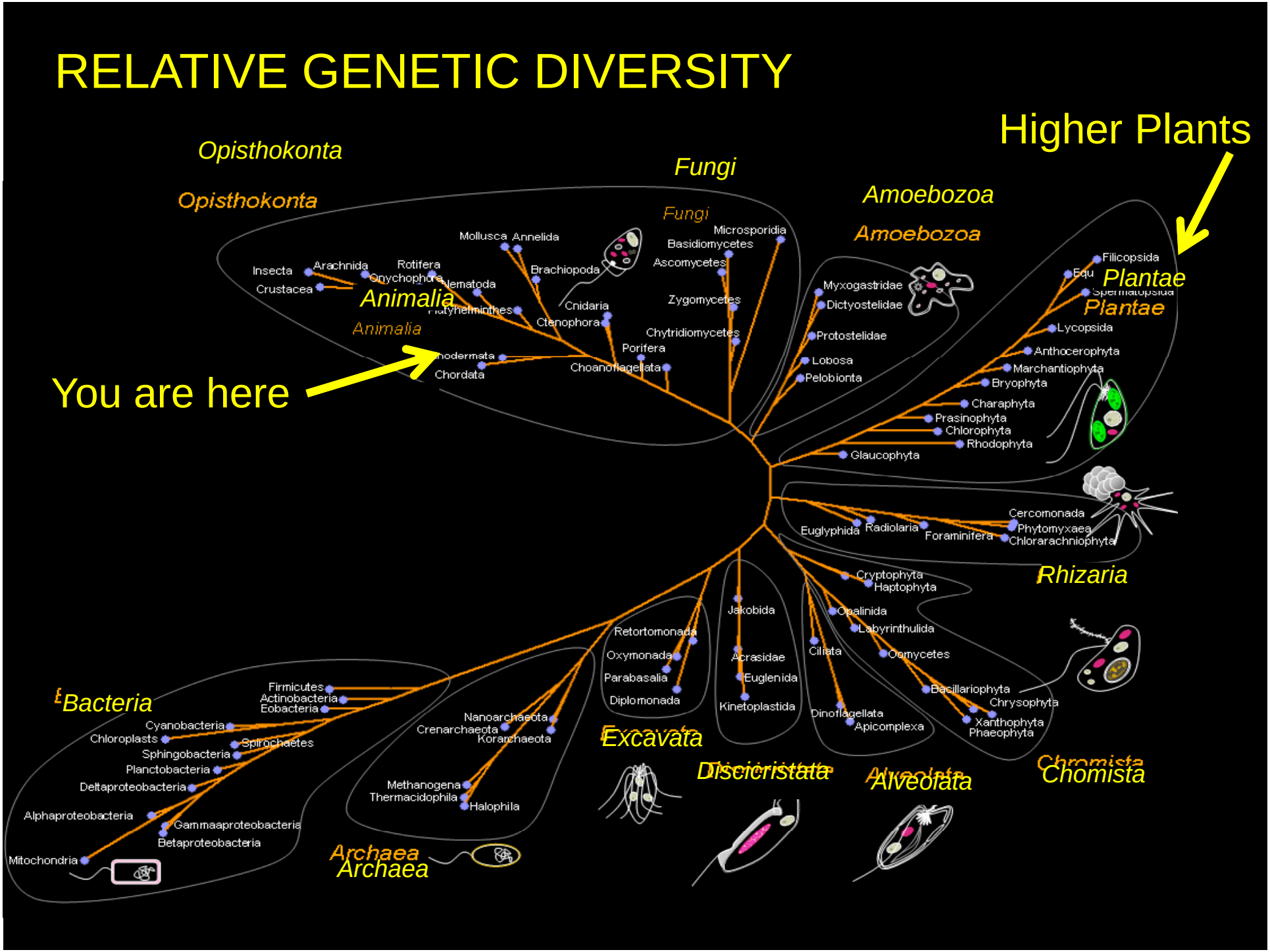


WGA = Whole Genome Amplification (using ϕ29 polymerase) – followed by pyrosequencing. (aka MDA – Multiple Displacement Amplification)

