NGS: a look into the future

COST Conference

BRATISLAVA 2015

Meta^{2}genomics

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Summary

- The need for meta-metagenomics
- The micro-bee
- Accuracy of metagenomics
- When is enough enough?
- Speeding up the process
- Comparing studies

Preamble

- Due to time constrains this is only an overview
- All the major points have been addressed
- Only some illustrative data will be provided
- A full description of all this work is being submitted for publication

The need for meta-metagenomics

Common trends

- There is a need to identify common trends across metagenomic studies
  - Economy
    - Do not repeat studies
  - Practical
    - Full reproducibility is rarely achievable (if ever)

Example: Maize rhizosphere

- We conducted studies at different locations, over different yearly cultivation cycles.
- Each study considered different conditions
  - Different times
  - Different location
  - Different maize cultivars
  - Different treatments
- Goal: identify cumulative effect of herbicides.
  - Each study led naturally to the next analysis

A bit of history

- Started with cultivable bacteria
- Moved to metagenomics using 16S-V6 (short read lengths)
- Test normal maize
- Test cotton
- Test herbicide resistant maize
- Test and compare additional herbicides
- Test herbicide combinations...
- <u>Each step must build on previous experience</u>

Scientific limitations

- One can not justify a new experiment before finishing the previous ones
- But then it must be done next year (with different climate)
- If cumulative effects are expected, then it must also be done on a new, virgin soil
- As years and locations change, so do environmental conditions

The trivial approach

- A possible solution
  - Repeat the experiment (e.g. include previous treatments) in all subsequent instances
  - Replicate the experiment on different soils at the same time
  - Replicate the experiment at different times
- Problems
  - Must use the same technology
  - Must repeat work already done
  - Must waste a lot of money

The not-so-trivial approach

- Try to reuse as much information as possible
  - Some experiments will need to be repeated in all cases (e.g. control)
  - Consider the possible impact of experimental conditions
    - Time
    - Location
    - Methods
    - Treatment
    - Etc...
  - Analyze heterogeneous data

The micro-bee

Bees

"Bienenwabe mit Eiern und Brut 5" by Waugsberg (talk · contribs) - Self-photographed. Licensed under CC BY-SA 3.0 via Wikimedia Commons

- Produce honey
- Pollinate plants
  - 60-80% of the world flowering plants and 35% of crop production depend on animal pollination
- Are terribly sensitive to pollution
  - Air pollution
  - Light pollution
  - Cell-phone radiation
  - Pesticide misuse
  - Global warming

"Bee covered in pollen" by Ragesoss - Own work. Licensed under CC BY-SA 3.0 via Wikimedia Commons

The micro-bee

- Framework:
  - **CBRN P35** EU-Africa cooperation project.
- Goal:
  - find an easy way to identify soil/water contamination
- Question:
  - is there a microbe species (or higher taxa) that can identify contamination?
- Premises:
  - Previous meta-genomic studies show that some phylogenetic groups tend to be consistently affected

The trivial approach

- Conduct experiments on as many locations as possible
- Repeat several years (to correct for climate changes)
- Test as many contaminants as possible
- Impoverish your funding agency

