Eliminating the Cost of Sex: Asexual Clonal Lineages Amidst Sexual Eukaryotic Microbes

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Joint work with T Chiang, J Koester, C Berthiaume, N Hiranuma, M Parker, V Iverson, R Morales, A Sarwate & E Armbrust

Quick review: genetics of sex

- Mitosis
  - all chromosomes duplicated
  - one division of a (typically) diploid cell
  - carefully allocate Chrs to the two (diploid) daughter cells
- Meiosis & Recombination
  - all chromosomes duplicated
  - homologous chromosomes pair (pairs of sister chromed pairs) 
  - *recombination* occurs between homologous pairs 
  - *then* cells divide, twice, yielding 4 haploid gametes, each with chromosomes that are a *mix* of maternal/paternal

Hardy-Weinberg Equilibrium

Very simple model of distribution of alleles in a population, assuming:

- sexually reproducing diploid organisms
- non-overlapping generations
- random mating
- infinite population size
- equal allele frequencies in both sexes
- no migration, mutation or selection

Hardy-Weinberg Equilibrium

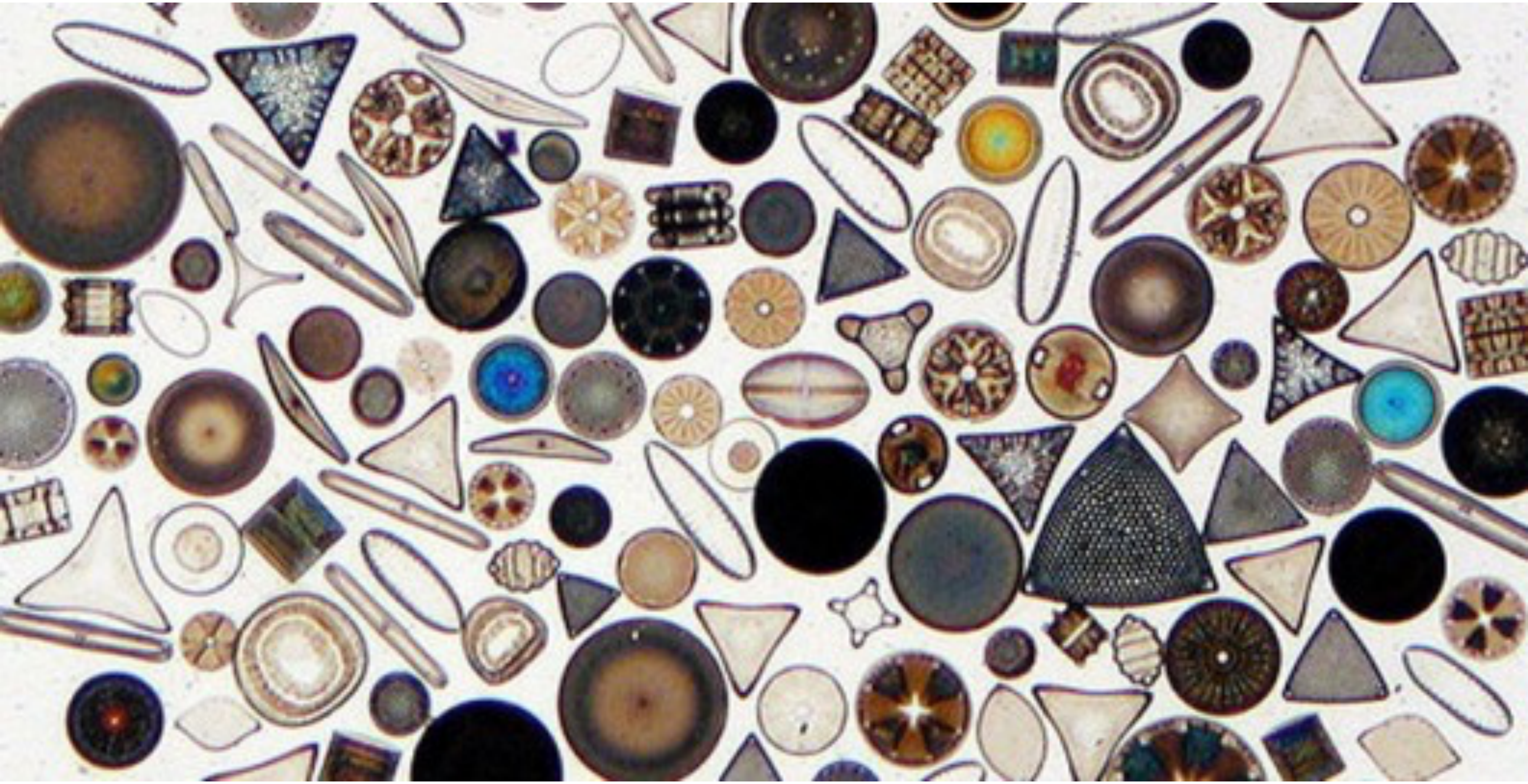
- Suppose 2 alleles, say A & a,exist at one site with population-wide frequencies of p and q=1-p
- What are frequencies of the *diploids AA, Aa, aa?*
  - *AA:* p^2
  - *Aa:* $2pq$
  - *aa:* q^2
  - *NB:* $p^2 + 2pq + q^2 = (p+q)^2 = 1$
- *And* assume no linkage, so adjacent site B & b, etc. will *independently* appear with analogous probabilities $\bar{p}^2, 2\bar{p}\bar{q}, \bar{q}^2$

Cost/Benefit of Sex

Sex is ancient, ubiquitous

+ Allows deleterious alleles to be shed (or not)
+ Accelerates mixing of alleles (for better or worse)
- BUT: Meiosis is complex, slow & expensive, Finding mates is hard, Only 1/2 of genes are passed on, …
- Ancient eukaryotic asexuals are rare, but theoretical support for benefit of sex is still debated

