Exploration of Shared SNPs in Thaps

trunc-qfiltered

July 26, 2017

Rambling exploration of SNP positions shared between two or more of the isolates. Code is included to document it thoroughly, (even if largely uninteresting to anyone else), and I will summarize it as I go.

Contents

1 History

This was added to SVN 1/26/2014; not sure when it was started, but earliest related emails I see are from 1/21/14.

```
r413 | ruzzo | 2014-01-26 08:22:37 -0800 (Sun, 26 Jan 2014) | 2 lines

adding shared-snp analysis.
```

2 Preliminaries

NOTE: Some comments in code and some parts of the text, especially specific numbers and general conclusions, are based on Unqfiltered, Chr1, Medium stringency (i.e., “[[2]]” below) analysis. The broad picture does not appear to change with other choices, but details do, and the text is neither fully parameterized nor fully updated, so proceed with caution.

Load utility R code; do setup:

```
source('../../../R/wlr.R') # load util code; path relative this folder or sibling in scripts/larrys

## Running as: ruzzo @ bicycle.cs.washington.edu; SVN Id, I miss you.  $Id: wlr.R  2017-07-21 or later $

setup.my.wd('shared-snps') # set working dir; UPDATE if this file moves, or if COPY/PASTE to new file
setup.my.knitr('figs-knitr/')
generic.setup('figs-mine/')
```

3 Major Analysis/Performance Parameters.

Choices here control how this file is processed, what data is analyzed, speed, etc. Set them carefully before running “make.” Major choices are:

1. WHICH SNP TABLES ARE LOADED??? The logical vector `load.tb` selects the desired combination of SNP tables to load, in the order `full.unfiltered`, `chr1.unfiltered`, `full.qfiltered`, `chr1.qfiltered`. E.g., `load.tb=(T, F, T, F)` loads *full* tables for *both* q- and un-qfiltered data. Primary analysis is only performed on one of them, but the others are retained for comparison/debugging.

2. WHICH MAIN ANALYSIS??? If multiple tables are loaded, which is used for the main analysis? Parameter `pri` is a permutation of 1:4, corresponding to `load.tb`; the first loaded table in that order becomes the analysis focus. The default `pri=c(1,2,3,4)` looks at un-q-filtered data in preference to q-filtered, and full tables in preference to Chr1 within each group.

   (Choice of data for the “Table 1” coverage summary in section 5 is independent of this; full genome data is prefered over Chr 1 for both q- and unq-filtered reads; change `tset.picker` calls near the end of that section to modify this.)

3. CLEAR CACHE??? `clear.cache=T` forces Knitr cache removal at the start of the run; especially important if the previous parameters have changed since the last run.

4. HOW MANY BOOTSTRAP REPLICATES??? The variable `nboot` is a major performance factor; 1000 reps takes several hours. Set to 5 for debug and quick look; 100 or more for final run.

5. TRUNCATE TABLES TO Chrs ONLY??? I.e., remove mitochondrial-, plastid-, and BD- contigs.

The following code chunk sets the first four parameters based on where it’s run. To prototype/debug on a laptop, faster is better—run on Chr1 with small `nboot`; when run on the linux servers, I typically do full genomes, more replicates. Just override them if these defaults don’t work for you.

```r
# for Makefile, params can be command line args, else base on system; see wlr.r for details.
# load.tb order: full.un, chr1.un, full.qfil,  chr1.qfil

params <- pick.params(
  mac    = list(load.tb=c(F,T,F,F), pri=1:4, clear.cache=F, nboot=  1, trunc.tables=T), # quick on lap
 #linux = list(load.tb=c(F,F,F,T), pri=1:4, clear.cache=F, nboot=  5, trunc.tables=T), # quick qfil on server
  linux = list(load.tb=c(T,F,T,F), pri=1:4, clear.cache=T, nboot=101, trunc.tables=T)  # full on server
)

# Alternatively, edit/uncomment the following to override the above as needed
#params<-pick.params(default=list(load.tb=c(T,T,T,T),pri=1:4,clear.cache=T,nboot=1000,trunc.tables=T))
print(params)

# $load.tb
# full.unf chr1.unf  full.qf  chr1.qf
#     TRUE    FALSE     TRUE    FALSE
#
# $pri
# [1] 3 4 1 2
#
# $clear.cache
# [1] TRUE
#
# $nboot
# [1] 101
#
# $trunc.tables
# [1] TRUE
```

CLEAR CACHE??!! Some code chunks use the knitr cache, but extent of cache consistency checks unknown. If in doubt, delete “cache/” (knitr’s) directory to force rebuild. T/F set in params above will/won’t force removal (actually, rename):

```r
decache(params$clear.cache)

# Rename of 'cache' to 'cache91396' returned TRUE .
```

If still in doubt, also manually remove “00common/mycache/” (mine).

Load the main SNP data file(s) based on the parameters set in section 3.

```r
# short names to keep the following chunk compact
tb <- params$load.tb
tset <- list(NULL, NULL, NULL, NULL) # tset = 'table set'
```

```r
# see wlr.R for load paths
if(tb[1]){tset[[1]] <- load.snp.tables(use.chr1.tables = FALSE, data.name='full.tables.01.26.14')}

# Loading full tables from ../../../data/ungit-data/full.tables.01.26.14.rda ...Loaded.
# ../00common/mycache/snp.tables.chr1.unqfiltered.rda saved.

if(tb[2]){tset[[2]] <- load.snp.tables(use.chr1.tables = TRUE , data.name='full.tables.01.26.14')}
if(tb[3]){tset[[3]] <- load.snp.tables(use.chr1.tables = FALSE, data.name='full.tables.02.25.15')}

# Loading full tables from ../../../data/ungit-data/full.tables.02.25.15.rda ...Loaded.
# ../00common/mycache/snp.tables.chr1.qfiltered.rda saved.
# Bandaiding qfiltered tables...

if(tb[4]){tset[[4]] <- load.snp.tables(use.chr1.tables = TRUE , data.name='full.tables.02.25.15')}
```

Grrr! I should have excluded non-Chr contigs from full genome runs. Rather than change tons of code below to add mask params, I’m just going to truncate the tables, as follows. (See notes in wlr.r::make.mask for assumptions.)

```
if(params$trunc.tables){
  for(i in 1:4){
    if(!is.null(tset[[i]])){
      first.mito <- match("mitochondria.fasta", tset[[i]][[7]]$Chr)
      if(!is.na(first.mito)){ # will be NA for Chr1 tables
        for(j in 1:7){
          # hmmm... slow; wonder whether head(tset[[i]][[j]],first.mito-1) is faster;
          # ok, simple tests suggest not: system.time(head(data.frame(1:1e7,1:1e7),5e6))
          tset[[i]][[j]] <- tset[[i]][[j]][1:(first.mito-1),]
        }
      }
    }
  }
} else {
    cat('***\n*** DID YOU *REALLY* WANT UNTRUNCATED TABLES???\n***\n')
}
```

The tersely-named `tset` list is sometimes convenient, but give them more descriptive names, too.

```
snp.tables.full.unfiltered <- tset[[1]]; names(tset)[1] <- 'snp.tables.full.unfiltered'
snp.tables.chr1.unfiltered <- tset[[2]]; names(tset)[2] <- 'snp.tables.chr1.unfiltered'
snp.tables.full.qfiltered  <- tset[[3]]; names(tset)[3] <- 'snp.tables.full.qfiltered'
snp.tables.chr1.qfiltered  <- tset[[4]]; names(tset)[4] <- 'snp.tables.chr1.qfiltered'
```

Main analysis may just use one of the potentially 4 table sets. Pick it according to the priority specified in section 3, using the shorter name ’snp.tables’ for this default choice.

```
snp.tables <- tset.picker(priority=params$pri, table.set=tset)
```

```
# Sanity check: unlike unqfiltered tables, bug in early code gave qfiltered ones different numbers
# of rows per strain, which breaks much code.  Verify this is no longer happening.
check.eq.nrows <- function(tables){
  if(!is.null(tables)){
    nrow.snp.tables <- unlist(lapply(tables,nrow))
    print(nrow.snp.tables)
    if(all(nrow.snp.tables == nrow.snp.tables[1])){
      cat('OK, all strains have same number of rows.\n')
    } else {
      cat('***\n*** Warning: Different strains have different numbers of rows! ***\n***\n')
    }
  }
}

dummy<-lapply(tset, check.eq.nrows)

#     1007     1012     1013     1014     1015     3367     1335
# 31301782 31301782 31301782 31301782 31301782 31301782 31301782
# OK, all strains have same number of rows.
#     1007     1012     1013     1014     1015     3367     1335
# 31301782 31301782 31301782 31301782 31301782 31301782 31301782
# OK, all strains have same number of rows.
```

Which tables have we got?:

```
# 'which.snp.tables' return summary of which tables, either as a char string (default), e.g.
# "Chr1-qfiltered", or as vector of 2 strings, e.g. c("full","unfiltered").
cat('This analysis uses: (', paste(unlist(lapply(tset,which.snp.tables)),collapse=', '), ') SNP tables.\n')

# This analysis uses: ( trunc-unfiltered, NULL, trunc-qfiltered, NULL ) SNP tables.

cat('Main shared SNP analysis focuses on', which.snp.tables(snp.tables), '\n')

# Main shared SNP analysis focuses on trunc-qfiltered
```

A LaTeX hack: I want which.snp.tables info in doc title/page headers, but it is unknown until now, so the following writes a command definition `\whichsnptables` into the .aux file, which is read during the *next* LaTeX run, when `\begin{document}` is processed:

```
\makeatletter
\immediate\write\@auxout{\noexpand\gdef\noexpand\whichsnptables{trunc-qfiltered}}
\makeatother
```

Subsequent analysis was initially all directed at Chr1. In general, I have *not* updated the discussion to reflect genome-wide analysis.

```
if(exists('snp.tables.chr1.qfiltered') && exists('snp.tables.chr1.unqfiltered')){
  # If have both, where is new unequal to old?
  uneq <- snp.tables.chr1.qfiltered[[1]]$Ref[1:chr1.len] != snp.tables.chr1.unqfiltered[[1]]$Ref[1:chr1.len]
  cat('Sum uneq:', sum(uneq,na.rm=T), '\n')
  cat('Sum NA:   ', sum(is.na(uneq)),  '\n')
  print(which(is.na(uneq))[1:10])
  seecounts(which(is.na(uneq))[1:4],snp.tables=snp.tables.qfiltered,debug=F)
}
```

In brief, “`snp.tables`” will be a list of 7 data frames, one per strain, giving read counts for each nucleotide at each position, SNP calls, etc.:

```
names(snp.tables)

# [1] "1007" "1012" "1013" "1014" "1015" "3367" "1335"

str(snp.tables[[1]])

# 'data.frame': 31301782 obs. of  16 variables:
#  $ snp   : int  0 0 0 0 0 0 0 0 0 0 ...
#  $ Chr   : chr  "Chr1" "Chr1" "Chr1" "Chr1" ...
#  $ Pos   : int  1 2 3 4 5 6 7 8 9 10 ...
#  $ Ref   : chr  "T" "C" "C" "A" ...
#  $ Cov   : num  0 2 3 4 4 4 7 8 9 10 ...
#  $ a     : num  0 0 0 0 0 0 0 0 0 0 ...
#  $ g     : num  0 0 0 0 0 0 0 0 0 0 ...
#  $ c     : num  0 0 0 0 0 0 0 0 0 0 ...
#  $ t     : num  0 0 0 0 0 0 0 0 0 0 ...
#  $ n     : num  0 0 0 0 0 0 0 0 0 0 ...
#  $ .match: num  0 2 3 4 4 4 7 8 9 10 ...
#  $ exon  : logi  FALSE FALSE FALSE FALSE FALSE FALSE ...
#  $ indel : logi  FALSE FALSE FALSE FALSE FALSE FALSE ...
#  $ chr   : Factor w/ 66 levels "BD1_7","BD10_65",..: 39 39 39 39 39 39 39 39 39 39 ...
#  $ pos   : int  1 2 3 4 5 6 7 8 9 10 ...
#  $ rawCov: num  1 3 4 5 7 7 10 12 13 15 ...
```

Just for background, also load the desert tables:

```
# from svn+ssh://ceg1.ocean.washington.edu/var/svn/7_strains/trunk/code/snpNB/data
#load('../../../data/ungit-data/des.rda')
load('../../../data/des.rda')
```

What’s the total length of all deserts in each strain? Big deserts (defined as “big.threshold” or longer)?

```
some.desert.stats <- function(big.threshold=0){
  desert.len <- unlist(lapply(des,function(x){sum(unlist(lapply(x,function(y){sum(y[,'Length'])})))}))
  bigdes.len <- unlist(lapply(des,function(x){sum(unlist(lapply(x,function(y){
                                                 sum(y[y[,'Length']>=big.threshold,'Length'])})))}))
  rbind(desert.len, desert.pct=round( desert.len / genome.length.constants()$genome.length.trunc * 100),
        bigdes.len, bigdes.pct=round( bigdes.len / genome.length.constants()$genome.length.trunc * 100))
}
some.desert.stats(big.threshold=50000)
```

```
#              tp1007   tp1012  tp1013  tp1014   tp1015 thapsIT   tp1335
# desert.len 11146526 11332566 5801763 9464213 11251426 6780300 10883723
# desert.pct       36       36      19      30       36      22       35
# bigdes.len  3495805  3936973   55365 3627235  3727061   57119  4046934
# bigdes.pct       11       13       0      12       12       0       13
```

I.e., looking at all deserts, about 1/3 of L-clade, 1/5 of H-clade are in deserts, whereas, looking at the largest deserts ($> 50k$), only about 12% in L-clade (and none in H-clade). Note that the rough stats above include artifactual "deserts" created by gaps in the reference sequence, large genomic deletions, etc. A more careful analysis of this is found in nc-snps.rnw.

