I) USING MOLECULAR PHYLOGENETICS TO CHARACTERIZE MICROBIAL BIODIVERSITY.

II) WHY INTERESTED?

III) WHY MICROBES DIFFERENT?
A) UNCULTURABLE - MANY
B) PHENOTYPE NOT. U. MEANINGFUL

IV) MOLECULAR PHYLOGENETICS

A) CAN CLONE EVEN IF NOT CULTURE
B) WHAT GENE?
C) WHY rRNA?
- RDP
- 2ARY ALIGN — SIGNATURE SEQS.
- PCR PRIMERS
- RIGHT RATE

D) HOW
- CLONE - PCR
- SEQUENCE
- ALIGN
- PHYLOGENY = PARSIMONY, DISTANCE, ML

1=Tree of Life
2=2ary
3=ALIGN w/ PRIMER
4=PCR ✓
5=

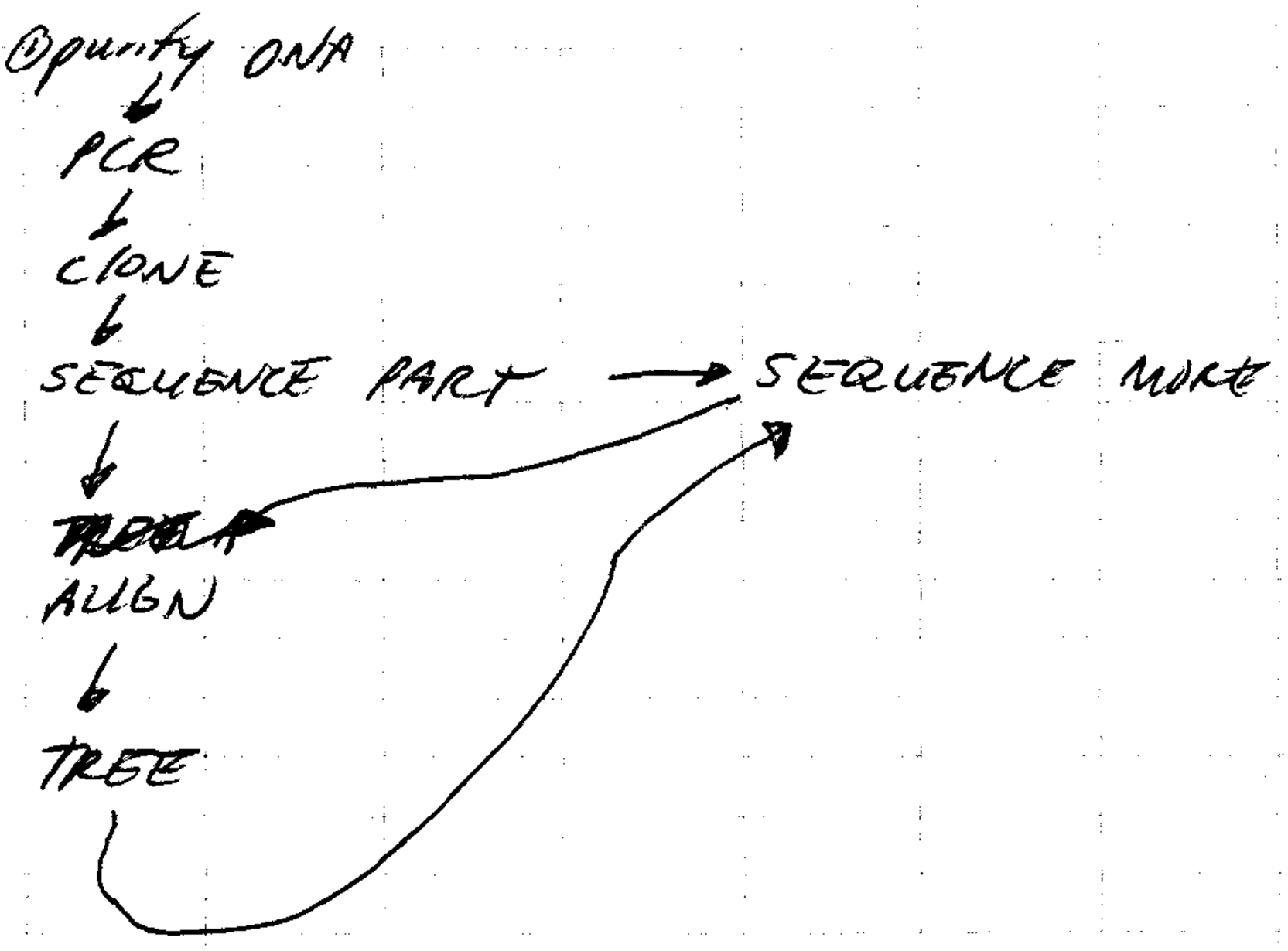
V) PAPERS

324 3343
328-44
324
845

PAPERS

II/ INTRO ETC.

A/ METHODS



I) Why interested in microbial diversity?

II) Why microbial diversity difficult?
A) Uncultureable

B) Phenotype not meaningful

III) So -- molecular phylogenetics

TREE OF LIFE

A) Revolutionized view of microbial evolution

~~Uncultureable~~
① TREE -
② ARCHAEA -
③ RAV - & OTHER UNCULTURABLE

Fig 1, 2
Fig 3 - X
Fig 4

B) So -- how make tree

① rRNA - translation
② conserved 2ary - ALIGN
③ signature sequences - PROBES
④ PCR easy
⑤ RATE GOOD
⑥ RDP*

Fig 5 - X
Fig 6
Fig 7

C) TREE
PARSIMONY, DISTANCE, ML

Olsen - Archaea, Archaea, Everywhere

① easy to overlook microbes

② count molecules ...

- DeLong et al - find lots of Archaea - 30% of SAM biomass
- so much that it's like overlooking 300 elephants in 1 km^2
- in terms of biodiversity like overlooking all eukaryotes

③ what are Archaea

- molecularly determined separate group of proks
- but thought to only be in v. harsh environments
  - acidic hotsprings
  - hydrothermal vents
  - saturated brines

④ previous studies

Fuhrman et al 1992
DeLong 1992
} widely distributed in oceans

⑤ physiology?

- two lineages in ocean ... see figure

ⓐ one branches off methanogen/halophile group

ⓑ one branches with thermophiles