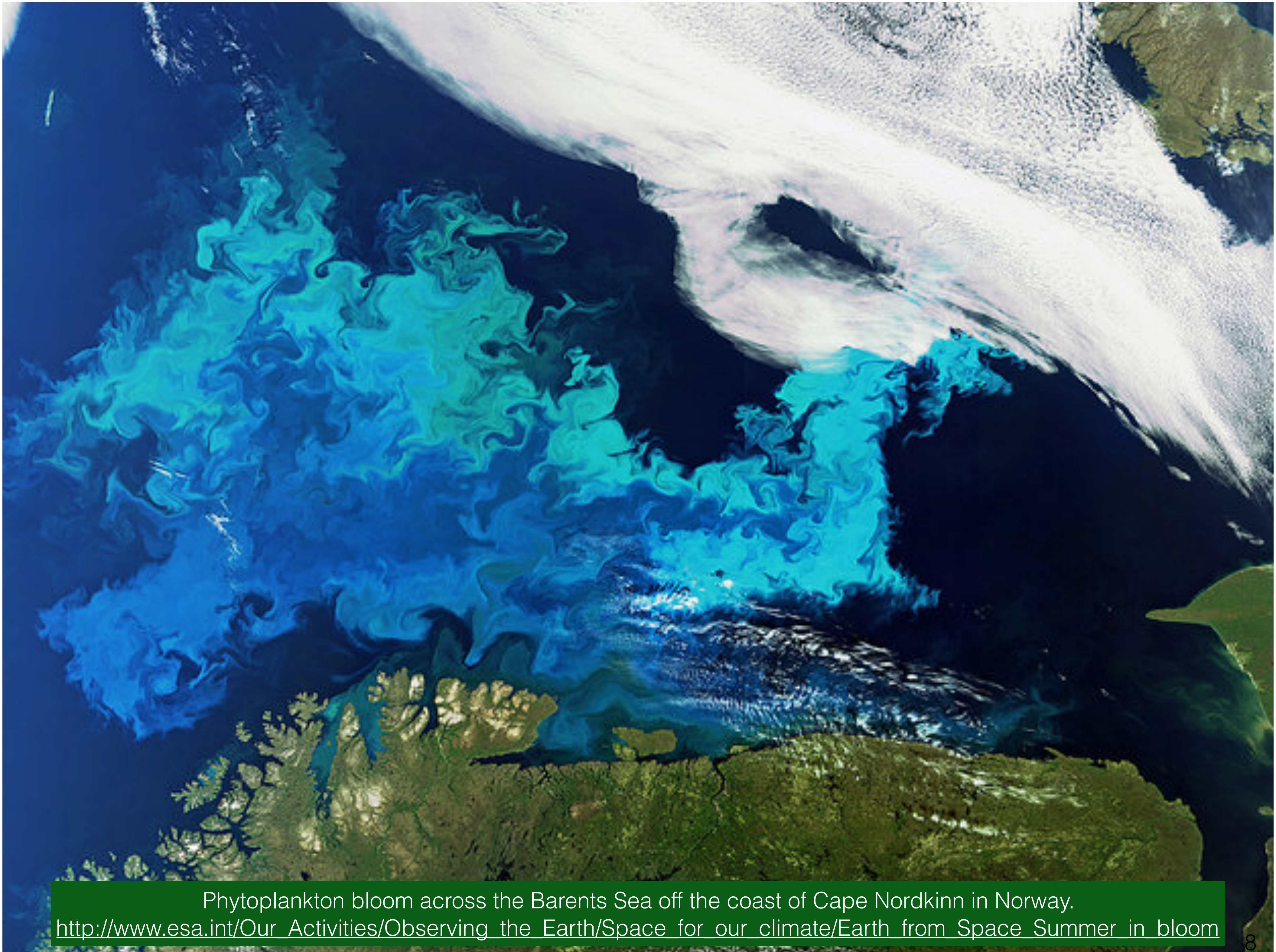
Diatoms



http://sarahjanemaki.com/diatoms-3/

Diatoms

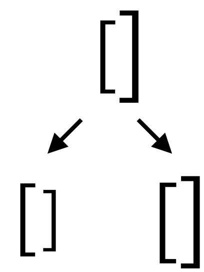
- First formally described in scientific literature by Danish naturalist Otto Friedrich Müller, 1783.
- Photosynthetic, unicellular, mostly aquatic, eukaryotes
- Plausibly about the same age as land plants, but at least an order of magnitude more species-rich
- Also noted for high in-species genetic diversity
- Estimated to contribute 20–40% of primary production
- Silica cell wall; they dominate oceanic SiO_2 cycling



Phytoplankton bloom across the Barents Sea off the coast of Cape Nordkinn in Norway.