4 Refined SNP Calls

4.1 Method

It is appropriate that SNP calls should be conservative, to avoid many false positives, but, when a position is called a SNP in one isolate, we often see a significant number of reads for the same non-reference nucleotide at that position in other isolates, even if they are not called as SNPs. On the other hand, we sometimes see a position called a SNP in two or more isolates, but with *different* pairs of nucleotides, potentially suggesting technical errors. Analysis in this section attempts to refine the SNP calls by looking for issues such as these by looking at all 7 isolates jointly, at each position called a SNP in any of them.

For a given strain, the following function returns a vector of 0:4 to indicate which nonreference nucleotide has the maximum read count at the corresponding position. The values 1..4 indicate that the max count occurred at A, G, C, T, resp. (Ties are resolved arbitrarily ($a < g < c < t$), which possibly deserves further attention.) The value 0 means all nonreference counts are below threshold, based *either* on absolute count *or* as a fraction of coverage. Default only excludes 0 counts.

```
nref.nuc.new <- function(strain=1, mask=T, thresh.count=0, thresh.rate=0.0){
        # get read count for max nonref nuc
        nref <- apply(snp.tables[[strain]][mask, c('a', 'g', 'c', 't')], 1, max)
        # where does nref count match a (g,c,t, resp) count
        as <- ifelse(nref == snp.tables[[strain]][mask,'a'],1,0)
        gs <- ifelse(nref == snp.tables[[strain]][mask,'g'],2,0)
        cs <- ifelse(nref == snp.tables[[strain]][mask,'c'],3,0)
        ts <- ifelse(nref == snp.tables[[strain]][mask,'t'],4,0)
        # most positions will show 3 zeros and one of 1:4, so max identifies max nonref count;
        # ties broken arbitrarily  (a<g<c<t)
        merge <- pmax(as,gs,cs,ts)
        # but if max nonref count is zero or below threshold, return 0
        merge[nref == 0 | nref < thresh.count] <- 0
        merge[nref/snp.tables[[strain]][mask,'Cov'] < thresh.rate] <- 0
        return(merge)
}
```

Get union and intersection of the sets of called SNPs. ("$snp" is 0/1.) Also, 5-way (L-clade) and 4-way (L-excluding Gyre).

```
# 4-way union/intersection
u4.snps <- snp.tables[[1]]$snp
i4.snps <- snp.tables[[1]]$snp
for(i in c(2,5,7)) {
        u4.snps <- pmax(u4.snps, snp.tables[[i]]$snp)
        i4.snps <- pmin(i4.snps, snp.tables[[i]]$snp)
}
# 5-way: add gyre
u5.snps <- pmax(u4.snps, snp.tables[[4]]$snp)
i5.snps <- pmin(i4.snps, snp.tables[[4]]$snp)
# 7-way
union.snps     <- pmax(u5.snps, snp.tables[[3]]$snp, snp.tables[[6]]$snp)
intersect.snps <- pmin(i5.snps, snp.tables[[3]]$snp, snp.tables[[6]]$snp)
nu4snps <- sum(u4.snps)
```

```
nu5snps <- sum(u5.snps)
ni4snps <- sum(i4.snps)
ni5snps <- sum(i5.snps)
nusnps  <- sum(union.snps)
nisnps  <- sum(intersect.snps)
c(n4u=nu4snps, n5u=nu5snps, n7u=nusnps, n4i=ni4snps, n5i=ni5snps, n7i=nisnps)

#    n4u    n5u    n7u    n4i    n5i    n7i
# 196296 197799 474613 128683  70687  15186
```

There are nusnps=474613 positions called as SNPs in one or more strains (but only nisnps=15186 that are shared among all 7). Note that the 4-way union is only modestly larger (1.5254229 times larger) than the 4-way intersection, emphasizing the inherent similarities among these SNP sets. The corresponding 5-way numbers show that Gyre adds relatively little to the 5-way union vs the 4-way union, whereas it removes a fair bit from the 5-way intersection. However, much of that loss is simply because Gyre has fewer called SNPs: only 89184 vs 128683 in the 4-way intersection, and they are highly concordant:

```
sum(snp.tables[[4]]$snp*i4.snps)/sum(snp.tables[[4]]$snp)

# [1] 0.7925973
```

So, a likely source of the Gyre's difference in called SNPs is technical (lower read coverage, higher read error rate) rather than biological.

Inclusion of the 2 H-clade members, however, causes more dramatic changes in both union and intersection numbers. I examine all these relationships in more detail below, but first I examine what I believe to be a significant source of technical error in these comparisons—erroneous SNP calls, especially false negative calls.

It is appropriate that SNP calls should be conservative, to avoid many false positives, but, if a position is called a SNP in one strain, we often see a significant number of reads for the same non-reference nucleotide at that position in other strains, even if they are not called as SNPs. For my purposes below, these will be considered "shared SNPs," based on three different levels of permissiveness. Note that, e.g., $\geq$ 84% of all positions have zero reads for any non-reference nucleotide, and only a small fraction have 2 or more non-reference reads:

```
nonmatch <- rbind(
  unlist(lapply(snp.tables,function(x){sum(x$Cov-x$.match == 0)})),
  unlist(lapply(snp.tables,function(x){sum(x$Cov-x$.match == 1)})),
  unlist(lapply(snp.tables,function(x){sum(x$Cov-x$.match == 2)})),
  unlist(lapply(snp.tables,function(x){sum(x$Cov-x$.match == 3)})),
  unlist(lapply(snp.tables,function(x){sum(x$Cov-x$.match >= 4)})),
  unlist(lapply(snp.tables,function(x){sum((x$Cov-x$.match)[union.snps==0] >= 4)}))
)/nrow(snp.tables[[1]])*100
rownames(nonmatch) <- c('% ==0','% ==1','% ==2','% ==3','% >=4', '% >=4, nonSNP')
nonmatch

#                     1007        1012        1013        1014        1015        3367        1335
# % ==0        97.71716831 97.35791400 95.45329400 97.29079003 97.18569697 95.89943474 96.48734376
# % ==1         1.48140448  1.75279158  3.01610304  2.08080805  1.88427930  2.58814338  2.54747477
# % ==2         0.11277633  0.12101867  0.22209918  0.18711714  0.13599226  0.21703876  0.19842640
# % ==3         0.05885927  0.05629072  0.09496264  0.10621121  0.06021063  0.09526295  0.06375675
# % >=4         0.62979162  0.71198502  1.21354113  0.33507358  0.73382084  1.20012017  0.70299831
# % >=4, nonSNP  0.08015518  0.12493857  0.25313575  0.04763946  0.13208513  0.28212771  0.13240460
```

Build a table of max non-reference nucleotides at each position in the union.snps set. The three criteria are

- [[1]]: any non-zero count at any coverage is considered significant
- [[2]]: (count $\geq$ 2 and count/coverage $\geq$ 0.05) is considered significant
- [[3]]: (count $\geq$ 4 and count/coverage $\geq$ 0.10) is considered significant

In all three cases, the nonref nucleotide must also be consistent across all strains passing that threshold; see below.

```
non.refs <- vector('list',4)
for(i in 1:4){
  non.refs[[i]] <- matrix(0, nrow=nusnps, ncol=7)
  colnames(non.refs[[i]]) <- names(snp.tables)
  rownames(non.refs[[i]]) <-
            paste(snp.tables[[1]]$chr[union.snps==1], ':', snp.tables[[1]]$pos[union.snps==1], sep='')
}
for(j in 1:7){
  non.refs[[1]][,j] <- nref.nuc.new(j, mask=union.snps==1, thresh.count=0, thresh.rate=0.00)
  non.refs[[2]][,j] <- nref.nuc.new(j, mask=union.snps==1, thresh.count=2, thresh.rate=0.05)
  non.refs[[3]][,j] <- nref.nuc.new(j, mask=union.snps==1, thresh.count=4, thresh.rate=0.10)
}
```

For comparison, I want to look at unfiltered SAMTools SNP calls. In complete opposition to the measures of consistency imposed above, I'm going to simply force this into the "non.refs" structure constructed above by imagining that any position called a SNP in any strain has its max nonref count on "A", so any given position called a SNP in any strain will automatically be declared "consistent." This will allow the tree-code, etc. given below to work in a uniform way (even though interpretation of the results is different.) Results will be jammed into a 4th component of the "non.refs" list; i.e., we have a 4th criterion:

- [[4]]: all called SNPs at a given position are considered "consistent."

As this case was a late addition to the analysis, the commentary throughout this document has not necessarily been updated to reflect that this case is distinct from the first three.

```
for(j in 1:7){
  non.refs[[4]][,j] <- snp.tables[[j]]$snp[union.snps==1]
}
```

```
str(non.refs[[4]])


#  num [1:474613, 1:7] 0 0 0 0 0 0 0 0 1 0 ...
#  - attr(*, "dimnames")=List of 2
#   ..$ : chr [1:474613] "Chr1:333" "Chr1:417" "Chr1:435" "Chr1:438" ...
#   ..$ : chr [1:7] "1007" "1012" "1013" "1014" ...
```

"non.refs" indicates, among those positions in the union of all called SNPS having any non-reference read count above the thresholds listed above, the non-ref nucleotide having the highest read count in each strain. If, for a given position, the max of this code is the same as the min (among non-zero values), then every strain having over-threshold nonref reads in that position, in fact has most non-reference reads on the *same* nucleotide. These are defined as the "consistent" SNPs.

```
find.consistent <- function(nr){
  nr.max <- apply(nr,1,max)
  nr.min <- apply(nr,1,function(x){ifelse(max(x)==0,0,min(x[x>0]))})
  return(nr.min == nr.max)
}
consistent  <- lapply(non.refs, find.consistent)
```

4.2 Save them

```
# wrap this in a data structure to be cached:
Description <- [2753 chars quoted with ''']

refined.snps <-
  list(Description=Description,

       Data=list(
         based.on.which.snp.tables=which.snp.tables(),
         number.union.snps=nusnps,
         number.intersection.snps=nisnps,
         non.ref.nucleotide=non.refs,
         consistent.snps=consistent),

       Code=list(
         get.snps = function(strain, stringency=2){
           # return nusnps x 1 Bool vector of consistent SNPs @ specified stringency & strain
           return(refined.snps$Data$consistent.snps[[stringency]] &
                  refined.snps$Data$non.ref.nucleotide[[stringency]][,strain] > 0)
         }
         ,
         get.snp.locs.char = function(strain, stringency=2){
           # return char vector of locations of consistent SNPs @ specified stringency & strain
           snps <- refined.snps$Code$get.snps(strain, stringency)
           return(names(snps)[snps])
         }
         ,
         get.snp.locs.df = function(strain, stringency=2){
           # return data frame (Chr/Pos) of locations of consistent SNPs @ specified stringency & strain
           snplist <- strsplit(refined.snps$Code$get.snp.locs.char(strain, stringency), ':', fixed=TRUE)
           # strsplit returns long list of 2-vectors, 1st=chr, 2nd=char position
           df <- data.frame(Chr=           unlist(lapply(snplist,function(x){return(x[1])})),
                            Pos=as.integer(unlist(lapply(snplist,function(x){return(x[2])}))),
                            stringsAsFactors = FALSE)
           return(df)
         }
       )
  )

# dont't clobber existing .rda, but save if absent.  (delete to re-save)
# result for trunc, unfiltered tables saved to "data" else "mycache"
if(which.snp.tables() == 'trunc-unfiltered'){
  rda.refined <- '../../../data/refined.snps-trunc-unfiltered.rda'
} else {
  rda.refined <- paste('../00common/mycache/refined.snps', which.snp.tables(), 'rda', sep='.')
}
if(file.exists(rda.refined)){
  cat('Pre-existing file', rda.refined, 'unchanged.\n')
} else {
  cat('Saving', rda.refined, '...')
  save(refined.snps, file=rda.refined, compress=TRUE)
  cat('Saved.\n')
}

# Saving ../00common/mycache/refined.snps.trunc-qfiltered.rda ...Saved.
```