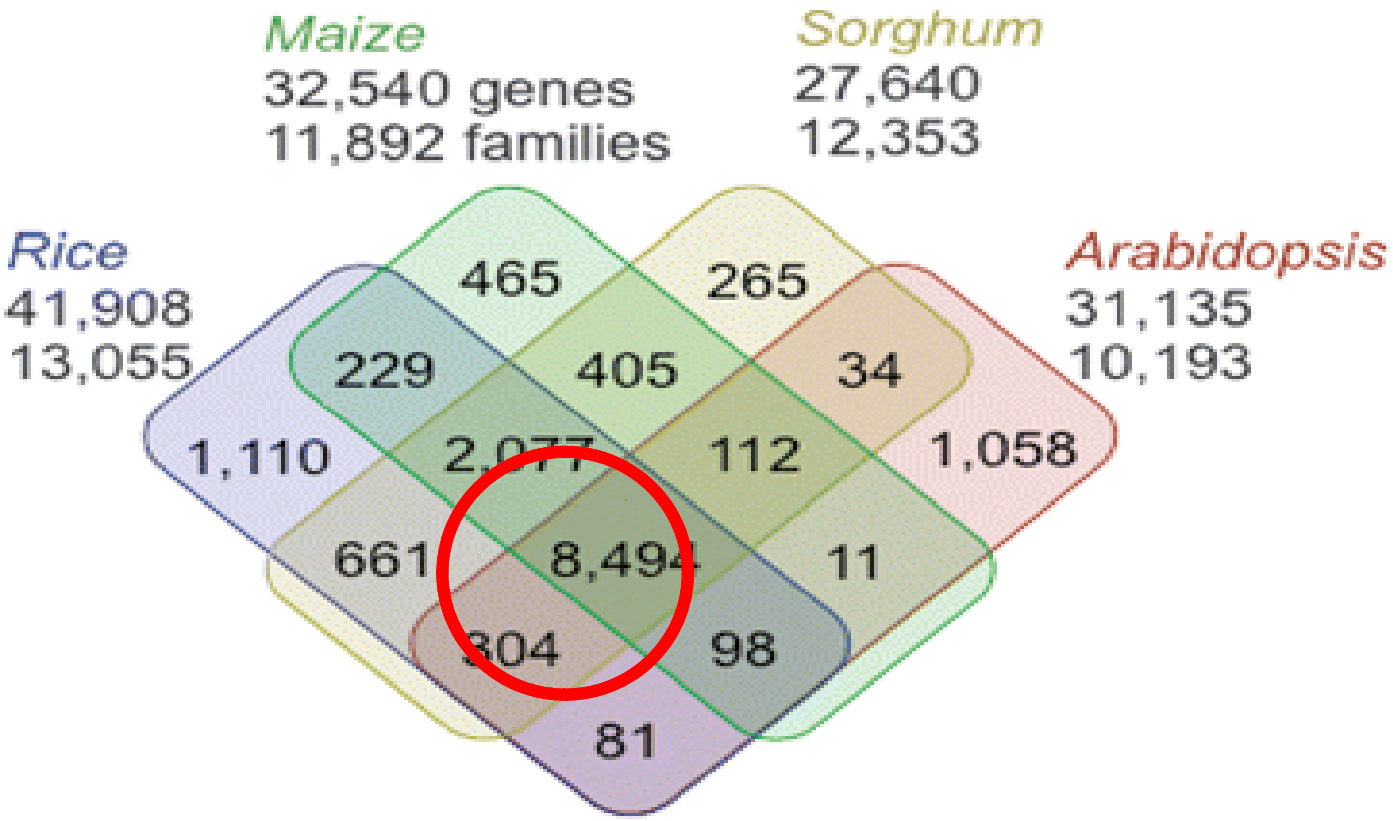


- Sequence genome and identify the unique genes by comparing with a closely related genome.
- Transform close species with unique genes of extinct plant.
- Genomically, plants are homogeneous.
- They differ from each other in small sets of genes.