http://www.esa.int/Our_Activities/Observing_the_Earth/Space_for_our_climate/Earth_from_Space_Summer_in_bloom

Sex and the Diatom

- Eukaryotes, normally diploid
- Most cell divisions are *mitotic*
  - 2 diploid daughter cells, each gets one valve (1/2 of cell wall)
  - New valve *inside* old ⇒ one daughter is smaller



- Occasionally undergo *meiosis*
  - Haploid gametes, die if they don't fuse with a partner (unlike yeast, e.g.)
- Triggers for sex are largely unknown; one is thought to be cell-size reduction – Auxospore (fertilized egg) outgrows its valve & makes new, larger ones

Thalassiosira pseudonana

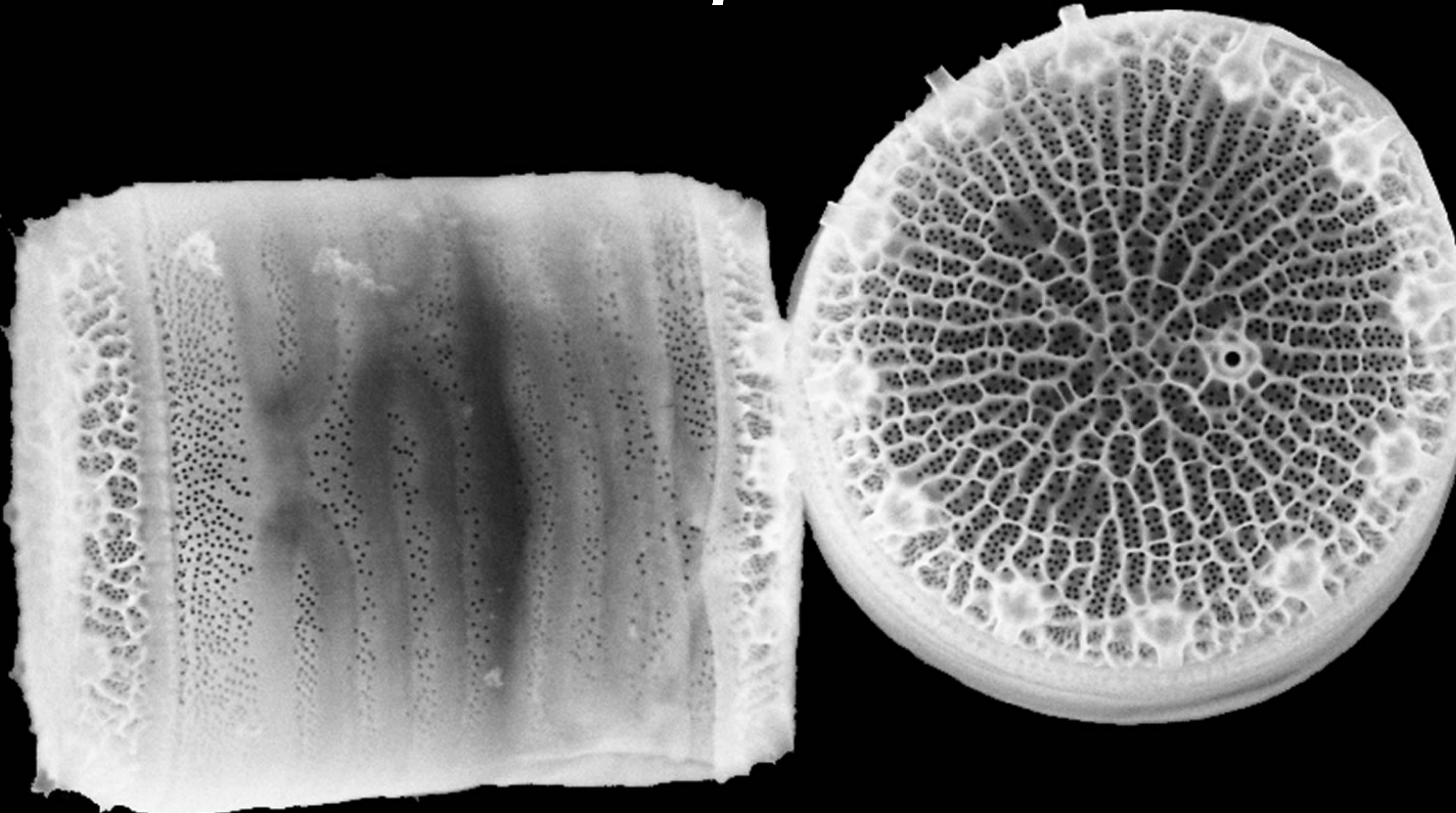
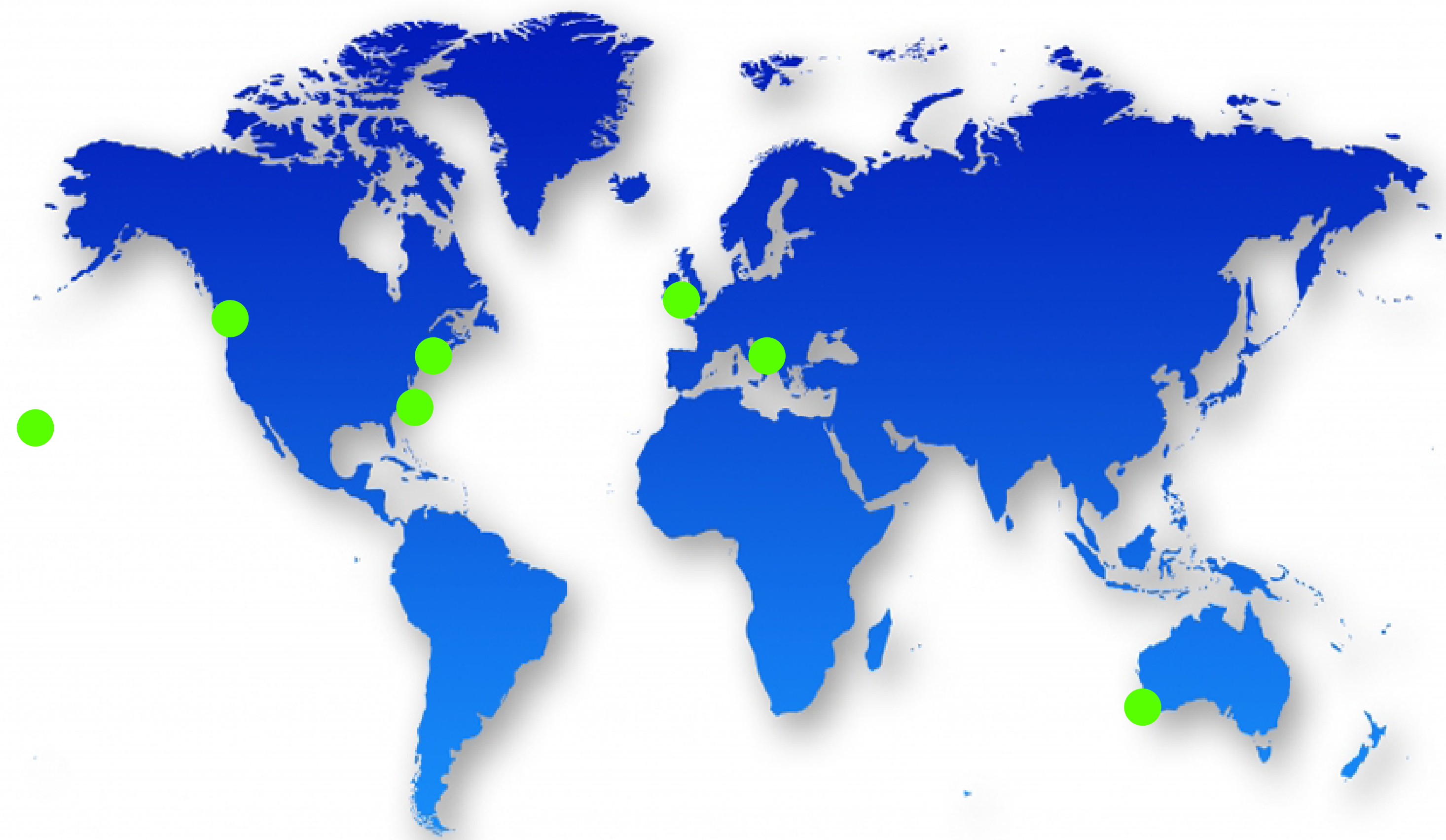