Knitr seems to be failing to format the long char string above, which says:

```
cat(refined.snps$Description)

# Contents of this .rda file:
#
#   * Description: this text
#
#   * Data -- 5 items defining refined SNPs, at 4 different stringency levels, as defined
#     in shared-snps.rnw:
#
#     * based.on.which.snp.tables: {"Chr1","full","trunc"}-{"unfiltered","qfiltered"},
#       depending on which snp tables were used to build this data. ("trunc" = all Chrs.)
#
#     * number.union.snps: the total number of SNPs (SAMtools calls) in the union of SNPs
#       across all 7 strains.
#
#     * number.intersection.snps: similar, for the 7-way intersection.
#
#       nusnps/nisnps are easily recalculated from the data below, but their inclusion
#       may be convenient, e.g., to quickly see if the .rda represents the full genome
#       (nusnps=488848), or the chr 1 subset (nusnps=47499); (redundant with "based.on...";
#       numbers above are for unfiltered, perhaps slightly different if qfiltered)
#
#     * non.ref.nucleotide: 4 arrays, each nusnps x 7, of values 0..4 (0..1 in the 4th
#       array).  In the 1st 3 arrays, 0 means the given position in the given strain did
#       not have nonreference read counts above the corresponding filtering threshold,
#       i.e., is NOT a refined SNP in that strain, whereas 1..4 mean that it did pass
#       threshold, for A,C,G,T resp.  In the 4th array, this value is just 1/0,
#       indicating is/is not a called SNP in that strain.
#
#     * consistent.snps: 4 Bool vectors of length nusnps flagging positions whose nonref
#       nucs (wrt to the 4 filtering criteria) are deemed *consistent* across
#       all 7 strains.  For the 1st 3, this means all nonzero entries of non.ref.nuc
#       are equal, i.e., nonref read counts passing threshold are on the SAME nonref
#       nucleotide in all strains having over-threshold counts.  Just for comparison
#       and uniformity of data structures, the 4th is all TRUE, i.e., union of SNPs
#       across all strains, without any regard for thresholds or consistency.
#
#       In short, the refined SNPs according to our medium filtering criteria are
#       strains/positions where consistent.snps[[2]]==TRUE and non.ref.nucleotide[[2]]>0.
#
#       Rownames in both non.ref.nucs and consistent define location, e.g. "Chr1:333".
#
#   * Code -- simple routines to extract refined SNPs in (potentially) convenient formats:
#
#     * get.snps(strain, stringency=2)
#       returns nusnps x 1 Bool vector of consistent SNPs @ specified stringency in
#       given strain
#
#     * get.snp.locs.char(strain, stringency=2)
#       returns n x 1 char vector of locations of consistent SNPs @ specified stringency
#       in given strain, e.g. "Chr1:1234", where n == sum(get.snps(...))
#
#     * get.snp.locs.df(strain, stringency=2){
#       As above, but returns data frame (char vector Chr, int vector Pos) with the same info.
```

```
str(consistent[[1]])

#  Named logi [1:474613] TRUE FALSE TRUE TRUE TRUE TRUE ...
#  - attr(*, "names")= chr [1:474613] "Chr1:333" "Chr1:417" "Chr1:435" "Chr1:438" ...
```

```
consistent.count <- unlist(lapply(consistent, sum)) ; consistent.count

# [1] 447177 469906 471171 474613

inconsistent.count <- consistent.count[4] - consistent.count; inconsistent.count

# [1] 27436  4707  3442     0

inconsistent.percent <- inconsistent.count/consistent.count[4]*100; inconsistent.percent

# [1] 5.7807098 0.9917554 0.7252224 0.0000000
```

I.e., of the 474613 positions in which a SNP is called, 447177 are consistent by my loose definition, and 471171 are consistent by my tightest definition. The increase in concordance supports the view that the loose definition is too loose. Perhaps misleadingly, these counts include positions that are "consistent SNPs" in only one strain; more below. (*TODO* I suspect, but have not yet systematically checked, that most of the rest are positions with low coverage and/or very low read counts on the mixture of non-reference nucleotides.)

4.3 Examples: Consistent

Here are a few (nonrandomly selected) prototypical consistent SNPs:

```
esnps <- names(consistent[[2]][consistent[[2]]])
esnps2 <- as.integer(unlist(lapply(strsplit(esnps[c(7,11:13,92)],':',fixed=TRUE),function(x){x[2]})))
seecounts(esnps2,snp.tables=snp.tables)

#     chr  pos Ref Strain  A   G  C  T SNP  exon indel nrf rat
# 1  Chr1  567   T
# 2                  1007  0   0  1 25   0  TRUE FALSE
# 3                  1012  0   0 14 39   1  TRUE FALSE
# 4                  1013  0   0 13 87   0  TRUE FALSE
# 5                  1014  0   1  0 23   0  TRUE FALSE
# 6                  1015  0   0  8 40   1  TRUE FALSE
# 7                  3367  0   0 16 38   1  TRUE FALSE
# 8                  1335  0   0  2 99   0  TRUE FALSE
# 9  Chr1 1053   A
# 10                 1007 25   0  0  4   0  TRUE FALSE
# 11                 1012 35   0  0 12   0  TRUE FALSE
# 12                 1013  2   1  0 32   0  TRUE FALSE
# 13                 1014  5   0  0  5   0  TRUE FALSE
# 14                 1015 29   0  0 15   1  TRUE FALSE
# 15                 3367  2   0  0  7   0  TRUE FALSE
# 16                 1335 56   0  0 39   1  TRUE FALSE
# 17 Chr1 1055   G
# 18                 1007  0  23  0  1   0  TRUE FALSE
# 19                 1012  0  37  0  6   0  TRUE FALSE
# 20                 1013  1  39  0  6   0  TRUE FALSE
# 21                 1014  0   6  0  2   1  TRUE FALSE
# 22                 1015  0  26  0 14   0  TRUE FALSE
# 23                 3367  0  12  0  0   0  TRUE FALSE
# 24                 1335  0  54  0 32   1  TRUE FALSE
# 25 Chr1 1176   G
# 26                 1007  1  53  0  0   0 FALSE FALSE
# 27                 1012  0  54  0  0   0 FALSE FALSE
# 28                 1013 19  56  0  0   0 FALSE FALSE
# 29                 1014  0  28  0  0   0 FALSE FALSE
# 30                 1015  3  85  0  0   0 FALSE FALSE
# 31                 3367  9   2  0  0   1 FALSE FALSE
# 32                 1335  0 156  0  0   0 FALSE FALSE
# 33 Chr1 8685   G
# 34                 1007  6  15  0  0   0  TRUE FALSE
# 35                 1012 10  23  0  0   0  TRUE FALSE
# 36                 1013 18  21  0  0   1  TRUE FALSE
# 37                 1014  4   8  0  0   0  TRUE FALSE
# 38                 1015 10  24  0  0   1  TRUE FALSE
# 39                 3367  0   4  0  0   0  TRUE FALSE
# 40                 1335  5  32  0  0   0  TRUE FALSE
```

4.4 Examples: Inconsistent

Here is a brief look at some *in*-consistent positions. E.g., Chr1:2013 shows nontrivial counts on 3 alleles in Wales, as do 2319, 3286, 5002, 5433, whereas 7878 shows a different alternate allele in Italy than in Wales.

```
unc <- names(consistent[[2]][!consistent[[2]]])
unc2 <- as.integer(unlist(lapply(strsplit(unc[1:10],':',fixed=TRUE),function(x){x[2]})))
seecounts(unc2,snp.tables=snp.tables)

#     chr   pos Ref Strain  A   G  C  T SNP  exon indel nrf rat
# 1  Chr1  2013   T
# 2                   1007  4   0  0 15   0  TRUE FALSE
# 3                   1012  6   0  0 21   0  TRUE FALSE
# 4                   1013  7  10  0  6   1  TRUE FALSE
# 5                   1014  1   0  0  6   0  TRUE FALSE
# 6                   1015 13   0  0 13   1  TRUE FALSE
# 7                   3367  7   0  0 25   0  TRUE FALSE
# 8                   1335 16   0  0 42   1  TRUE FALSE
# 9  Chr1  2319   C
# 10                  1007  0  28 10  0   1  TRUE FALSE
# 11                  1012  0  43 17  0   1  TRUE FALSE
# 12                  1013 13  15  9  0   1  TRUE FALSE
# 13                  1014  0  18  6  0   1  TRUE FALSE
# 14                  1015  0  53 20  0   1  TRUE FALSE
# 15                  3367  4   0 24  0   0  TRUE FALSE
# 16                  1335  0 118 28  0   1  TRUE FALSE
# 17 Chr1  3286   T
# 18                  1007  4   0  1 10   0  TRUE FALSE
# 19                  1012  7   0  3 32   0  TRUE FALSE
# 20                  1013 34   0 30  1   1  TRUE FALSE
# 21                  1014  4   0  4 10   0  TRUE FALSE
# 22                  1015 11   0  6 31   0  TRUE FALSE
# 23                  3367  5   0 29  0   0  TRUE FALSE
# 24                  1335 14   0  3 55   0  TRUE FALSE
# 25 Chr1  5002   T
# 26                  1007  0  14  0  7   0  TRUE FALSE
# 27                  1012  0  20  0 19   1  TRUE FALSE
# 28                  1013 18  10  0 22   0  TRUE FALSE
# 29                  1014  0   5  0  2   0  TRUE FALSE
# 30                  1015  0  18  0 12   1  TRUE FALSE
# 31                  3367  0   0  0 31   0  TRUE FALSE
# 32                  1335  0  46  0 44   0  TRUE FALSE
# 33 Chr1  5178   G
# 34                  1007  0  20  0  0   0  TRUE FALSE
# 35                  1012  0  32  0  0   0  TRUE FALSE
# 36                  1013 47   9  0  0   1  TRUE FALSE
# 37                  1014  0  13  0  0   0  TRUE FALSE
# 38                  1015  0  30  0  0   0  TRUE FALSE
# 39                  3367 32  19  0  0   1  TRUE FALSE
# 40                  1335  0  38  0  2   0  TRUE FALSE
# 41 Chr1  5433   G
# 42                  1007  0  40  0  3   0  TRUE FALSE
# 43                  1012  0  53  0  5   0  TRUE FALSE
# 44                  1013 16  29  0  7   1  TRUE FALSE
# 45                  1014  9   8  0  0   1  TRUE FALSE
# 46                  1015  6  53  0  2   0  TRUE FALSE
# 47                  3367  8  37  0  0   0  TRUE FALSE
# 48                  1335  6  72  0  2   0  TRUE FALSE
# 49 Chr1  7858   C
# 50                  1007  0   0 42  0   0  TRUE FALSE
# 51                  1012  0   0 35  0   0  TRUE FALSE
# 52                  1013  0   0 81  8   0  TRUE FALSE
# 53                  1014  0   0 12  0   0  TRUE FALSE
# 54                  1015  0   0 71  0   0  TRUE FALSE
# 55                  3367 20   0  2  0   1  TRUE FALSE
# 56                  1335  0   0 83  0   0  TRUE FALSE
# 57 Chr1  8974   C
# 58                  1007  0   1  5  0   0  TRUE FALSE
# 59                  1012  0   2 13  0   0  TRUE FALSE
# 60                  1013  9  15  2  0   1  TRUE FALSE
# 61                  1014  0   1  1  0   0  TRUE FALSE
# 62                  1015  0   1  9  0   0  TRUE FALSE
# 63                  3367  2   0  1  0   0  TRUE FALSE
# 64                  1335  0  11 30  0   0  TRUE FALSE
# 65 Chr1 10099   T
# 66                  1007 16   0  0 24   0  TRUE FALSE
# 67                  1012 45   0  0 26   0  TRUE FALSE
# 68                  1013  0   2  6 55   0  TRUE FALSE
# 69                  1014 32   0  0 11   0  TRUE FALSE
# 70                  1015 38   0  0 37   0  TRUE FALSE
# 71                  3367  0   1  0  7   0  TRUE FALSE
# 72                  1335 52   0  0 61   1  TRUE FALSE
# 73 Chr1 15154   A
# 74                  1007 13   0  0  0   0 FALSE FALSE
# 75                  1012 37   0  0  1   0 FALSE FALSE
# 76                  1013  2   0 35  7   1 FALSE FALSE
# 77                   1014 10    0   0   0    0 FALSE FALSE
# 78                   1015 24    0   0   0    0 FALSE FALSE
# 79                   3367  3    0   0  12    1 FALSE FALSE
# 80                   1335 47    0   0   3    0 FALSE FALSE
```