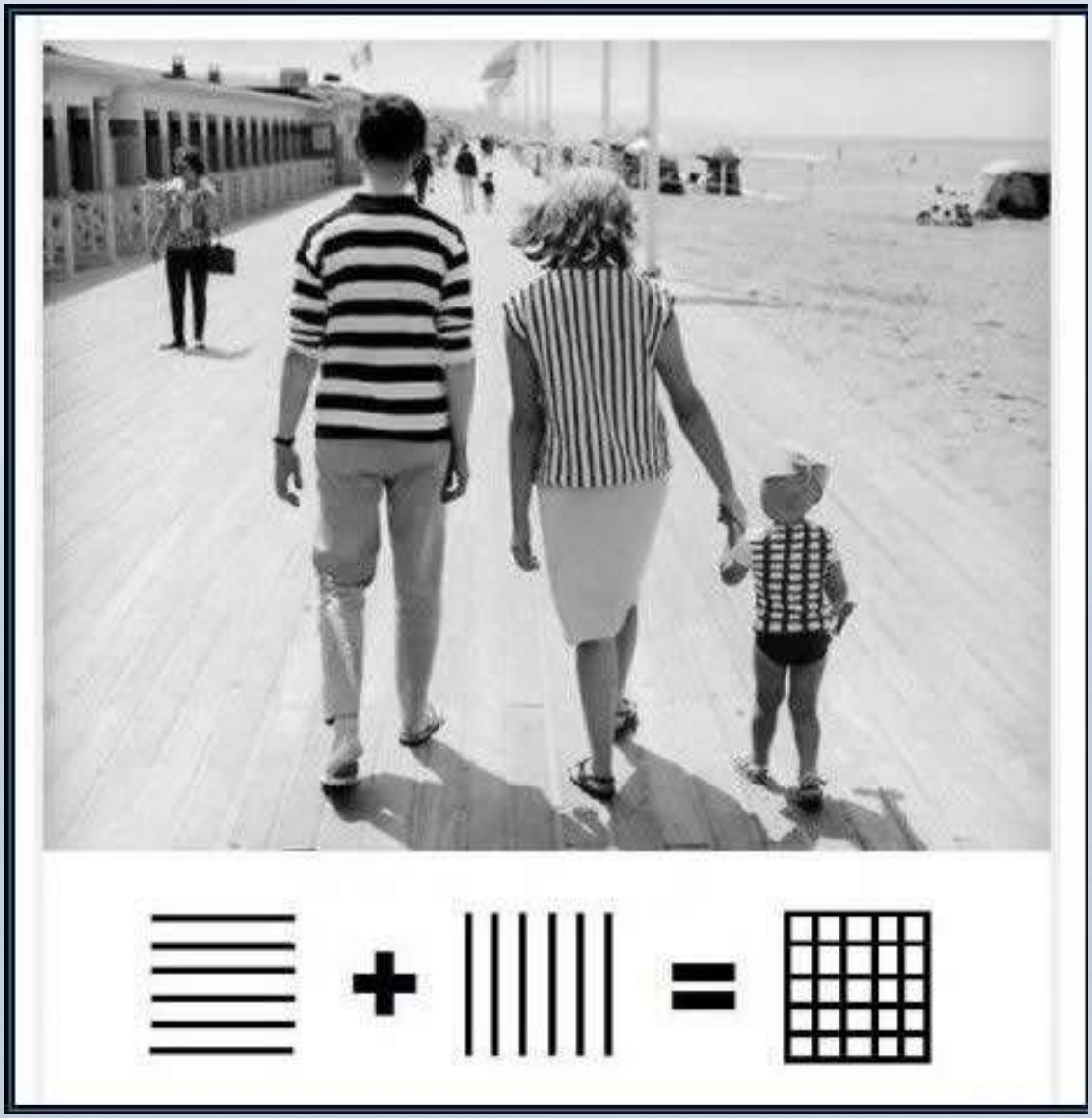
The not-so-trivial approach

- Collect as many previous studies as possible
- Compare them
- Identify a species -or taxonomic group- that is consistently affected by aggressive treatments
- Develop a simple test for changes in the micro-bee population.

Data sources

- Heterogeneous data from different experiments and authors
  - Pesticide treatments
  - Grassland soils
  - Maize cultures
  - Cotton cultures
  - Etc...
- Retrieved from SRA
- Original analyses must be replicated
  - At least to the extent required by our goal

Measuring accuracy



The problem

- Taxonomy assignment is based on similarity
  - Different species differ in ~3%
  - 97% similarity → same species
- Knowledge limits
  - Not all bacterial sequences are known
- Practical limits
  - Some species are known to be indistinguishable by some methods
- how many species can we identify?

Measuring accuracy

- Cluster all sequences known at 97% similarity
  - Clusters gives the maximum number of groups that can be unequivocally identified
  - Singleton clusters give the maximum number of species that can be identified
- Must be checked for each method
  - Reference sequence
  - Clustering/identification method (blast, uclust, RDP, Rtax, etc...)
  - Etc...