Photo: N. Kröger

Thalassiosira pseudonana

- A marine diatom
- Named long ago
- In continuous culture since 1958
- “Cosmopolitan” = found all over the world
- First diatom genome sequence (Sanger-based, 2004)
    - Diploid, ~ 32 Megabases, “SNP” every 100-200bp

Re-Sequenced 7 Isolates



Biogeography – correlate diversity with geography

Re-Sequenced 7 Isolates

- Goal:
  - Biogeography – correlate diversity with geography
- Findings:
  - There is (almost) no geographical diversity!
  - 5 of 7 are nearly identical, genetically
  - Down to the level of sharing heterozygous positions
  - Why? They are obligate asexual clones!
  - And they rapidly colonized the world's oceans
  - The other 2: we see nothing that contradicts sex and Hardy-Weinberg (tho sex has never been observed)

Non-reference Read to Coverage ≈ Alternate Alleles

R = nonreference / coverage

Aligned Reads

C T G C T G G T A A T A G G A A G G C T A T
C T G C T G G T A A T A G G A A G G C T A T
C T G C T G G T A A T A G G A A G G C T A T
C T G C T G G T A A T A G G A A G G C T A T
C T G C T G G T A A T A G G A A G G C T A T
G T G C T G G T A A T A G G A A G G C T A T
G T G C T G G T A A T A G G A A G G C T A T
G T G C T G G T A A T A G G A A G G C T A T
G T G C T G G T A A T A G G A A G G C T A T
G T G C T G G T A A T A G G A A G G C T A T

Reference

C T G C T G G T A A T A G G A A G G C T A G

R = 1/2 0 1

Non-reference Read to Coverage is Imperfect Proxy

R = nonreference / coverage

Aligned Reads

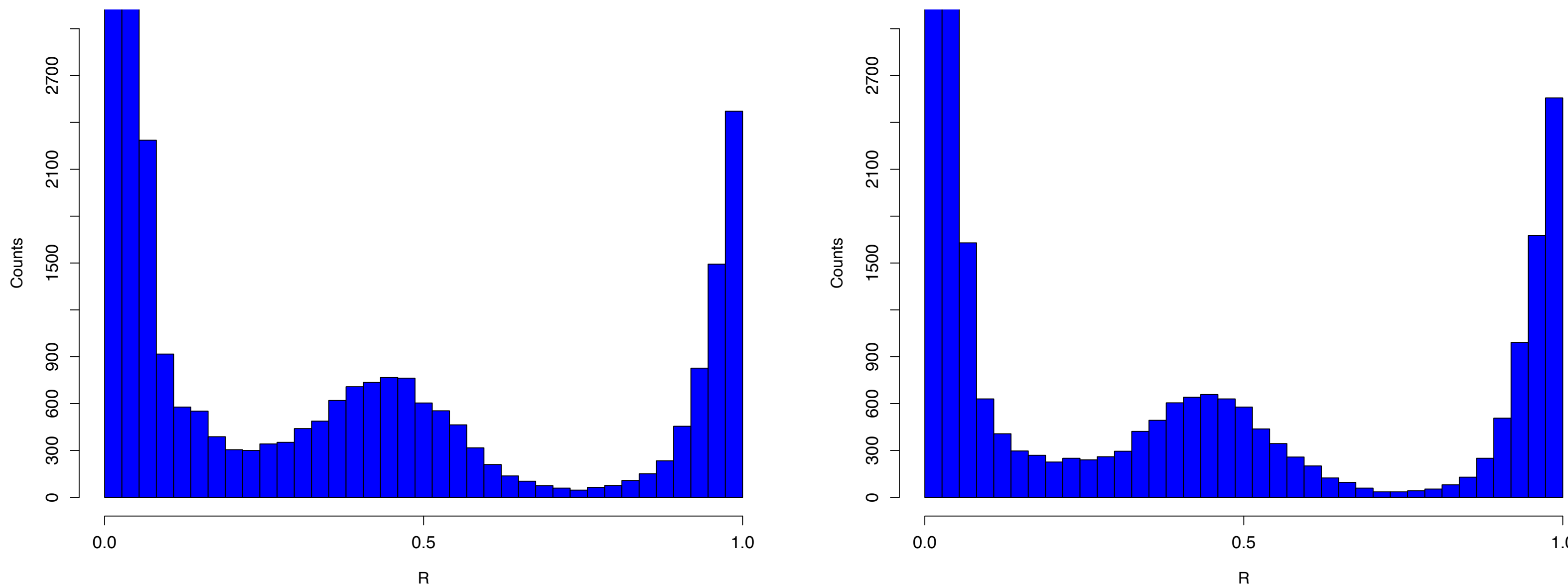
```
CTGCTGGTAATAGGAAGGCTAT
CTGCTGGTAATAGGAAGGCTAT
CTGCTGGTAATAGGAAGGCTAT
CTGCTGGTAATAGGAAGGCTAT
CTGCTGGTAATAGGAAGGCTAT
CTGCTGGTTATAGGAAGGCTAT
CTGCTGGTAATAGGAAGGCTAT
GTGCTGGTAATAGGAAGGCTAT
GTGCTGGTAATAGGAAGGCTAT
GTGCTGGTAATAGGAAGGCTAC
```