4.5 Examples: Homozygous nonref

And at some *homozygous nonreference* positions (defined to be those with nonref fraction > 0.75):

```
hnr <- lapply(snp.tables, function(x){x$.match/x$Cov < 0.25})        # find them
hnr <- lapply(hnr, function(x){ifelse(is.na(x),FALSE,x)})            # remove NA
unlist(lapply(hnr,sum))                                               # count per strain

#   1007   1012   1013   1014   1015   3367   1335
#  16069  14356 120037  11436   6862 142515   1854
```

Hmm, in L-clade, excluding the ref isolate (1335) this tracks time-in culture to some degree; Maybe many of these are in hemizygous regions. Next two chunks lifted from nc-snps to get tables for hemi-deletion.

```
cnv.chronly <- load.cnv.tables('../../../data/cnv.txt', chrs.only=TRUE)

str(cnv.chronly)

# 'data.frame': 1956 obs. of  11 variables:
#  $ strain   : Factor w/ 7 levels "IT","tp1007",..: 3 3 3 3 3 3 3 3 3 3 ...
#  $ chr      : Factor w/ 65 levels "BD1_7","BD10_65",..: 38 38 38 38 38 38 38 38 38 38 ...
#  $ start    : int  10601 112001 215001 358901 536501 554801 673401 781801 806901 853201 ...
#  $ end      : int  13500 116500 221100 370300 538600 559300 685000 787400 811100 855600 ...
#  $ length   : int  2900 4500 6100 11400 2100 4500 11600 5600 4200 2400 ...
#  $ filtered : logi  FALSE FALSE FALSE TRUE FALSE FALSE ...
#  $ type     : Factor w/ 1 level "CNVnator": 1 1 1 1 1 1 1 1 1 1 ...
#  $ cov_ratio: num  0.63738 1.54893 1.65381 0.00204 0.68486 ...
#  $ dup_frac : num  0.41188 0.00908 0.01178 0.97997 0.0211 ...
#  $ iStart   : num  10601 112001 215001 358901 536501 ...
#  $ iEnd     : num  13500 116500 221100 370300 538600 ...

cnv.chronly[c(1:4,nrow(cnv.chronly)+c(-1,0)),]                   ## first/last few rows

#      strain   chr  start    end length filtered     type  cov_ratio   dup_frac   iStart     iEnd
# 1    tp1012  Chr1  10601  13500   2900    FALSE CNVnator 0.63738000 0.41187900    10601    13500
# 2    tp1012  Chr1 112001 116500   4500    FALSE CNVnator 1.54893000 0.00907677   112001   116500
# 3    tp1012  Chr1 215001 221100   6100    FALSE CNVnator 1.65381000 0.01178470   215001   221100
# 4    tp1012  Chr1 358901 370300  11400     TRUE CNVnator 0.00204431 0.97997300   358901   370300
# 1955 tp1335 Chr24 259901 278000  18100    FALSE CNVnator 1.41458000 0.38091100 31264334 31282433
# 1956 tp1335 Chr24 286901 289800   2900    FALSE CNVnator 1.74941000 0.74228100 31291334 31294233
```

```
get.cnv.dels <- function(cov.thresh.lo = 0.0,
                         cov.thresh.hi = 0.8,
                         cnv,
                         snp.tables = NULL,
                         DEBUG = FALSE
){
  # build list of 7 Bool vectors of genome length, with i-th == T iff
  #  * i-th pos is 'NA' in genome seq (if snp.tables are provided), or
  #  * in CNVnator call for coverage in half-open [cov.thresh.lo, hi), and
  #  * not marked 'filtered' by CNVnator
  cnv.deletions <- vector(mode='list',7)                   # make list of bool vectors
  if(is.null(snp.tables)){
    # if no tables, assume full
    t.len <- genome.length.constants()$genome.length.trunc
  } else {
    t.len <- nrow(snp.tables[[1]])
  }
  for(st in 1:7){
    if(is.null(snp.tables)){
      cnv.deletions[[st]] <- logical(t.len)                         # all F
    } else  {
      cnv.deletions[[st]] <- is.na(snp.tables[[st]]$Pos[1:t.len])   # NA positions in genome
    }
  }
  strain.names <- c(paste('tp10',c('07',12:15),sep=''),'IT','tp1335')
  names(cnv.deletions) <- strain.names
  for(i in 1:nrow(cnv)){
    if(!cnv$filtered[i] &&
       cnv$cov_ratio[i] >= cov.thresh.lo &&
       cnv$cov_ratio[i] <  cov.thresh.hi)
    {
      if(DEBUG){
        print(cnv[i,])
        print(as.character(cnv$strain[i]))
      }
      # following ASSUMES no CNVnator call crosses a chromosome bdry, & that
      # t.len ends at chr end (typically chr1 or chr24)
      if(cnv$iEnd[i] <= t.len){
        cnv.deletions[[as.character(cnv$strain[i])]][cnv$iStart[i]:cnv$iEnd[i]] <- TRUE
      }
    }
  }
  return(cnv.deletions)
}

# sanity check:
cnv.dels.38 <- get.cnv.dels(0.3, 0.8, cnv.chronly, snp.tables = NULL)
unlist(lapply(cnv.dels.38,sum)) # does it match low.length.38 in tic ?

#  tp1007  tp1012  tp1013  tp1014  tp1015      IT  tp1335
# 1672500 1781500 1383600 1313700  988400  320900 1453000

# 1672500 1781500 1399400 1313700 988400 336500 1453000 <== low.length.38 from tic (circa page 8)
# 1672500 1781500 1399400 1313700 988400 336500 1453000 <== low.length.38 from tic (pg9, 6/28/17)
rm(cnv.dels.38)
```

Slight discrepancy in H-clade that I should hunt down, but basically OK. (hmm; maybe untrunc tbls.)

```
# the ones we want for the current analysis:
hemi.masks <- get.cnv.dels(0.3, 0.8, cnv.chronly, snp.tables=snp.tables)

rbind(
  homnr        = unlist(lapply(hnr,sum)),
  hemi         = unlist(lapply(hemi.masks, sum)),
  homnr.unhemi = unlist(lapply(list(1,2,3,4,5,6,7), function(i){sum(hnr[[i]] & !hemi.masks[[i]])}))
)

#                 1007    1012    1013    1014    1015   3367    1335
# homnr          16069   14356  120037   11436    6862 142515    1854
# hemi         1834990 1940024 1527725 1472095 1134652 480817 1596965
# homnr.unhemi    9650    7347  111674   10091    5113 140185    1829
```

```
# based on the thought that hnr in 1335 may reflect errors in the ref seq,
# are they shared with others?
unlist(lapply(hnr, function(x){sum(x & hnr[[7]])}))                # hnr shared with 1335

# 1007 1012 1013 1014 1015 3367 1335
#  517  592  748  362  617  793 1854

# answer: around 300 in each strain, of 558 in NY, genomewide,
# so that seems like a plausibly important factor.

hnr.lclade <- hnr[[1]] | hnr[[2]] | hnr[[4]] | hnr[[5]] | hnr[[7]]  # union over L-clade
sum(hnr.lclade)                                                     # count all in L-clade
```

```
# [1] 31723

sum(hnr[[3]] | hnr[[6]])                                            # present in H-clade

# [1] 188637

sum(hnr[[3]] & hnr[[6]])                                            # shared in H-clade

# [1] 73915

# look at a few in L-clade
w.hnr.l <- which(hnr.lclade)
seecounts(w.hnr.l[1:10],snp.tables=snp.tables)

#     chr  pos Ref Strain  A   G   C  T SNP  exon indel nrf rat
# 1  Chr1 1559   A
# 2                  1007  7   0   0 24   0  TRUE FALSE
# 3                  1012 11   0   0 37   0  TRUE FALSE
# 4                  1013  9   0   0  5   0  TRUE FALSE
# 5                  1014  4   0   0 16   0  TRUE FALSE
# 6                  1015 47   0   0 35   0  TRUE FALSE
# 7                  3367  0   0   0  0   0  TRUE FALSE
# 8                  1335 60   0   0 50   0  TRUE FALSE
# 9  Chr1 1575   G
# 10                 1007 24   7   0  0   0  TRUE FALSE
# 11                 1012 42  13   0  0   0  TRUE FALSE
# 12                 1013 17  16   0  0   0  TRUE FALSE
# 13                 1014 15   4   0  0   0  TRUE FALSE
# 14                 1015 43  31   0  0   1  TRUE FALSE
# 15                 3367  0   2   0  0   0  TRUE FALSE
# 16                 1335 34  74   0  0   0  TRUE FALSE
# 17 Chr1 1893   C
# 18                 1007  0   0  14 32   0  TRUE FALSE
# 19                 1012  0   0  38 52   0  TRUE FALSE
# 20                 1013  0   0  95 14   0  TRUE FALSE
# 21                 1014  0   0   5 31   0  TRUE FALSE
# 22                 1015  0   0  47 44   0  TRUE FALSE
# 23                 3367  0   0  29  0   0  TRUE FALSE
# 24                 1335  0   0  68 85   0  TRUE FALSE
# 25 Chr1 2223   A
# 26                 1007 25  13   0  0   0  TRUE FALSE
# 27                 1012 13  12   1  0   0  TRUE FALSE
# 28                 1013  5  24   0  0   0  TRUE FALSE
# 29                 1014  0   4   0  0   0  TRUE FALSE
# 30                 1015 19  22   0  0   1  TRUE FALSE
# 31                 3367 15   3   0  0   0  TRUE FALSE
# 32                 1335 33  22   0  0   0  TRUE FALSE
# 33 Chr1 2319   C
# 34                 1007  0  28  10  0   1  TRUE FALSE
# 35                 1012  0  43  17  0   1  TRUE FALSE
# 36                 1013 13  15   9  0   1  TRUE FALSE
# 37                 1014  0  18   6  0   1  TRUE FALSE
# 38                 1015  0  53  20  0   1  TRUE FALSE
# 39                 3367  4   0  24  0   0  TRUE FALSE
# 40                 1335  0 118  28  0   1  TRUE FALSE
# 41 Chr1 2502   A
# 42                 1007 14   2   0  0   0 FALSE FALSE
# 43                 1012 17   6   0  0   0 FALSE FALSE
# 44                 1013  6  13   0  0   0 FALSE FALSE
# 45                 1014  1   6   0  0   0 FALSE FALSE
# 46                 1015 20   7   0  0   0 FALSE FALSE
# 47                 3367  3   3   0  0   0 FALSE FALSE
# 48                 1335 29  17   0  0   0 FALSE FALSE
# 49 Chr1 2573   C
# 50                 1007  0   0  11 28   1  TRUE FALSE
# 51                 1012  0   0  30 50   1  TRUE FALSE
# 52                 1013  0   0 231 12   0  TRUE FALSE
# 53                 1014  0   0   4 18   1  TRUE FALSE
# 54                 1015  0   0  50 38   1  TRUE FALSE
# 55                 3367  0   0  71  0   0  TRUE FALSE
# 56                 1335  0   0  62 75   1  TRUE FALSE
# 57 Chr1 3938   G
# 58                 1007 12  20   0  0   0  TRUE FALSE
# 59                 1012  9  22   0  0   0  TRUE FALSE
# 60                 1013 35  19   0  0   0  TRUE FALSE
# 61                 1014  8   2   0  0   0  TRUE FALSE
# 62                 1015 25  53   0  0   0  TRUE FALSE
# 63                 3367 14  13   0  0   0  TRUE FALSE
# 64                 1335 59  42   0  0   0  TRUE FALSE
# 65 Chr1 4876   G
# 66                 1007  0   1   0  0   0 FALSE FALSE
# 67                 1012  1   4   0  0   0 FALSE FALSE
# 68                 1013  0   0   0  0   0 FALSE FALSE
# 69                 1014  1   0   0  0   0 FALSE FALSE
# 70                 1015  0   3   0  0   0 FALSE FALSE
# 71                 3367  4   4   0  0   0 FALSE FALSE
# 72                 1335  2   2   0  0   0 FALSE FALSE
# 73 Chr1 4938   T
# 74                 1007  0  43   0 23   1 FALSE FALSE
# 75                 1012  0  63   0 48   1 FALSE FALSE
# 76                 1013  0  83   0  2   0 FALSE FALSE
# 77                 1014  0  27   0  4   1 FALSE FALSE
# 78                 1015  0  75   0 47   1 FALSE FALSE
# 79                 3367  0  19   0 12   1 FALSE FALSE
# 80                 1335  0  57   0 59   1 FALSE FALSE

# one of those is a little weird:
xx<-snp.tables[[1]][149457,]
for (i in 2:7){xx <- rbind(xx,snp.tables[[i]][149457,])}
row.names(xx)<-names(snp.tables)
# My guess is that Chr/Pos/Ref are left as NA if coverage is zero.
xx

#      snp  Chr    Pos  Ref Cov a g c t n .match  exon indel  chr    pos rawCov
# 1007   0 <NA>     NA <NA>   0 0 0 0 0 0      0 FALSE FALSE <NA>     NA      0
# 1012   0 <NA>     NA <NA>   0 0 0 0 0 0      0 FALSE FALSE <NA>     NA      0
# 1013   0 <NA>     NA <NA>   0 0 0 0 0 0      0 FALSE FALSE <NA>     NA      0
# 1014   0 Chr1 149457    G   0 0 0 0 0 0      0 FALSE FALSE Chr1 149457      1
# 1015   0 <NA>     NA <NA>   0 0 0 0 0 0      0 FALSE FALSE <NA>     NA      0
# 3367   0 <NA>     NA <NA>   0 0 0 0 0 0      0 FALSE FALSE <NA>     NA      0
# 1335   0 Chr1 149457    G   0 0 0 0 0 0      0 FALSE FALSE Chr1 149457      1
```