Similarity classification

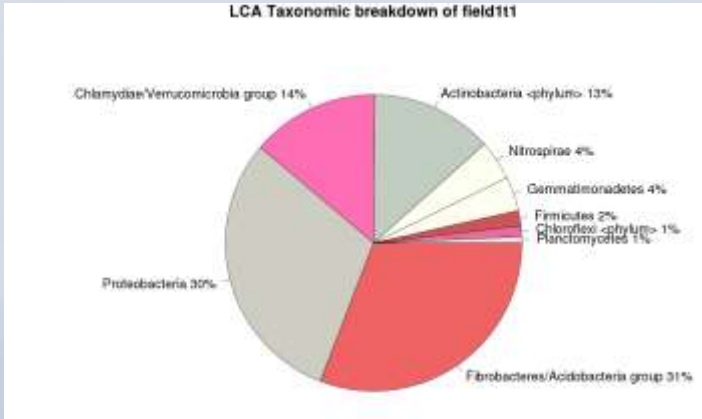
- VAMPS 16S rRNA hyper-variable regions 97% (subset)

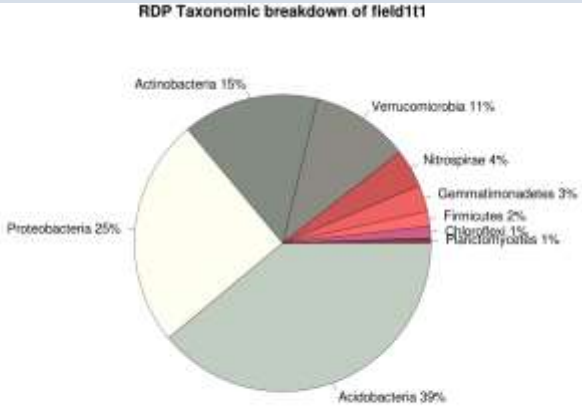
Region	N seqs	Avg. Len.	Clusters
V3	118982	76	34951
V3V5	203487	362	34700
SSU	401607	900	24276

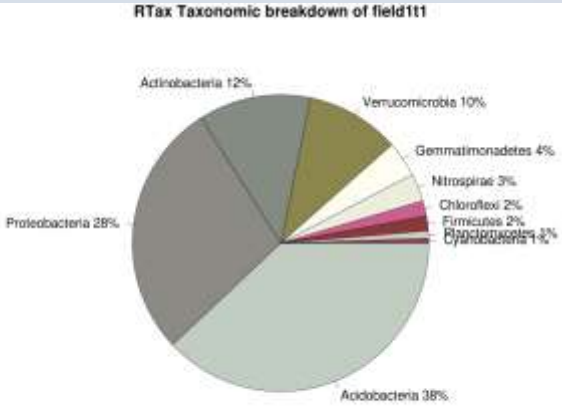
NOTES:
SSU includes non-hyper-variable regions
More sequences or more length do not imply greater power

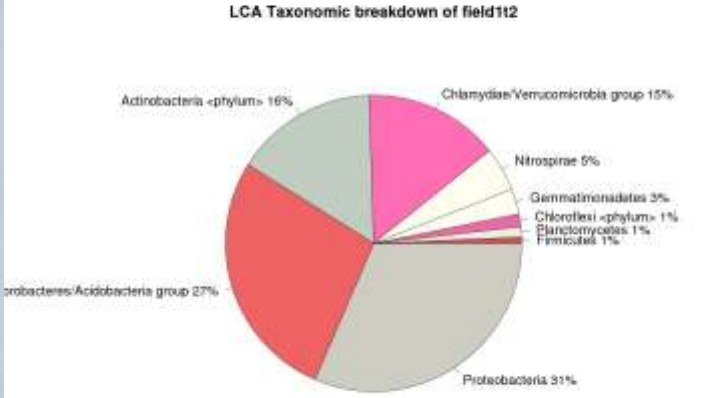
What if I do not use similarity?