Reference

```
CTGCTGGTAATAGGAAGGCTAG
```

R = 3/10 ~0 9/10

Allelic-Distribution of Two Isolates



Wales, UK - CCMP1013

Venice, Italy - CCMP3367

Blue: Histogram of R-value distribution (Chr 1)

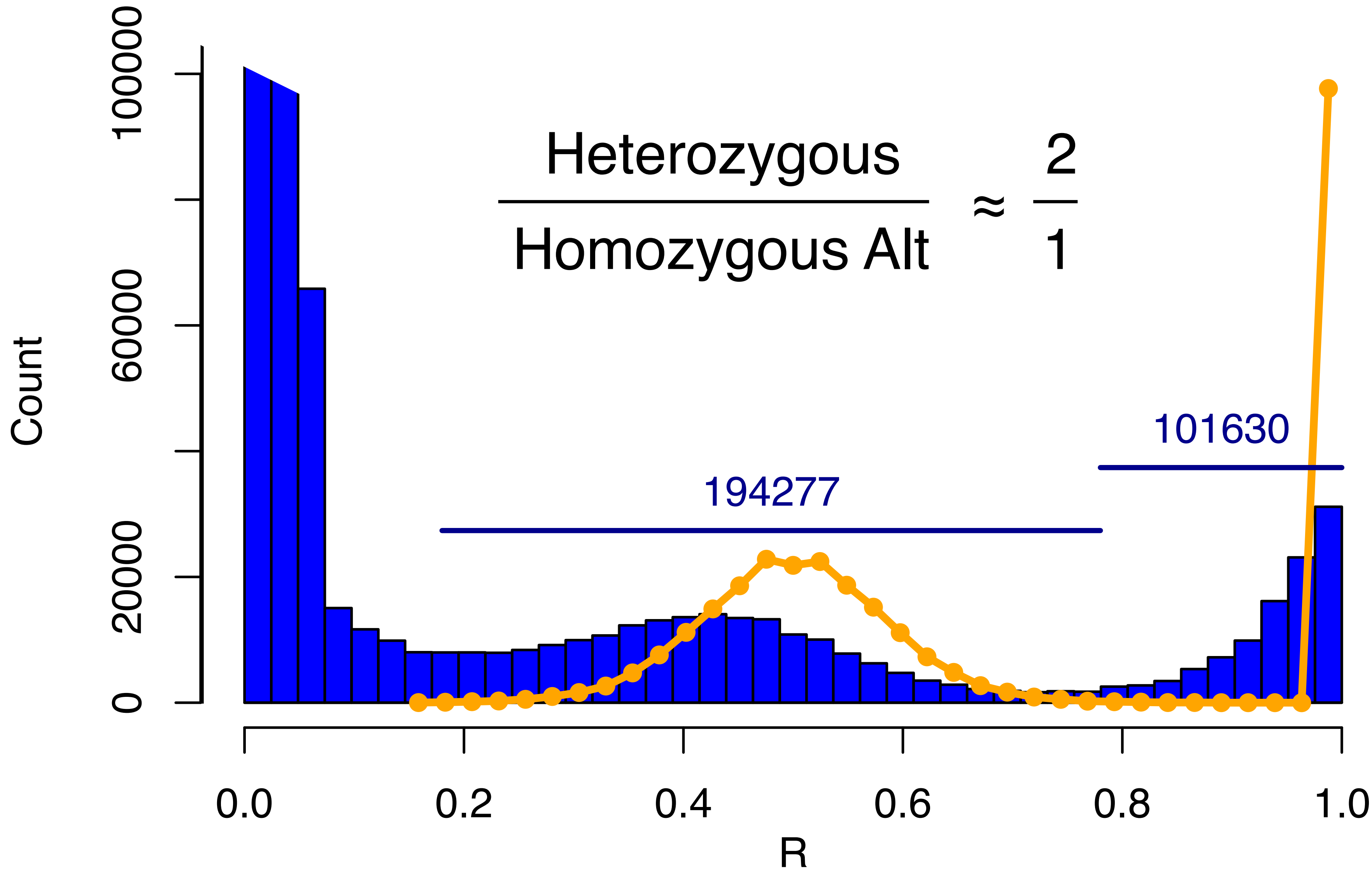
$R \approx 0$: homozygous, reference

$R \approx 1$: homozygous, non-reference

Why ≠ 0/1?
Seq & map errs

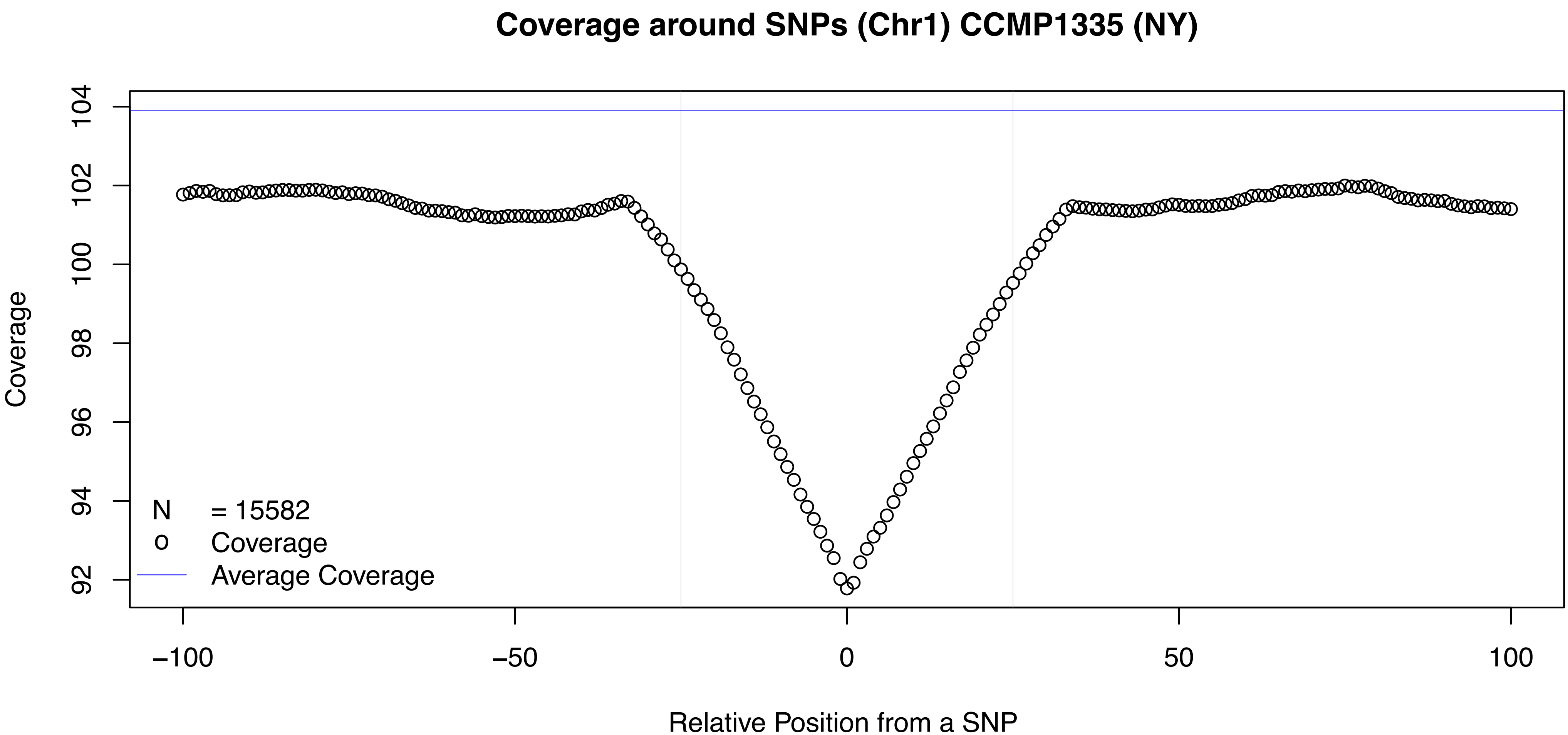
$R \approx 0.5$: heterozygous (ref + non-ref) Why ≠ .5? Sampling