5 Table 1 stats

Here is a brief summary of per-strain SNP counts, pairwise overlaps, and other conveniently available stats, such as those shown in Table 1 of the paper.

```
snp.counts     <- matrix(NA,7,4)
snp.pctofny    <- matrix(NA,7,4)
snp.pctofself <- matrix(NA,7,4)
snp.inter   <- matrix(NA,7,7)
snp.union   <- matrix(NA,7,7)
rownames(snp.counts)     <- names(snp.tables)
rownames(snp.pctofny)    <- names(snp.tables)
rownames(snp.pctofself) <- names(snp.tables)
rownames(snp.inter)   <- names(snp.tables)
colnames(snp.inter)   <- names(snp.tables)
rownames(snp.union)   <- names(snp.tables)
colnames(snp.union)   <- names(snp.tables)
for(stringency in 1:4){
  cat('\nStringency', stringency, ifelse(stringency==4,'(i.e. raw SAMTools SNP calls)',''),
      ':\n--------------\n')
  for(i in 1:7){
    f.snps.i <- refined.snps$Code$get.snps(i, stringency)
    snp.counts[i,stringency] <- sum(f.snps.i)
    for(j in i:7){
      f.snps.j <- refined.snps$Code$get.snps(j, stringency)
      snp.inter[i,j] <- sum(f.snps.i & f.snps.j)
      snp.union[i,j] <- sum(f.snps.i | f.snps.j)
    }
  }
  snp.pctofny  [,stringency] <- snp.inter[,7]/snp.counts[7,stringency]
  snp.pctofself[,stringency] <- snp.inter[,7]/snp.counts[ ,stringency]
  cat('Union Counts:\n');                  print(snp.union)
  cat('Intersect Counts:\n');              print(snp.inter)
  cat('Intersect as percent of union:\n'); print(snp.inter/snp.union*100,digits=3)
}

#
# Stringency 1  :
# --------------
# Union Counts:
#        1007   1012   1013   1014   1015   3367   1335
# 1007 184621 190979 363297 196256 197762 354191 199128
# 1012     NA 187793 364751 198002 198919 355526 200266
# 1013     NA     NA 296795 356666 366717 391621 364222
# 1014     NA     NA     NA 165741 196847 347035 195294
# 1015     NA     NA     NA     NA 191668 357845 198939
# 3367     NA     NA     NA     NA     NA 283086 355107
# 1335     NA     NA     NA     NA     NA     NA 187044
# Intersect Counts:
#        1007   1012   1013   1014   1015   3367   1335
# 1007 184621 181435 118119 154106 178527 113516 172537
# 1012     NA 187793 119837 155532 180542 115353 174571
# 1013     NA     NA 296795 105870 121746 188260 119617
# 1014     NA     NA     NA 165741 160562 101792 157491
# 1015     NA     NA     NA     NA 191668 116909 179773
# 3367     NA     NA     NA     NA     NA 283086 115023
# 1335     NA     NA     NA     NA     NA     NA 187044
# Intersect as percent of union:
#      1007 1012  1013  1014  1015  3367  1335
# 1007  100   95  32.5  78.5  90.3  32.0  86.6
# 1012   NA  100  32.9  78.6  90.8  32.4  87.2
# 1013   NA   NA 100.0  29.7  33.2  48.1  32.8
# 1014   NA   NA    NA 100.0  81.6  29.3  80.6
# 1015   NA   NA    NA    NA 100.0  32.7  90.4
# 3367   NA   NA    NA    NA    NA 100.0  32.4
# 1335   NA   NA    NA    NA    NA    NA 100.0
#
# Stringency 2  :
# --------------
# Union Counts:
#        1007   1012   1013   1014   1015   3367   1335
# 1007 181682 189049 374070 191223 196135 364611 195371
# 1012     NA 186199 376272 194060 197053 366680 196729
# 1013     NA     NA 304444 356961 378452 408556 373836
# 1014     NA     NA     NA 137771 193813 346571 187896
# 1015     NA     NA     NA     NA 190384 369360 195610
# 3367     NA     NA     NA     NA     NA 290844 364209
# 1335     NA     NA     NA     NA     NA     NA 180709
# Intersect Counts:
#        1007   1012   1013   1014   1015   3367   1335
# 1007 181682 178832 112056 128230 175931 107915 167020
# 1012     NA 186199 114371 129910 179530 110363 170179
# 1013     NA     NA 304444  85254 116376 186732 111317
# 1014     NA     NA     NA 137771 134342  82044 130584
# 1015     NA     NA     NA     NA 190384 111868 175483
# 3367     NA     NA     NA     NA     NA 290844 107344
# 1335     NA     NA     NA     NA     NA     NA 180709
# Intersect as percent of union:
```

```
#      1007  1012  1013  1014  1015  3367  1335
# 1007  100  94.6  30.0  67.1  89.7  29.6  85.5
# 1012   NA 100.0  30.4  66.9  91.1  30.1  86.5
# 1013   NA    NA 100.0  23.9  30.8  45.7  29.8
# 1014   NA    NA    NA 100.0  69.3  23.7  69.5
# 1015   NA    NA    NA    NA 100.0  30.3  89.7
# 3367   NA    NA    NA    NA    NA 100.0  29.5
# 1335   NA    NA    NA    NA    NA    NA 100.0
#
# Stringency 3  :
# --------------
# Union Counts:
#        1007   1012   1013   1014   1015   3367   1335
# 1007 169346 183708 363625 176590 190884 354428 188236
# 1012     NA 179828 368821 185220 192949 359446 191771
# 1013     NA     NA 296287 330697 371321 403242 364437
# 1014     NA     NA     NA  88328 187158 319824 176609
# 1015     NA     NA     NA     NA 184704 362666 191220
# 3367     NA     NA     NA     NA     NA 283373 355133
# 1335     NA     NA     NA     NA     NA     NA 171199
# Intersect Counts:
#        1007   1012   1013  1014   1015   3367   1335
# 1007 169346 165466 102008 81084 163166  98291 152309
# 1012     NA 179828 107294 82936 171583 103755 159256
# 1013     NA     NA 296287 53918 109670 176418 103049
# 1014     NA     NA     NA 88328  85874  51877  82918
# 1015     NA     NA     NA    NA 184704 105411 164683
# 3367     NA     NA     NA    NA     NA 283373  99439
# 1335     NA     NA     NA    NA     NA     NA 171199
# Intersect as percent of union:
#      1007  1012  1013  1014  1015  3367  1335
# 1007  100  90.1  28.1  45.9  85.5  27.7  80.9
# 1012   NA 100.0  29.1  44.8  88.9  28.9  83.0
# 1013   NA    NA 100.0  16.3  29.5  43.7  28.3
# 1014   NA    NA    NA 100.0  45.9  16.2  47.0
# 1015   NA    NA    NA    NA 100.0  29.1  86.1
# 3367   NA    NA    NA    NA    NA 100.0  28.0
# 1335   NA    NA    NA    NA    NA    NA 100.0
#
# Stringency 4 (i.e. raw SAMTools SNP calls) :
# --------------
# Union Counts:
#        1007   1012   1013   1014   1015   3367   1335
# 1007 161103 176738 343873 171675 185741 336599 180313
# 1012     NA 166089 346766 176177 186459 339458 182312
# 1013     NA     NA 247737 302322 352586 386037 339669
# 1014     NA     NA     NA  89184 179976 295574 162912
# 1015     NA     NA     NA     NA 174701 345396 184068
# 3367     NA     NA     NA     NA     NA 240413 331982
# 1335     NA     NA     NA     NA     NA     NA 153901
# Intersect Counts:
#        1007   1012   1013  1014   1015   3367   1335
# 1007 161103 150454  64967 78612 150063  64917 134691
# 1012     NA 166089  67060 79096 154331  67044 137678
# 1013     NA     NA 247737 34599  69852 102113  61969
# 1014     NA     NA     NA 89184  83909  34023  80173
# 1015     NA     NA     NA    NA 174701  69718 144534
# 3367     NA     NA     NA    NA     NA 240413  62332
# 1335     NA     NA     NA    NA     NA     NA 153901
# Intersect as percent of union:
#      1007  1012  1013  1014  1015  3367  1335
# 1007  100  85.1  18.9  45.8  80.8  19.3  74.7
# 1012   NA 100.0  19.3  44.9  82.8  19.8  75.5
# 1013   NA    NA 100.0  11.4  19.8  26.5  18.2
# 1014   NA    NA    NA 100.0  46.6  11.5  49.2
# 1015   NA    NA    NA    NA 100.0  20.2  78.5
# 3367   NA    NA    NA    NA    NA 100.0  18.8
# 1335   NA    NA    NA    NA    NA    NA 100.0

vs.stringency <- cbind(snp.counts, matrix(NA,7,1), round(snp.counts[,1:3]/snp.counts[,4]*100,1))
colnames(vs.stringency) <- c('[[1]]', '[[2]]', '[[3]]', '[[4]]', '----', '[[1]]%', '[[2]]%', '[[3]]%')

# SNPs vs filtering stringency (raw counts and as % of [[4]]).  Medium filter
# adds 10-20% in most cases.  Big exception is Gyre, where low coverage,
# high err rate and SAMTools conservatism seemed to seriously undercall:
print(vs.stringency)

#       [[1]]  [[2]]  [[3]]  [[4]] ---- [[1]]% [[2]]% [[3]]%
# 1007 184621 181682 169346 161103   NA  114.6  112.8  105.1
# 1012 187793 186199 179828 166089   NA  113.1  112.1  108.3
# 1013 296795 304444 296287 247737   NA  119.8  122.9  119.6
# 1014 165741 137771  88328  89184   NA  185.8  154.5   99.0
# 1015 191668 190384 184704 174701   NA  109.7  109.0  105.7
# 3367 283086 290844 283373 240413   NA  117.7  121.0  117.9
# 1335 187044 180709 171199 153901   NA  121.5  117.4  111.2

# Intersect NY as % of self (vs stringency):
print(snp.pctofself*100, digits=3)

#       [,1]  [,2]  [,3]  [,4]
# 1007  93.5  91.9  89.9  83.6
# 1012  93.0  91.4  88.6  82.9
# 1013  40.3  36.6  34.8  25.0
# 1014  95.0  94.8  93.9  89.9
# 1015  93.8  92.2  89.2  82.7
# 3367  40.6  36.9  35.1  25.9
# 1335 100.0 100.0 100.0 100.0

# Intersect NY as % of NY (vs stringency):
print(snp.pctofny*100, digits=3)

#       [,1]  [,2]  [,3]  [,4]
# 1007  92.2  92.4  89.0  87.5
# 1012  93.3  94.2  93.0  89.5
# 1013  64.0  61.6  60.2  40.3
# 1014  84.2  72.3  48.4  52.1
# 1015  96.1  97.1  96.2  93.9
# 3367  61.5  59.4  58.1  40.5
# 1335 100.0 100.0 100.0 100.0
```