RELATIVE GENETIC DIVERSITY

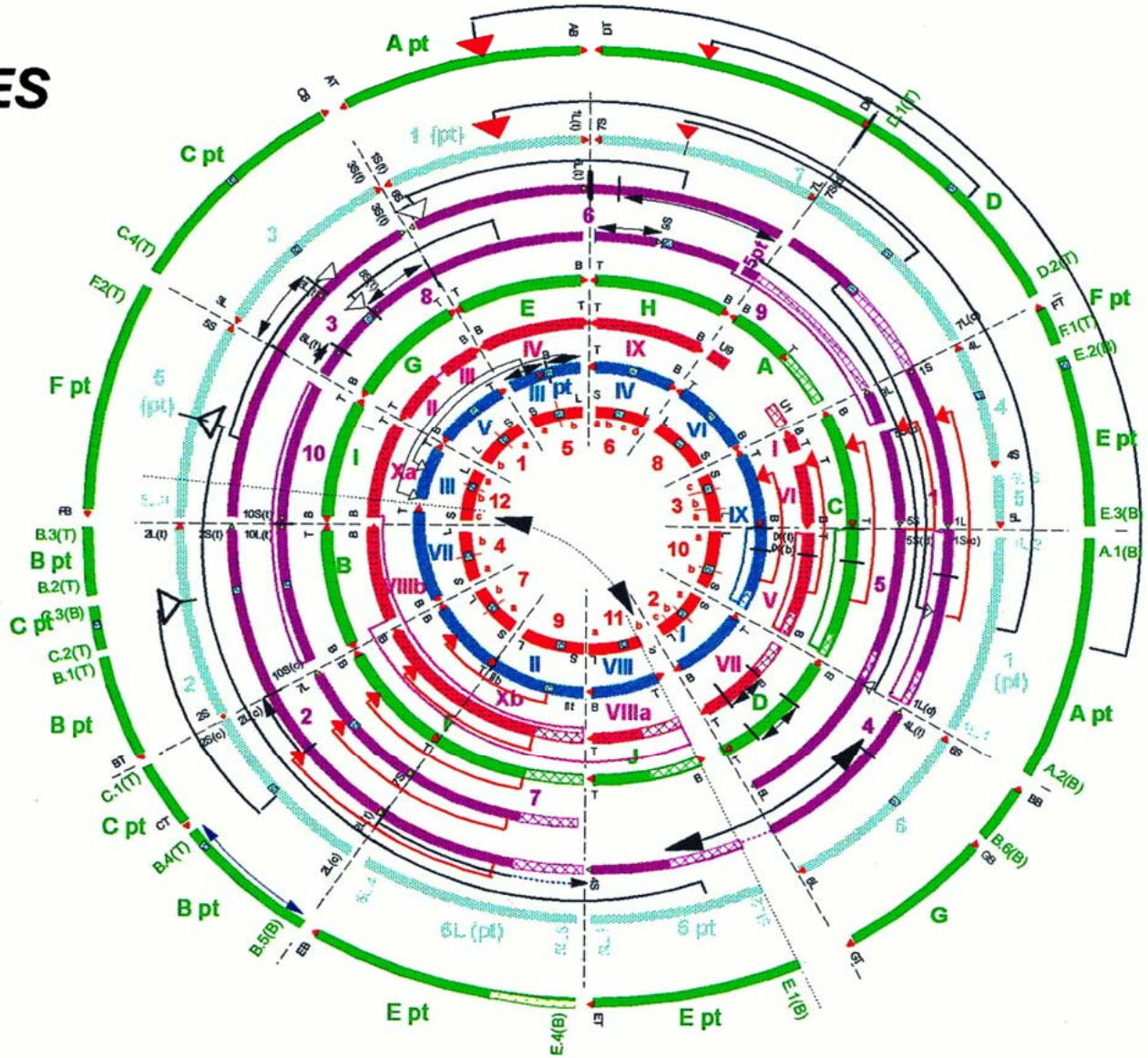


Most plant genes are common to all (or most) plants



65-83% of gene families are in common

GRASS GENOMES



Oats

Triticeae

Maize

Sorghum

Sugar cane

Foxtail millet

Rice

Heterologous cDNA Microarray data

Same species	90-100% hybridization
Same Genus	70-90% (est.)
Same Family	47-70% (est.)
More distant	23-47%