Matches H-W Expectation

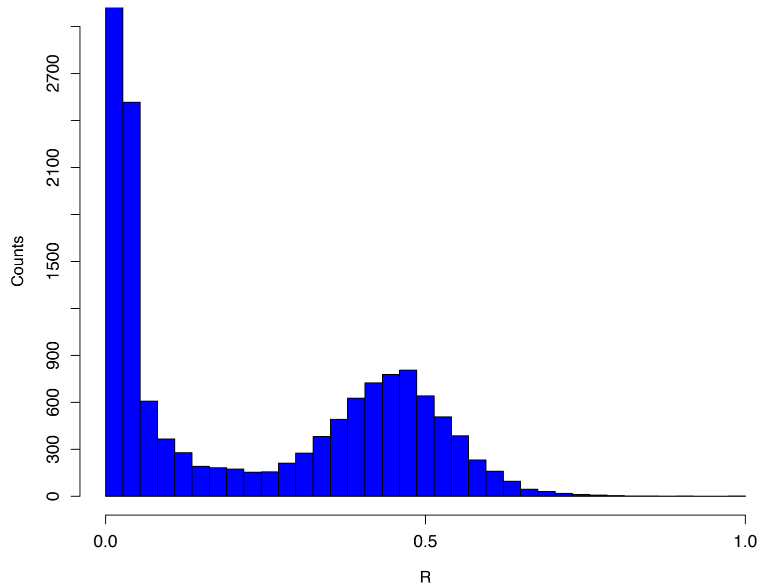


blue = Wales; orange = simple theoretical H-W model

A Digression: Mapping Bias



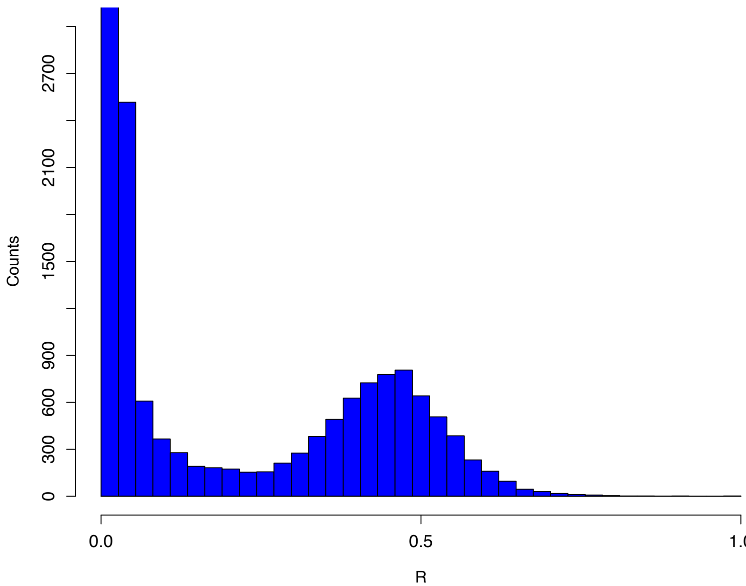
New York does not match HWE



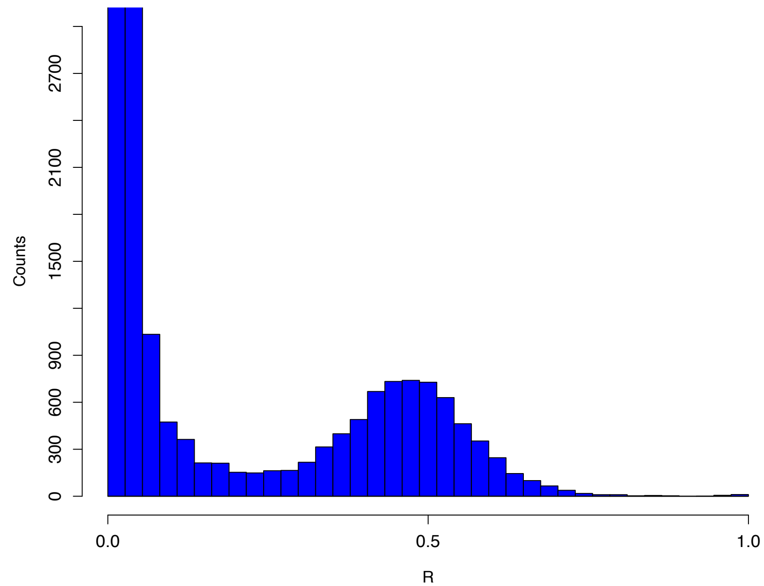
New York, USA - CCMP1335

- Missing homozygous non-reference peak at $R = 1$
- NY is the reference strain

New York does not match HWE...nor does Washington



New York, USA - CCMP1335

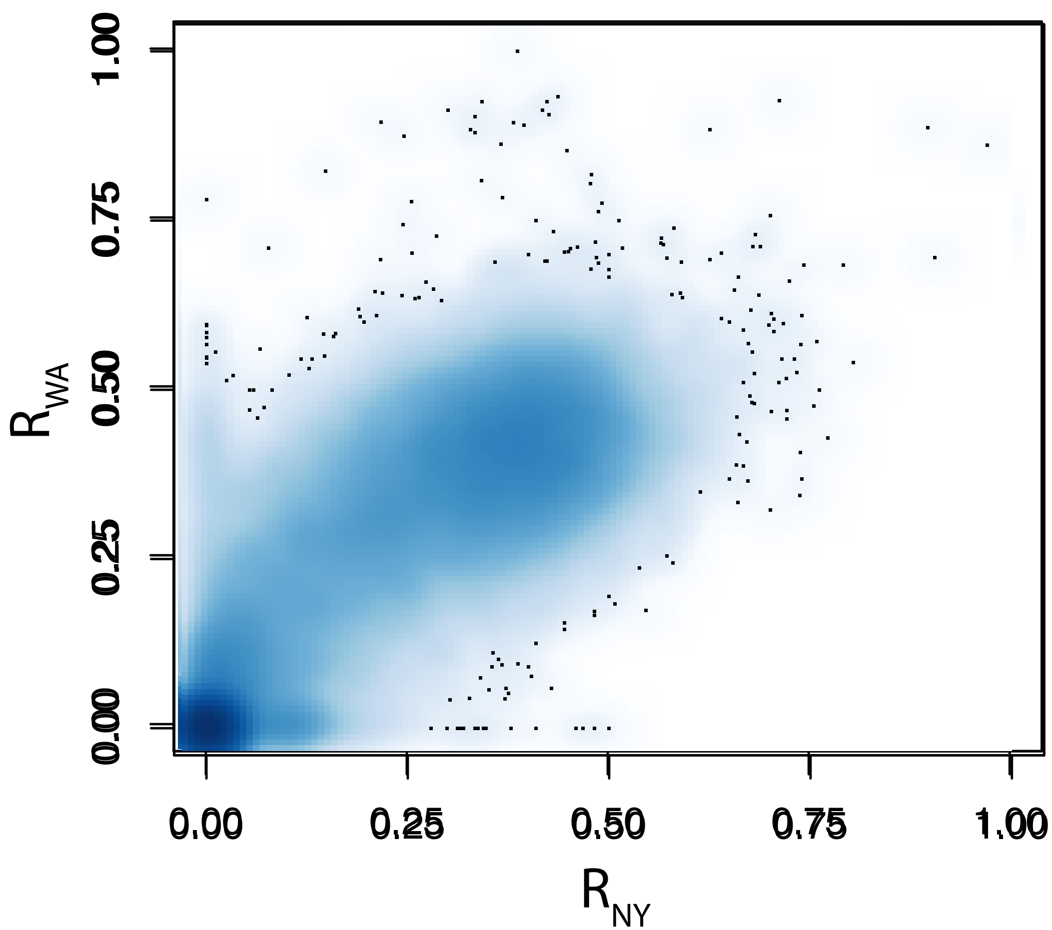


Washington, USA - CCMP1015

- Missing homozygous non-reference peak at $R = 1$

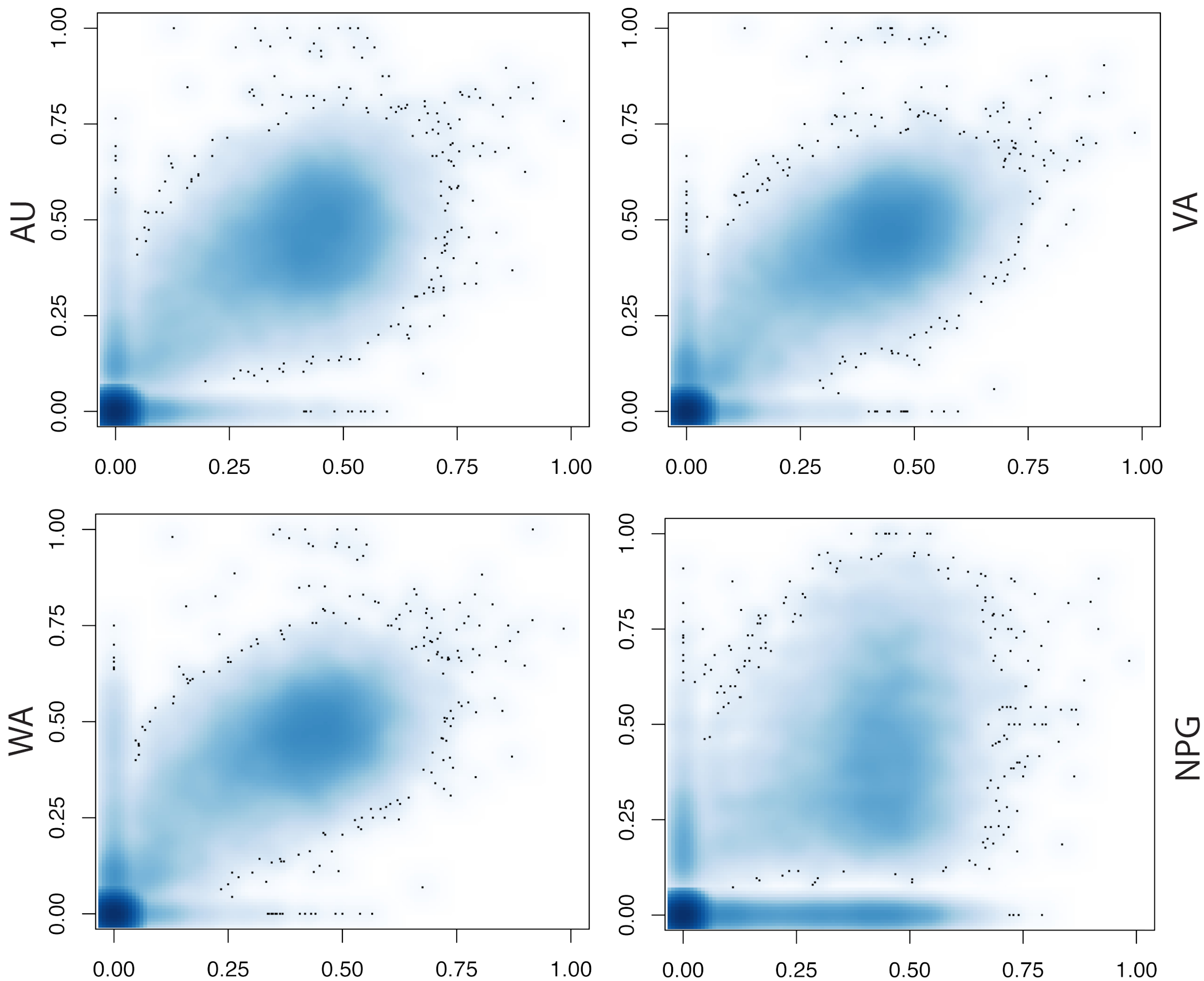
- NY is the reference strain
- WA is not!

Not only the same distribution but the same heterozygous positions



- 10K randomly selected genomic positions
- Plotted the R-value of NY against WA
- Concentration of sites are in two locations: (0,0) & $(\frac{1}{2}, \frac{1}{2})$
- There are no points at (0,1) & (1,0)

Strong agreement of heterozygous positions



Heterozygous concordance with the reference is at least 96%.

Heterozygous concordance implies extreme departure from HWE

We have detected:

1. Clonal cultures from 5 dispersed regions
2. $\geq 96\%$ concordance in SNPs

What is the probability that this population is in Hardy-Weinberg Equilibrium along with the above constraints?