Quick look at coverage. Are there any NA?:

```
nacount <- NULL
for(i in 1:4){
  if(!is.null(tset[[i]])){
    nacount <- rbind(nacount,
                     unlist(lapply(tset[[i]], function(x){sum(is.na(x$Cov))}))
                     )
    rownames(nacount)[nrow(nacount)] <- names(tset)[i]
  }
}
nacount

#                             1007 1012 1013 1014 1015 3367 1335
# snp.tables.full.unfiltered    0    0    0    0    0    0    0
# snp.tables.full.qfiltered     0    0    0    0    0    0    0
```

Seemingly no. What's average in unq- vs q-filtered:

```
snp.tables.unqfil <- tset.picker(c(1,2), table.set = tset)
snp.tables.qfil   <- tset.picker(c(3,4), table.set = tset)
cov.unqfil <- unlist(lapply(snp.tables.unqfil, function(x){mean(x$Cov)}))
cov.qfil   <- unlist(lapply(snp.tables.qfil,   function(x){mean(x$Cov,na.rm=T)}))
cov.both <- rbind(cov.unqfil,cov.qfil,cov.qfil/cov.unqfil)
i <- 1
if(!is.null(snp.tables.unqfil)){
  rownames(cov.both)[i] <- which.snp.tables(snp.tables.unqfil)
  i <- i+1
}
if(!is.null(snp.tables.qfil)){
  rownames(cov.both)[i] <- which.snp.tables(snp.tables.qfil)
  i <- i+1
}
if(i==3){
  rownames(cov.both)[i] <- 'Ratio'
}
cat('Mean Coverage:\n'); cov.both

# Mean Coverage:
#                        1007       1012       1013       1014       1015       3367        1335
# trunc-unfiltered 37.0555484 70.8060724 69.6610432 33.1009373 61.5365159 64.0284488 107.7425968
# trunc-qfiltered  28.2750286 51.3249686 45.4036337 13.7261052 48.7880005 44.8042054  81.8823765
# Ratio             0.7630444  0.7248668  0.6517794  0.4146742  0.7928301  0.6997547   0.7599815
```

5.1 Table 1 Data

Throw together the conveniently-available Table 1 data, *in Table 1 row order*:

```
# if coverage unavailable, build NA vector
if(!is.null(cov.unqfil)){cov.unqfilv <- cov.unqfil} else {cov.unqfilv <- rep(NA,times=7)}
if(!is.null(cov.qfil  )){cov.qfilv   <- cov.qfil  } else {cov.qfilv   <- rep(NA,times=7)}
t1data.df <- data.frame(
  id        = st.locs(1:7, id=T, loc=F, date=F),
  loc       = st.locs(1:7, id=F, loc=T, date=F),
  date      = st.locs(1:7, id=F, loc=F, date=T),
  cov.unq   = cov.unqfilv,
  cov.q     = cov.qfilv,
  SNPs.4    = snp.counts[,4],
  SNPs.2    = snp.counts[,2],
  olap.ny.4 = snp.pctofny[,4]*100,
  olap.ny.2 = snp.pctofny[,2]*100
)
t1row.order <- c(7,1,2,5,3,6,4)
print(t1data.df[t1row.order,],digits=3)

#            id             loc date cov.unq cov.q SNPs.4 SNPs.2 olap.ny.4 olap.ny.2
# 1335 CCMP1335        New York 1958   107.7  81.9 153901 180709     100.0     100.0
# 1007 CCMP1007        Virginia 1964    37.1  28.3 161103 181682      87.5      92.4
# 1012 CCMP1012    W. Australia 1965    70.8  51.3 166089 186199      89.5      94.2
# 1015 CCMP1015     Puget Sound 1985    61.5  48.8 174701 190384      93.9      97.1
# 1013 CCMP1013           Wales 1973    69.7  45.4 247737 304444      40.3      61.6
# 3367 CCMP3367           Italy 2007    64.0  44.8 240413 290844      40.5      59.4
# 1014 CCMP1014 N. Pacific Gyre 1971    33.1  13.7  89184 137771      52.1      72.3
```

6 Shared-SNPs P-Value

Text of the main paper quotes a "p-value" for the observed degree of SNP sharing in L-clade (and/or L-clade excluding Gyre) under a null model that these isolates were sampled from a population globally in Hardy-Weinberg equilibrium. Details of this analysis are as follows.

6.1 SNP Concordance

Arbitrarily pick one isolate, say, A, as the "template". Arbitrarily pick a heterozygous (aka "SNP") position in A. Let p_1, and $q_1 = 1 - p_1$ be the frequencies in the overall population of the two nucleotides observed at that position in A.

(Positions having 3 or 4 nucleotide variants segregating in the population are assumed to be negligibly rare.) Under the HWE null model, a second isolate B will also be heterozygous at the same position with probability $2p_1q_1 \leq 1/2$. Similarly, this position will be heterozygous in a third isolate C with the same probability, *independently*, and so on for isolates D and E. Overall, (assuming HWE) the probability that a heterozygous position in A is simultaneously heterozygous in the other 4 isolates is at most $1/2^4 = 1/16$. Continuing, suppose we pick a second heterozygous position in A, *on a different chromosome* with allele frequencies $p_2, q_2 = 1 - p_2$, say. Again assuming HWE, this position will be a SNP in all of B, C, D and E with probability $(2p_2q_2)^4 \leq 1/16$, and this is independent of the first position, since segregation on different chromosomes is unlinked. Repeat this at 24 heterozygous positions in A, one per chromosome. Then, the number of five-way concordant positions observed should be dominated by the number observed when sampling from a binomial distribution with parameters $n = 24$ and $p = 1/16$, i.e., we expect at most $1/16 = 6.25\%$ of positions to agree, or at most $24/16 = 1.5$ five-way concordant positions in total. In sharp contrast, choosing CCMP 1014 (North Pacific Gyre) as the template, we see many more five-way concordant positions than predicted under these assumptions:

```
gyre.count <- sum(snp.tables[[4]]$snp)
# NOTE: what we now calle "refined" SNPs were once called "filtered" SNPs and I have NOT tried
# to update variable names and annotation in the code below to reflect the terminology change...
# 'unfil.' => unfiltered for consistency; see below.
unfil.fiveway.count   <- sum( snp.tables[[4]]$snp * i4.snps)
unfil.fiveway.percent <- unfil.fiveway.count / gyre.count * 100
unfil.p.value <- pbinom(floor(unfil.fiveway.count/gyre.count*24)-1, 24, 1/16, lower.tail = FALSE)
consistency.comparison <-
  data.frame(
    fiveway.count   = unfil.fiveway.count,
    fiveway.percent = unfil.fiveway.percent,
    p.value         = unfil.p.value
  )
consistency.comparison

#   fiveway.count fiveway.percent      p.value
# 1         70687        79.25973 4.142632e-19
```

Namely, 89184 positions are called as SNPs in CCMP1014, of which 70687 or 79.2597327% are also called as SNPs in *all four* other L-clade isolates. 79.2597327% of 24 is 19.0223358, and the probability of seeing 19 or more "Heads" in 24 flips of a biased coin with $P(\text{Heads}) \leq 1/16$, i.e., our p-value under the HWE null hypothesis, is at most: $4.1426317 \times 10^{-19}$ based on this simple binomial model. This is obviously strong evidence against the null hypothesis.

This analysis is potentially overly-simplistic in four respects, addressed below.

1. "$2pq \leq 1/2$" is conservative. Neutral theory predicts that most variant nucleotides are rare in the population, so $2pq \ll 1/2$ is to be expected. This should make our quoted p-value very conservative.

2. Effect of Erroneous SNP calls. We base our analysis on *predicted* (by SAMTOOLS) heterozygous positions, not absolute-truth, which may affect our conclusions. However,

   - False negatives in A are irrelevant, since we never examine those positions. (This is the motivation for using CCMP1014 as the template; it has the lowest predicted SNP rate, likely due to a high false negative rate in that sequencing run. As noted elsewhere, it had the lowest coverage and lowest sequence quality of the 7 isolates, both of which impare SNP calling.)

   - False negatives in $BCDE$ make such positions appear *non*-concordant. For our purpose, this makes our statistic more conservative since it can only deflate a statistic that we argue is nevertheless unexpectedly large.

   - False positive calls in A are conservatively treated, as well: barring simultaneous false-positive calls in all of $BCDE$, such a position will appear non-concordant, again deflating the statistic. The *false* positive rates in B, C, D and E are unknown, but cannot exceed SAMTOOLS *total* positive rate, which is below 1% in all 7 isolates, suggesting a simultaneous $BCDE$ false positive rate $< 10^{-8}$, which will have a negligible effect.

- A potentially more serious issue is a true positive in A aligned to false positives in BCD and/or E. (I.e., a position that is polymorphic in the population and heterozygous in A, under the HWE null model is likely to be homozygous for one of the two alleles in one or more of $BCDE$; false positive SNP calls in all of those isolates would make the site appear concordant, i.e., provide evidence against the null model.) However, (a) my impression is that SAMTOOLS is more prone to false negative calls than to false positive calls (see Section 4), and (b) we would need a high rate of false positives to turn a truely heterozygous but non-concordant A call into a false "concordant" call—I'd expect at most half (especially given point 1 above) of $BCDE$ to be heterozygous, but all would need to be falsely declared heterozygous. Such a high false positive rate on $BCDE$ seems unlikely (see previous bullet), and would likely be counterbalanced by a similarly increased rate of false positives on A, which, as noted, tend to deflate our statistic (previous bullet again).
- Systematic errors. If there were, say, a sequence-context-dependent bias in the DNA sequencing, mapping and/or SNP-calling that tended to suggest (or hide) a SNP at some position, we're going to systematically over- (or under-) estimate concordant SNPs across isolates. The discordance of called SNPs between the L- and H-clades and within the H-clade suggests that this is not a major problem, but it is worth noting as a possibility.

3. Discordant nucleotides at "concordant" SNP positions. A "shared" SNP at a given position might be, say, G/C in one isolate vs T/C in another, reflecting an unexpected tri-allelic position in the population or a technical sequencing error. It is inappropriate to count such a "shared" SNP position as evidence against the null hypothesis, since it isn't clear that it is truely shared. Instead, I will identify such inconsistent positions, based on the "stringency [[2]]" criteria established above, and treat each as non-concordant. I.e., a position will be considered to be a "5-way concordant SNP" if and only if it was called as a SNP by SAMTOOLS (independently) in all 5 L-clade isolates, *and* shows the same dominant non-reference nucleotide in all 5, according to criteria [[2]] above. As it turns out, this correction has a very minor effect on the resulting p-value:

```
# 'unfil.' => Ignoring "consistency";  'fil.' => Filtering for "consistency":
fil.fiveway.count   <- sum((snp.tables[[4]]$snp * i4.snps)[union.snps == 1] & consistent[[2]])
fil.fiveway.percent <- fil.fiveway.count / gyre.count * 100
fil.p.value <- pbinom(floor(fil.fiveway.count/gyre.count*24)-1, 24, 1/16, lower.tail = FALSE)
# append new stats to previous table for easy comparison
consistency.comparison <-
  rbind(consistency.comparison,
        data.frame(
          fiveway.count   = fil.fiveway.count,
          fiveway.percent = fil.fiveway.percent,
          p.value         = fil.p.value
        )
  )
rownames(consistency.comparison) <- c('unfiltered', 'consistency.filtered')
consistency.comparison

#                      fiveway.count fiveway.percent      p.value
# unfiltered                   70687        79.25973 4.142632e-19
# consistency.filtered         69941        78.42326 1.976512e-17
```

In particular, it removes 0.8% of five-way consistent positions (only 746 of 70687 positions), and still shows a highly significant p-value.