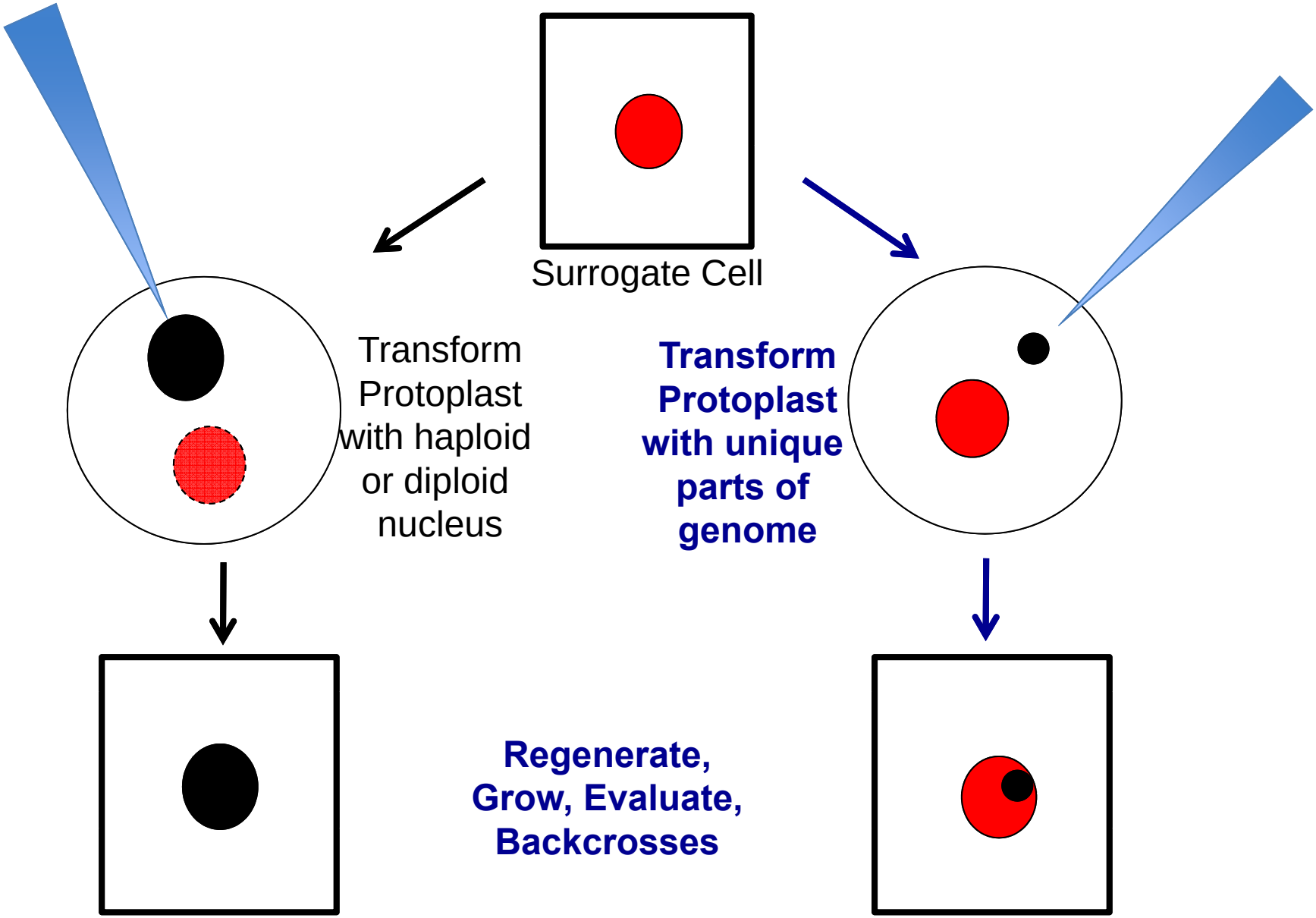
- Majority of genes are the same in plants, especially those that are closely related.

Changes in some lead to phenotypic species diversity.

Use surrogate parent for growing new cells.

Add unique genes from extinct plant.

Resurrecting an Extinct Plant



CONCLUSIONS

- Herbaria fail to protect biomolecules.
- Freezing is the best method to preserve biomolecules.
- Environmental ice acts as a trap for ancient biomolecules and organisms.
- Given preserved cells and/or nucleic acids & advancing technologies, it may be possible to resurrect extinct plant species.

RECOMMENDATIONS

- Herbaria should freeze parts of each sample.
- Freezing at -20°C is as good at -80°C.
- Samples for all threatened and rare plants should be collected and frozen.
- Environmental ice should be used as a a source for extinct plant samples.
- Resurrection of an extinct plant with a small genome and living relatives should be attempted.

Thank You

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BGSU