Simple Binomial Model

$$\dots$$

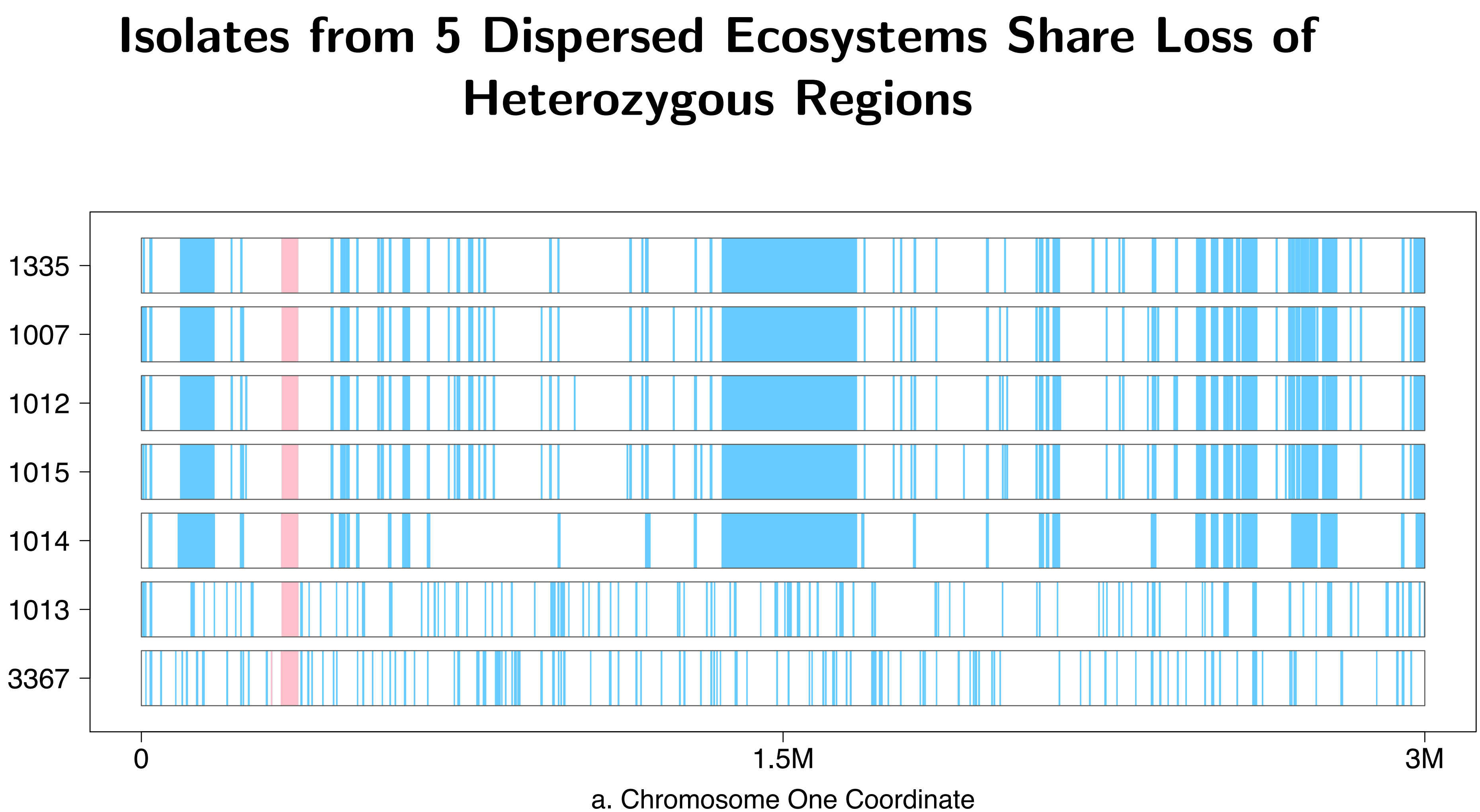
Using 1 heterozygous position per chromosome to avoid linkage.

$$p \leq 1.2 \times 10^{-29}$$

Data Summary

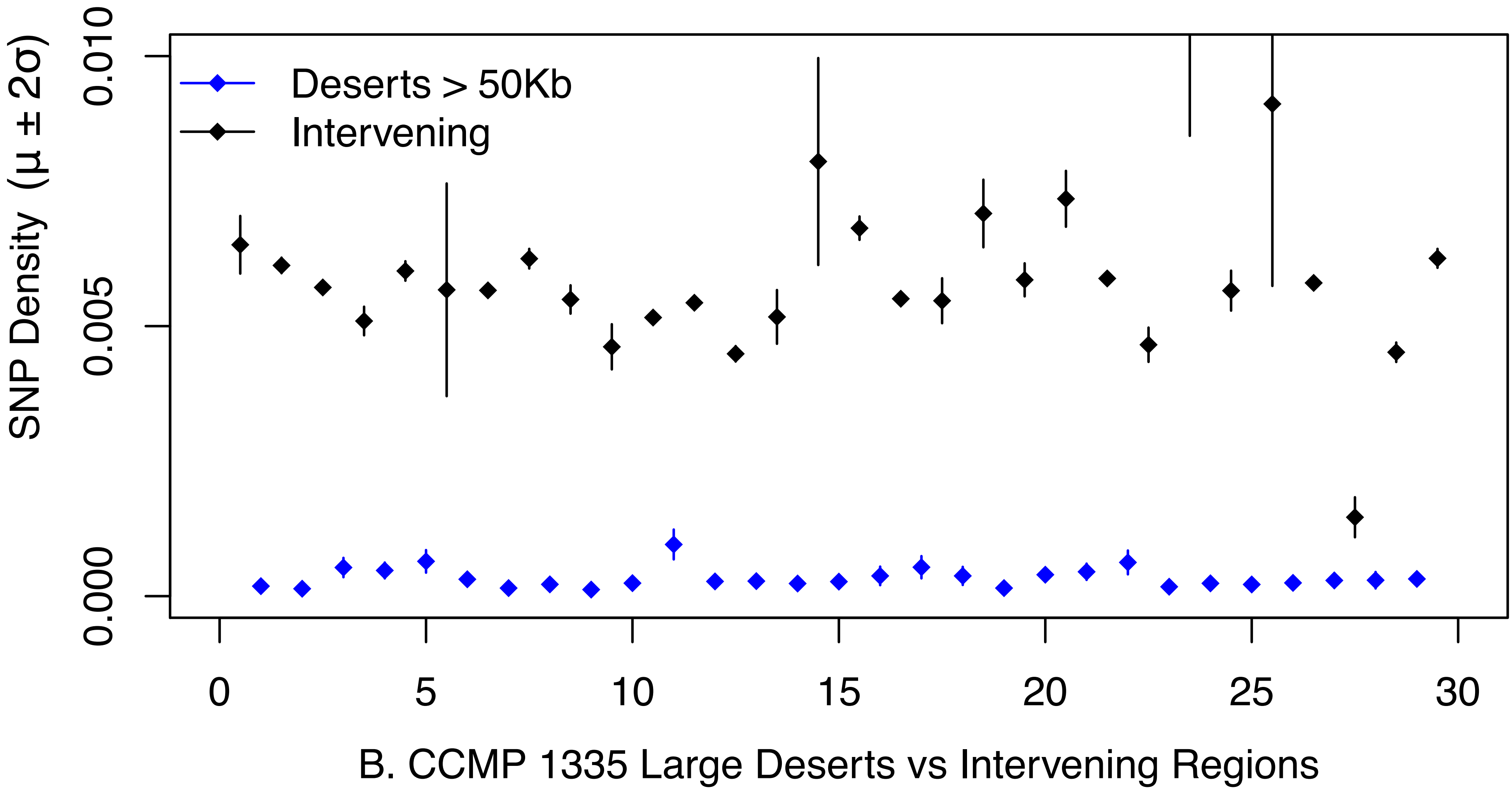
- 5 of 7 isolates share 96% or their SNPs
- Essentially no mixing of alleles as expected under Hardy-Weinberg, e.g. heterozygous positions are abundant in all individuals but (almost) never re-assort, (almost) never result in homozygous but non-reference positions, …
- Estimated crossover rate in the 5 is ~20x lower than in the other 2
- CONCLUSION: they are ***obligate asexuals***, reproducing exclusively by mitotic cell division for ~1000 years