4. "$P(E[X]) \neq E[P(X)]$". I'm expressing this poorly, but finding the p-value based on the *expected* number of concordant positions is somewhat non-standard. A more typical set-up would use the *actual* value of some statistic, then calculate the probability of observing a value that extreme (or more extreme) under the null model. The fundamental problem is that we have thousands of SNPs, but I don't see an easy way to use more than 24 of them at a time, because potential genetic linkage seemingly destroys statistical independence, which is key to most simple analyses. A somewhat more formal, but still non-standard, approach is the following. Suppose we randomly sample one SNP per chromosome and count the number X of them that are 5-way concordant. What I outlined above calculated the p-value based on $E[X]$, the expected value of X, i.e., $P(E[X])$. Alternatively, we can calculate $E[P(X)]$, the expected p-value. (They are not the same.) In effect, this averages the p-values that

would be seen over many different randomly-sampled sets of 24 SNPs. This is not difficult to calculate. First, the probability that we would observe $0 \leq i \leq 24$ concordant positions in a sample of 24, given that 78.42% of positions are concordant follows this binomial distribution:

```
x.equals.i.distribution <- dbinom(0:24, 24, fil.fiveway.percent/100)
print(x.equals.i.distribution, digits=3)

#  [1] 1.04e-16 9.04e-15 3.78e-13 1.01e-11 1.92e-10 2.80e-09 3.22e-08 3.01e-07 2.32e-06 1.50e-05
# [11] 8.18e-05 3.78e-04 1.49e-03 5.00e-03 1.43e-02 3.46e-02 7.07e-02 1.21e-01 1.71e-01 1.96e-01
# [21] 1.78e-01 1.23e-01 6.12e-02 1.93e-02 2.93e-03
```

Second, the p-value corresponding to $0 \leq i \leq 24$ observed concordant positions also follows a different binomial distribution:

```
p.val.of.x.equals.i <- c(1, pbinom(0:23, 24, 1/16, lower.tail = F))
print(p.val.of.x.equals.i, digits=3)

#  [1] 1.00e+00 7.88e-01 4.48e-01 1.87e-01 5.95e-02 1.49e-02 3.01e-03 4.99e-04 6.90e-05 8.02e-06
# [11] 7.89e-07 6.60e-08 4.72e-09 2.87e-10 1.49e-11 6.59e-13 2.46e-14 7.66e-16 1.98e-17 4.14e-19
# [21] 6.88e-21 8.70e-23 7.88e-25 4.56e-27 1.26e-29
```

Finally, the expected (or “average”) p-value is just the weighted average of the latter values, weighted by the former:

```
e.of.p.of.x <- sum(x.equals.i.distribution * p.val.of.x.equals.i)
e.of.p.of.x

# [1] 6.808209e-10
```

This is still highly significant, but weaker than the $P(E[X])$ analysis, basically because $X < E[X]$ has a fair probability of occurring, and the corresponding p-value $P(X)$ rises rapidly as X declines.

Another way to look at the numbers:

```
pvdf <- data.frame(x.density=x.equals.i.distribution,
                   x.cdf=cumsum(x.equals.i.distribution),
                   pval.of.x=p.val.of.x.equals.i)
print(pvdf, digits=4)

#    x.density     x.cdf pval.of.x
# 1  1.037e-16 1.037e-16 1.000e+00
# 2  9.043e-15 9.147e-15 7.875e-01
# 3  3.780e-13 3.871e-13 4.476e-01
# 4  1.008e-11 1.046e-11 1.869e-01
# 5  1.922e-10 2.027e-10 5.950e-02
# 6  2.795e-09 2.998e-09 1.490e-02
# 7  3.217e-08 3.517e-08 3.010e-03
# 8  3.007e-07 3.358e-07 4.994e-04
# 9  2.322e-06 2.658e-06 6.899e-05
# 10 1.500e-05 1.766e-05 8.015e-06
# 11 8.181e-05 9.947e-05 7.887e-07
# 12 3.784e-04 4.779e-04 6.603e-08
# 13 1.490e-03 1.968e-03 4.716e-09
# 14 4.999e-03 6.967e-03 2.875e-10
# 15 1.428e-02 2.124e-02 1.493e-11
# 16 3.459e-02 5.584e-02 6.590e-13
# 17 7.072e-02 1.266e-01 2.456e-14
# 18 1.210e-01 2.475e-01 7.662e-16
# 19 1.710e-01 4.185e-01 1.977e-17
# 20 1.963e-01 6.148e-01 4.143e-19
# 21 1.783e-01 7.931e-01 6.877e-21
# 22 1.235e-01 9.165e-01 8.701e-23
# 23 6.119e-02 9.777e-01 7.884e-25
# 24 1.934e-02 9.971e-01 4.556e-27
# 25 2.929e-03 1.000e+00 1.262e-29
```

E.g., row 9 in that table says that the concordance rate (78%) is so high that a sample of 24 SNPs will almost always have 9 or more five-way concordant positions (probability of fewer is only 2.658e-06), while under the null model, seeing 9 or more is very unlikely (probability at most 6.899e-05). ***AM I OFF-BY-ONE INTERPRETING ROW 9 HERE??***

6.2 Notes

In earlier drafts, an analog of the above analysis was based on the concordance of *refined* SNPs. This now seems to me to be questionable, since the "refined" SNP calling makes SNPs called across L-clade non-independent. OTOH, the above analysis seems valid: SAMTOOLS was run on each isolate independently, and likewise "criterion [[2]]" is evaluated independently in each strain, and is being used here solely to remove SNP predictions, not to add them. "Systematic errors" as outlined above remain a potential problem, but again discordance with/within H-clade suggests that this is of limited concern.

For completeness, I did a similar analysis including a sample of H-clade comparisons: Gyre vs Italy, NY vs Italy, NY vs Italy+Wales, and of Italy vs Wales. As expected, none of these show a statistically significant p-value, although the $\approx 40\%$ concordance in the 2-way comparisons, while $< 1/2$ as predicted, is a bit higher than I expected based on "neutral theory implies many rare variants." (I did not bother to include "criterion[[2]] filtering" in these calculations.)

```
# 'gi.twoway' => gyre vs italy 2-way concordance;
# 'ni.twoway' => new york vs italy 2-way concordance;
# not bothering with criterion[[2]] filtering
gi.twoway.count   <- sum(snp.tables[[4]]$snp * snp.tables[[6]]$snp)
gi.twoway.percent <- gi.twoway.count / gyre.count * 100
gi.p.value <- pbinom(floor(gi.twoway.count/gyre.count*24)-1, 24, 1/2, lower.tail = FALSE)
ny.count <- sum(snp.tables[[7]]$snp)
ni.twoway.count   <- sum(snp.tables[[7]]$snp * snp.tables[[6]]$snp)
ni.twoway.percent <- ni.twoway.count / ny.count * 100
ni.p.value <- pbinom(floor(ni.twoway.count/ny.count*24)-1, 24, 1/2, lower.tail = FALSE)
niw.threeway.count   <- sum(snp.tables[[7]]$snp * snp.tables[[6]]$snp * snp.tables[[3]]$snp)
niw.threeway.percent <- niw.threeway.count / ny.count * 100
niw.p.value <- pbinom(floor(niw.threeway.count/ny.count*24)-1, 24, 1/4, lower.tail = FALSE)
it.count <- sum(snp.tables[[6]]$snp)
iw.twoway.count   <- sum(snp.tables[[6]]$snp * snp.tables[[3]]$snp)
iw.twoway.percent <- iw.twoway.count / it.count * 100
iw.p.value <- pbinom(floor(iw.twoway.count/it.count*24)-1, 24, 1/2, lower.tail = FALSE)
consistency.comparison <-
  rbind(consistency.comparison,
        data.frame(
          fiveway.count   = c(gi.twoway.count,   ni.twoway.count,   niw.threeway.count,   iw.twoway.count),
          fiveway.percent = c(gi.twoway.percent, ni.twoway.percent, niw.threeway.percent, iw.twoway.percent),
          p.value         = c(gi.p.value,        ni.p.value,        niw.p.value,          iw.p.value)
        )
  )
colnames(consistency.comparison)[1:2] <- c('552232way.count', '552232way.percent') # old col names misleading
rownames(consistency.comparison)[3:6] <- c('gyre.vs.italy', 'new.york.vs.italy',    # new rows
                                           'ny.vs.it.plus.wales', 'it.vs.wales')
consistency.comparison

#                      552232way.count 552232way.percent      p.value
# unfiltered                     70687          79.25973 4.142632e-19
# consistency.filtered           69941          78.42326 1.976512e-17
# gyre.vs.italy                  34023          38.14922 9.242052e-01
# new.york.vs.italy              62332          40.50136 9.242052e-01
# ny.vs.it.plus.wales            35796          23.25911 7.533516e-01
# it.vs.wales                   102113          42.47399 8.462719e-01
```

6.3 P-Value: The Bottom Line

So, what to say in the body of the paper? $E[P(X)]$ is highly significant, and conservative, but complex to explain. $P(E[X])$ is simpler to explain, but may be criticized as misleading if we aren't very careful in that explanation. I'm slightly leaning towards the last option, but want to sleep on it and draft the key sentence or two before settling.

7 Sharing

The following analysis looks at the sharing patterns among the consistent SNPs. I assume that shared SNPs reflect shared ancestry, and that SNPs accumulate slowly over time. Then, in outline, the story is consistent with what we have seen in other analyses—there seem to be 3 groups: 1013 (Wales) in one, 3367 (Italy) in another, and the other 5 in a third, with some hints as to the order of divergence. A caveat is that in a sexual population, non-shared SNPs do not immediately imply non-shared ancestry; they may merely reflect Hardy-Weinberg capturing a homozygous state in one isolate vs the other. (Or read errors, etc.) Thus, if we are right that the H-isolates retain sex, then the large number of "private" SNPs in H may be at least partially due to HWE.

Analysis is broken into cases based on how many strains share a particular SNP.

7.1 Code

To categorize SNPs by sharing patterns, first convert the 7-way consistent sharing pattern into a 7-bit binary number, and tabulate based on that:

```
# convert (n x 7) 0-1 matrix to n vector of 0-127
tobin <- function(x){
  bin <- integer(nrow(x)) # initialized to 0
  for(i in 1:7){
    bin <- bin*2 + as.integer(x[,i]>0)
  }
  return(bin)
}

# get full set of patterns
snp.pattern.all <- lapply(non.refs,tobin)
# prune to just the consistent ones
snp.pattern <- snp.pattern.all
for(i in 1:3){
  snp.pattern[[i]][!consistent[[i]]] <- NA
}

# analogous to built-in ``table'' but simpler.  Count entries in an integer
# vector sharing values in a (smallish) range.  Result is a 2-column matrix with
# the shared values in col 1 and count of occurrences of that value in col 2.
# Out-of-range values cause subscript error.
mytable <- function(vec, therange=range(vec,na.rm=T)){
  counts <- matrix(0,nrow=therange[2]-therange[1]+1,ncol=2,dimnames=list(NULL,c('val','count')))
  counts[1:nrow(counts),1] <- therange[1]:therange[2]
  for(i in 1:length(vec)){
    if(!is.na(vec[i])){
      counts[vec[i]-therange[1]+1,2] <- counts[vec[i]-therange[1]+1,2] + 1
    }
  }
  return(counts)
}

pattern.counts <- lapply(snp.pattern, function(x){mytable(x,c(0,127))})
```

To display the results, build a data frame whose i-th row, $0 \leq i \leq 127$ shows one of the 128 possible sharing patterns, with counts of the numbers of consistent, shared SNPs with that pattern according to criteria c1-c3.

```
tobitvec <- function(x){
  bitvec <- integer(7)
  for(i in 7:1){
    bitvec[i] <- x %% 2
    x <- x %/% 2
  }
  return(bitvec)
}

flg <- function(x){
  return(ifelse(x==1,'X',''))
}

pat.summary <- function(listOfTbls){
  mydf <- data.frame(pat=0:127,sharedBy=NA,
                     tp1007='',tp1012='',tp1013='',tp1014='',tp1015='',tp3367='',tp1335='',
                     count1=NA,count2=NA,count3=NA,count4=NA,stringsAsFactors=F)

  for(i in 1:128){
    bvec <- tobitvec(i-1)
    mydf[i,'sharedBy']=sum(bvec)
    mydf[i,'tp1007']=flg(bvec[1])
    mydf[i,'tp1012']=flg(bvec[2])
    mydf[i,'tp1013']=flg(bvec[3])
    mydf[i,'tp1014']=flg(bvec[4])
    mydf[i,'tp1015']=flg(bvec[5])
    mydf[i,'tp3367']=flg(bvec[6])
    mydf[i,'tp1335']=flg(bvec[7])
  }

  for(i in 1:length(listOfTbls)){
    tbl <- listOfTbls[[i]]
    if(!is.null(tbl)){
      mydf[,9+i] <- tbl[,2]  ## count1/2/3/4 are columns 10/11/12/13 in mydf
      #for(j in 1:length(tbl)){
      #  k <- as.integer(rownames(tbl)[j]);
      #  mydf[k+1,9+i] <- tbl[j]  ## count1/2/3 are columns 10/11/12
      #}
    }
  }

  mydf$pat <-as.octmode(mydf$pat)  # display bit pattern in octal
  return(mydf)
}

pat.summaries <- pat.summary(pattern.counts)
```