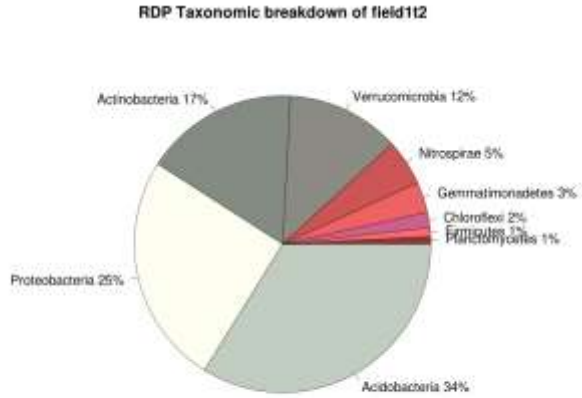
Blast 97% LCA **RDP** **RTax**

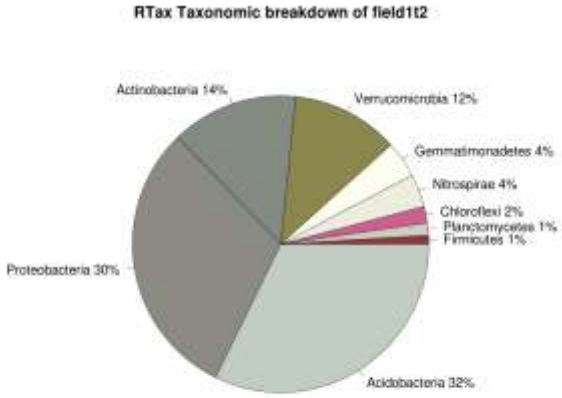




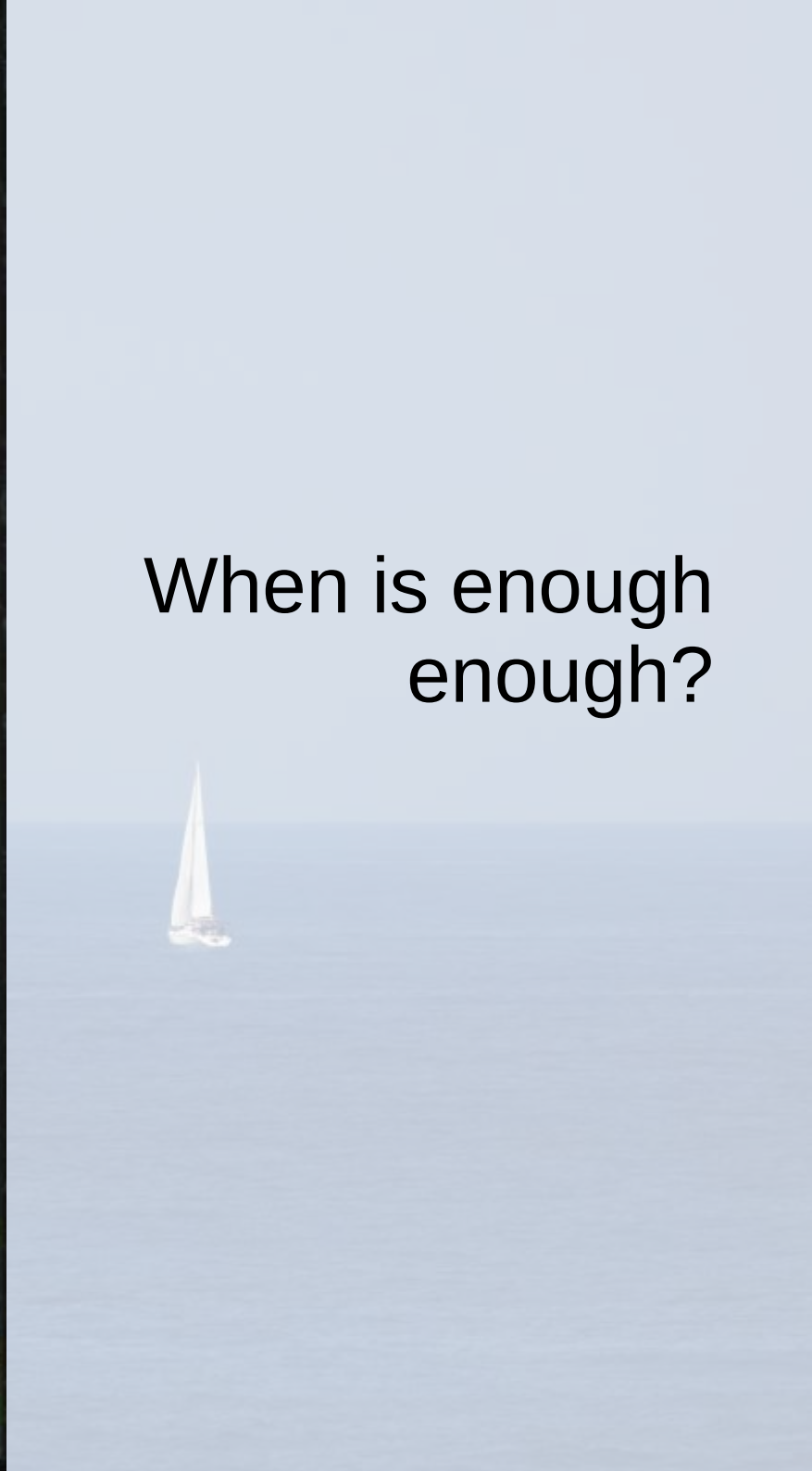






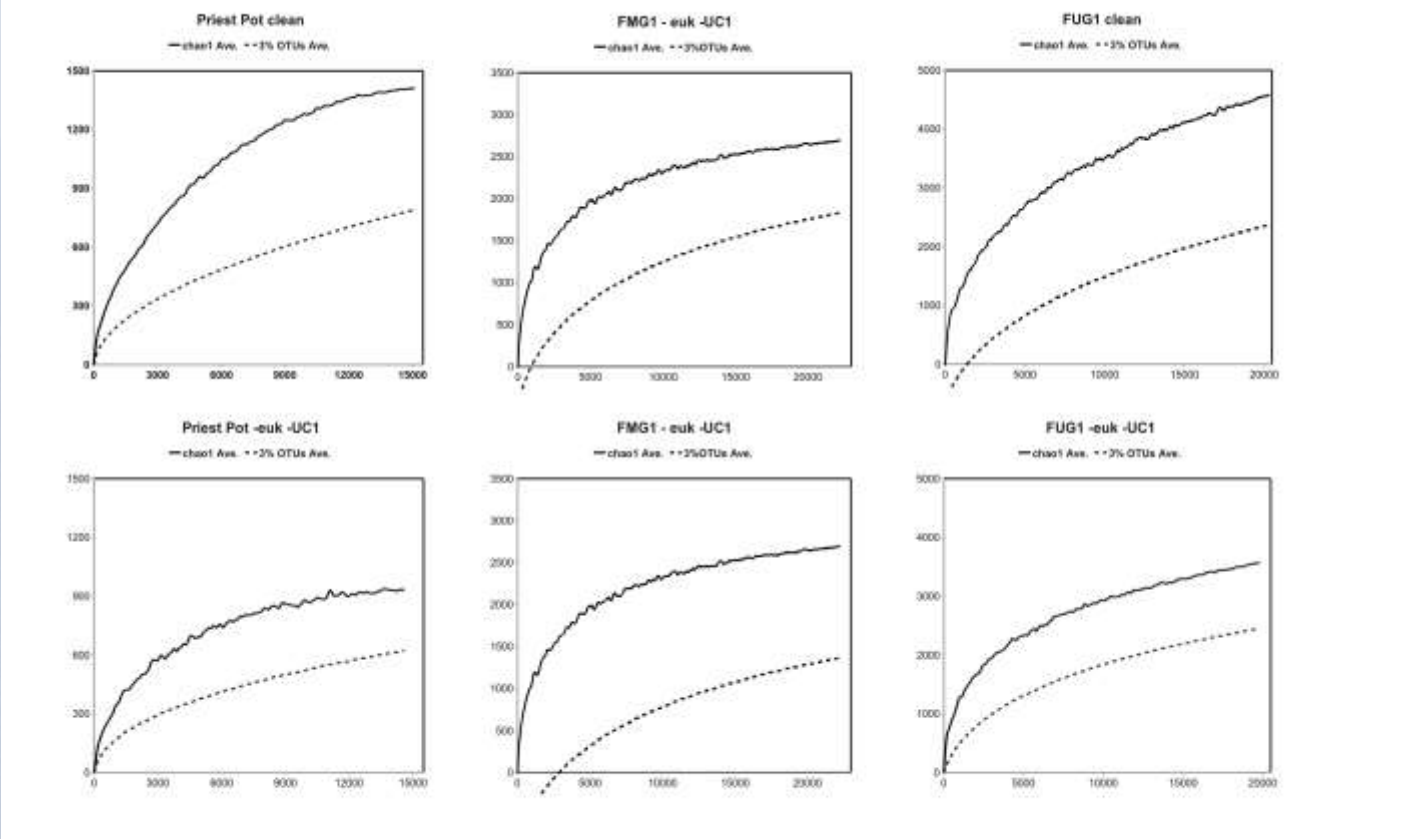


When is enough enough?



Identifying genetic biodiversity

- Saturating OTUS requires ~400.000 reads
- Saturating CHAO1/ACE requires ~40.000
- We need to know the shape of the distribution



Adjusting curves

- Most current methods use a standard curve (e.g. lognormal log mean=1, log sd=1)
- Does this reflect reality?