Spacial uniformity of heterozygous sites reveals evolutionary history

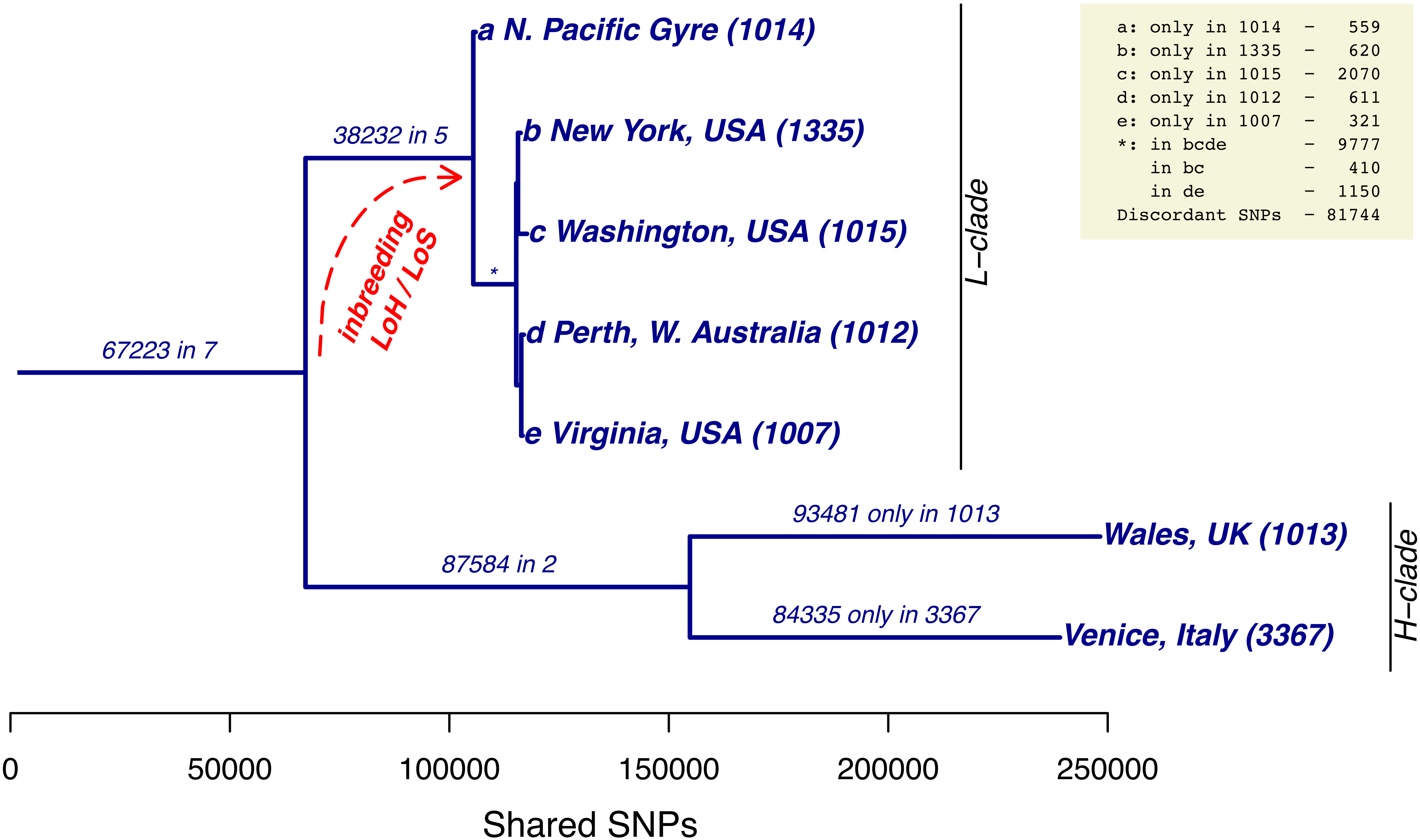


The blue regions are nearly homozygous regions (aka SNP deserts)

LoH Events Happened Nearly Simultaneously



T. Pseudonana History?



Summary

- A population bottleneck / inbreeding caused a loss of heterozygosity in a sub-population of T. pseudonana
- A functional loss of sex occurred within this sub-population
- This obligate asexual lineage spread across the world's oceans
- At least 5 CCMP isolates are descendants of this lineage
- At least 2 CCMP isolates maintain sexual reproduction in the wild

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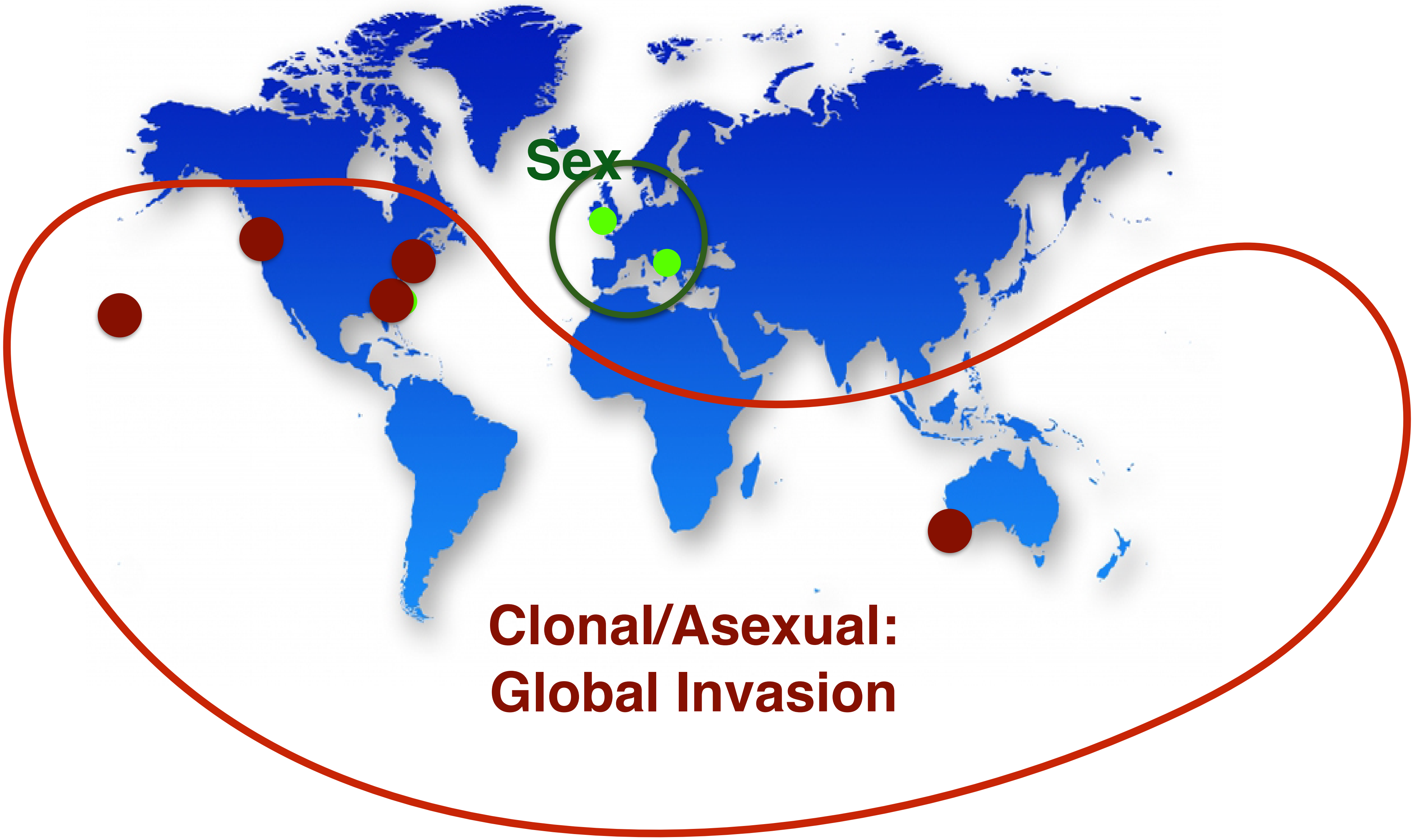

Implications

- A clonal global dispersal implies the existence of a general purpose genotype for *T. pseudonana*.
- It is unlikely that this type of obligate asexuality is unique to a single marine microbe.
- Environmental isolates may be biased in favor of such genotypes explaining the lack of sexual reproduction in culture despite attempted induction.
- This clonal sub-population is susceptible to global disruption by disease or environmental perturbations.
- Counters the classical assumption - genetic diversity correlates with geographic distance (biogeography).

Acknowledgements

- Collaborators:
  T Chiang, J Koester, C Berthiaume, N Hiranuma, M Parker, V Iverson, R Morales, A Sarwate & E Armbrust

- Funding:

Gordon and Betty
MOORE
FOUNDATION

Systematic DNA Loss in Culture

(⇒ No Contamination)