⑥ other

ⓐ lots of microdiversity -

ⓑ lots of macrodiversity -

⑥ DeLong et al

Method

① collect H_2O
② filter different sizes
③ purify DNA
④ PCR
⑤ clone & sequence parts
③ purify rRNA
④ probe w/ Archaeal & Eukaryal probes

phylogeny
sequence now from some

Results

① 18-30% of total picoplankton rRNA was Archaeal
② see Table 1 for other areas

① 14 clones
② 9/14 similar to hyperthermophiles Crenarchaeota
③ 5/14 similar to Euryarchaeotal
④ max. likelihood supports assignment --- see Tree

Discussion

① high abundance in deep, cold H_2O suggests Archaea important part of biosphere
② only few areas looked at
③ implications for Archaeal evolution
④ what about physiology

Barns et al

① Intro
- can't cultivate many microbes
- seq. based phylogeny
- isolate genes from environment
- can use as probes to look at morphotype/abundance

② Method
ⓐ DNA
- collect sediment from Mud Volcano area
  - 3x9 m pool
  - some boiling
  - sediment black
  - high iron
- isolate DNA
- PCR - using euk & arch specific primers
- clone

ⓑ Trees
- align to RDP
- check chimera
  - ⓐ ARB Soft LSADT
  - ⓑ Phylip NJ
  - ⓒ Paup parsimony } Bootstrapped
  - ⓓ fastDNAml ML

③ Results
- ⓐ All in Crenarchaeal clade
- ⓑ trees mostly similar
- ⓒ but pJP 27 & 78 signatures of both suggest NOT either
- ⓓ bootstraps mostly high
- ⓔ transversion only same results so unlikely base composition problem

Barns et al

Discussion

① Great diversity - probably didn't cover all

② Representatives of major clades

③ Some w/ no clade

④ much erratic behavior

Problems, Etc

① phylogenetic placement questionable for some
② are the sequences artifacts?
③ sampling bias (PCR, clone, ...)

Future

① use to characterize morphology
② other probes
③ more areas
④ bacteria?
⑤ eukaryotes?