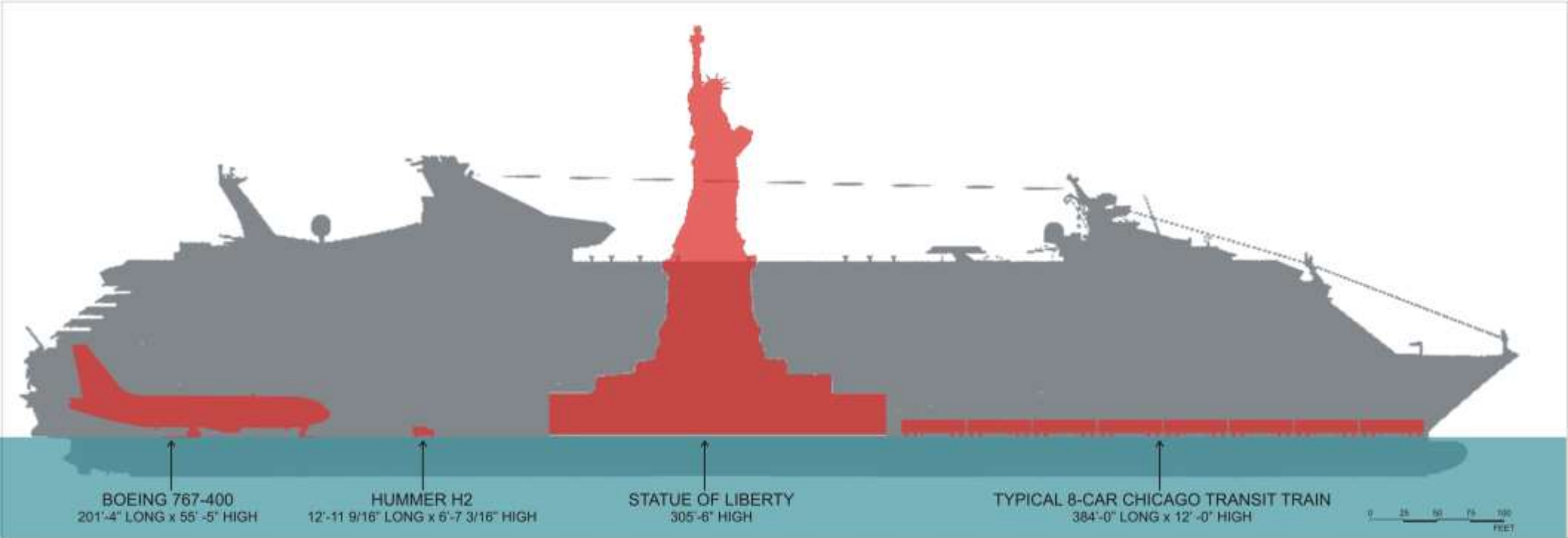
Dataset	Log mean	Log SD
FMG1 (Nacke et al.)	1.08	1.15
UPG1	1.34	0.78
UPG3	0.94	1.18
PriestPot (Quince et al.)	0.93	1.39
r143_s2 (Huse et al.)	1.411	1.94
Zaragoza Avg (Valverde et al)	1.77	1.71
ZC1	1.30	1.31
ZC2	1.85	1.61
ZG1	2.14	1.36
...	...	...

Speeding up

Test and compare alternatives

- Taxonomical classification
  - BLAT / BOWTIE
  - Similarity algorithms
  - RDP
  - Rtax
- Select appropriate sample size
  - Compare with saturated studies
    - Illumina
  - Consider curve fitting: rely on preliminary studies
  - Allow for experimental error

Comparing experiments



The problem

- Taxonomical comparisons are hard
  - Huge amounts of categorical data
  - Many non-shared groups
  - Various hierarchical levels
- We need a systematic approach to compare taxonomic hierarchies
  - How similar are two populations?
  - Are cladistic differences significant?

TaxFrac

- A novel approach to taxonomic comparison using full-knowledge
  - Consider all cladistic levels
  - Define a comparison metric
  - Define a statistical validation method
- Answer the question
  - “how similar are two populations?”

Item-level validation

- Two basic questions:
  - How similar are two populations?
  - Are differences significant?
- Road blocks:
  - How variable are specific sub-populations?
  - Dealing with undetectable sub-populations?
- Approaches
  - Subsampling (good for a single experiment)
  - Compare many studies (required for cross-experimental comparison)
  - Ignore method-specific discrepancies

So, what?

- The more data we collect the better
- Metagenomics is still young
- Probably any conclusion we make now will need to be reviewed in the future
- But we can start to consider it right now.

Thanks

NGS: a look into the future

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