7.2 Sanity Checks

Some sanity checking: table sums equal to number of consistent positions?

```
all(consistent.count == apply(pat.summaries[,10:13],2,sum))

# [1] TRUE
```

More sanity checking: visually inspect a pattern with small counts, specifically pattern 12, i.e., consistent SNPs shared by only strains 1014 and 1015 (2nd and 3 rows from bottom, binary code $12 = 2^3 + 2^2$). There are only 10 such positions on Chr1. Chr1 2524239 has pattern 12 under criteria c1 and c2 but not c3; Chr1 1088766 has in c2 only. Both look good. Neither position is a *called* SNP except in 1015. However, all but 1 nonreference read agree with the called SNP (the exception being one read in Wales). Both 1014 and 1015 have at least 2 non-reference reads, comprising at least 5% of coverage, and in both strains, those reads are on the same non-reference base, satisfying criterion c2. The other strains have higher coverage and/or lower non-reference counts, so they do not satisfy c2. Position 2524239 also satisfies c1, but not c3, since 2 reads out of 35 is below the 10% threshold. (It is pattern 4 inder c3, i.e., a SNP private to 1015.) Position 1088766 is also pattern 4 under c3 (2 reads out of 56 in 1335 is below both thresholds), and it is not consistent under c1, since the single A read in 1013 is discordant with the other non-reference reads.

```
unlist(lapply(snp.pattern,function(x){sum(x==12,na.rm=T)}))

# [1] 133 139 132 417

sp1 <- snp.pattern[[1]]==12
sp2 <- snp.pattern[[2]]==12
```

```
sp3 <- snp.pattern[[3]]==12
sp4 <- snp.pattern[[4]]==12
c(sum(sp1,na.rm=T), sum(sp2,na.rm=T), sum(sp3,na.rm=T), sum(sp4,na.rm=T))

# [1] 133 139 132 417

r1 <- rownames(non.refs[[1]])[which(sp1)]
r2 <- rownames(non.refs[[2]])[which(sp2)]
r3 <- rownames(non.refs[[3]])[which(sp3)]
r4 <- rownames(non.refs[[4]])[which(sp4)]

r2

#   [1] "Chr1:1799155"     "Chr2:713075"      "Chr2:1464209"     "Chr2:2406031"
#   [5] "Chr2:2480466"     "Chr2:2480532"     "Chr2:2480838"     "Chr2:2483322"
#   [9] "Chr2:2488863"     "Chr2:2489189"     "Chr2:2490933"     "Chr2:2492886"
#  [13] "Chr2:2492887"     "Chr2:2497794"     "Chr2:2500122"     "Chr2:2503000"
#  [17] "Chr2:2507585"     "Chr2:2507680"     "Chr2:2510117"     "Chr2:2513923"
#  [21] "Chr2:2515103"     "Chr2:2516669"     "Chr2:2516751"     "Chr2:2518558"
#  [25] "Chr2:2518653"     "Chr2:2518980"     "Chr2:2519285"     "Chr2:2519288"
#  [29] "Chr2:2519718"     "Chr2:2520984"     "Chr2:2521271"     "Chr2:2522648"
#  [33] "Chr2:2524223"     "Chr2:2524439"     "Chr2:2525160"     "Chr2:2525463"
#  [37] "Chr2:2527281"     "Chr2:2527916"     "Chr2:2528472"     "Chr2:2528769"
#  [41] "Chr2:2529076"     "Chr2:2529140"     "Chr2:2529684"     "Chr2:2530064"
#  [45] "Chr2:2530216"     "Chr2:2530239"     "Chr2:2530294"     "Chr2:2530768"
#  [49] "Chr2:2530896"     "Chr2:2531114"     "Chr2:2531498"     "Chr2:2531567"
#  [53] "Chr2:2532173"     "Chr2:2532365"     "Chr2:2533028"     "Chr2:2533171"
#  [57] "Chr2:2533440"     "Chr2:2534441"     "Chr2:2535121"     "Chr2:2535122"
#  [61] "Chr2:2535314"     "Chr2:2535493"     "Chr2:2535503"     "Chr2:2535509"
#  [65] "Chr2:2535862"     "Chr2:2536242"     "Chr2:2537201"     "Chr2:2537864"
#  [69] "Chr2:2537917"     "Chr2:2538072"     "Chr2:2538498"     "Chr2:2539318"
#  [73] "Chr2:2543595"     "Chr2:2545615"     "Chr2:2545798"     "Chr2:2546865"
#  [77] "Chr2:2546991"     "Chr2:2547055"     "Chr2:2547086"     "Chr2:2547120"
#  [81] "Chr2:2547155"     "Chr2:2547212"     "Chr2:2547248"     "Chr2:2547318"
#  [85] "Chr2:2547554"     "Chr2:2547938"     "Chr2:2547944"     "Chr2:2548131"
#  [89] "Chr2:2549281"     "Chr2:2551574"     "Chr2:2551930"     "Chr2:2554708"
#  [93] "Chr2:2554860"     "Chr2:2555005"     "Chr2:2555203"     "Chr2:2555820"
#  [97] "Chr3:192441"      "Chr3:496665"      "Chr4:1086589"     "Chr4:1393682"
# [101] "Chr4:1747983"     "Chr4:2314475"     "Chr5:7509"        "Chr5:141375"
# [105] "Chr5:1071721"     "Chr6:1330532"     "Chr7:399475"      "Chr7:1736991"
# [109] "Chr7:1813303"     "Chr8:556556"      "Chr10:54351"      "Chr10:95217"
# [113] "Chr10:947088"     "Chr11a:344258"    "Chr11b:75778"     "Chr12:214112"
# [117] "Chr12:458461"     "Chr12:507608"     "Chr13:96361"      "Chr13:375598"
# [121] "Chr14:284131"     "Chr15:417704"     "Chr16a:39914"     "Chr16a:177501"
# [125] "Chr16a:206719"    "Chr16a:394030"    "Chr17:461465"     "Chr19a_19:300076"
# [129] "Chr19a_19:303090" "Chr19c_29:64170"  "Chr19c_29:64811"  "Chr19c_29:65720"
# [133] "Chr20:230994"     "Chr20:486431"     "Chr20:519835"     "Chr22:380816"
# [137] "Chr23:190382"     "Chr23:274291"     "Chr24:114599"

c1 <- as.integer(unlist(lapply(strsplit(r1[1:min(20,length(r1))],':',fixed=TRUE),function(x){x[2]})))
c2 <- as.integer(unlist(lapply(strsplit(r2[1:min(20,length(r2))],':',fixed=TRUE),function(x){x[2]})))
c3 <- as.integer(unlist(lapply(strsplit(r3[1:min(20,length(r3))],':',fixed=TRUE),function(x){x[2]})))
c4 <- as.integer(unlist(lapply(strsplit(r4[1:min(20,length(r4))],':',fixed=TRUE),function(x){x[2]})))

c1

#  [1]  614335  914018 1317406 2388286   62676  713075 2406031 2480466 2480838 2481998 2483322
# [12] 2488863 2489189 2490933 2492887 2497794 2500122 2503000 2507585 2507680

c2

#  [1] 1799155  713075 1464209 2406031 2480466 2480532 2480838 2483322 2488863 2489189 2490933
# [12] 2492886 2492887 2497794 2500122 2503000 2507585 2507680 2510117 2513923

c3

#  [1]  371484  518347 1210354 2209068 2264683 2898352 1276745 1464904 1464905 1766966 2347253
# [12] 2406031 2480532 2480838 2483322 2488863 2489189 2490933 2497794 2507585
```

```
c4

#  [1]  518347  691730  767408 1049906 1390437 2072951 2254059 2254789 2264683 2823796 2898352
# [12] 2998868   77394   77407  155680  761325  968120 1182096 1222176 1264023

seecounts(c2,snp.tables=snp.tables)

#      chr     pos Ref Strain   A  G   C   T SNP  exon indel nrf rat
# 1   Chr1 1799155   C
# 2                      1007   0  0  10   1   0  TRUE FALSE
# 3                      1012   0  0  16   1   0  TRUE FALSE
# 4                      1013   0  0  10   0   0  TRUE FALSE
# 5                      1014   0  0   8   2   0  TRUE FALSE
# 6                      1015   0  0  12   3   1  TRUE FALSE
# 7                      3367   1  0   1   1   1  TRUE FALSE
# 8                      1335   0  0   7   1   0  TRUE FALSE
# 9   Chr1  713075   T
# 10                     1007   0  0   0  37   0  TRUE FALSE
# 11                     1012   0  0   0  90   0  TRUE FALSE
# 12                     1013   0  0   0  65   0  TRUE FALSE
# 13                     1014   0  0   0  32   0  TRUE FALSE
# 14                     1015   0  0   0  84   0  TRUE FALSE
# 15                     3367   0  0   0  53   0  TRUE FALSE
# 16                     1335   0  0   0 109   0  TRUE FALSE
# 17  Chr1 1464209   T
# 18                     1007   0  0   0  22   0 FALSE FALSE
# 19                     1012   0  0   0  38   0 FALSE FALSE
# 20                     1013   0  0   0  22   0 FALSE FALSE
# 21                     1014   0  0   0  12   0 FALSE FALSE
# 22                     1015   0  0   0  30   0 FALSE FALSE
# 23                     3367   0  0   0  39   0 FALSE FALSE
# 24                     1335   0  0   0  81   0 FALSE FALSE
# 25  Chr1 2406031   C
# 26                     1007   0  0  18   0   0  TRUE FALSE
# 27                     1012   0  0  23   0   0  TRUE FALSE
# 28                     1013   0  0  46   0   0  TRUE FALSE
# 29                     1014   0  0  13   0   0  TRUE FALSE
# 30                     1015   0  0  34   0   0  TRUE FALSE
# 31                     3367   0  0  29   0   0  TRUE FALSE
# 32                     1335   0  0  68   0   0  TRUE FALSE
# 33  Chr1 2480466   A
# 34                     1007  26  0   0   0   0  TRUE FALSE
# 35                     1012  42  0   0   0   0  TRUE FALSE
# 36                     1013  39  0   0   0   0  TRUE FALSE
# 37                     1014   9  0   0   0   0  TRUE FALSE
# 38                     1015  49  0   0   0   0  TRUE FALSE
# 39                     3367  32  0   0   0   0  TRUE FALSE
# 40                     1335  77  0   0   0   0  TRUE FALSE
# 41  Chr1 2480532   G
# 42                     1007   0 25   0   0   0  TRUE FALSE
# 43                     1012   0 27   0   0   0  TRUE FALSE
# 44                     1013   0 43   0   0   0  TRUE FALSE
# 45                     1014   0  1   0   0   0  TRUE FALSE
# 46                     1015   0 23   0   0   0  TRUE FALSE
# 47                     3367   0 23   0   0   0  TRUE FALSE
# 48                     1335   0 71   0   0   0  TRUE FALSE
# 49  Chr1 2480838   T
# 50                     1007   0  0   0   8   0  TRUE FALSE
# 51                     1012   0  0   0  12   0  TRUE FALSE
# 52                     1013   0  0   0  24   0  TRUE FALSE
# 53                     1014   0  0   0   6   0  TRUE FALSE
# 54                     1015   0  0   0  15   0  TRUE FALSE
# 55                     3367   0  0   0   9   0  TRUE FALSE
# 56                     1335   0  0   0  81   0  TRUE FALSE
# 57  Chr1 2483322   A
# 58                     1007  22  0   0   0   0  TRUE FALSE
# 59                     1012  23  0   0   0   0  TRUE FALSE
# 60                     1013  52  0   0   0   0  TRUE FALSE
# 61                     1014  24  0   0   0   0  TRUE FALSE
# 62                     1015  55  0   0   0   0  TRUE FALSE
# 63                     3367  37  0   0   0   0  TRUE FALSE
# 64                     1335  82  0   0   0   0  TRUE FALSE
# 65  Chr1 2488863   C
# 66                     1007   0  0  26   0   0 FALSE FALSE
# 67                     1012   0  0  34   0   0 FALSE FALSE
# 68                     1013   0  0  27   0   0 FALSE FALSE
# 69                     1014   0  0  11   0   0 FALSE FALSE
# 70                     1015   0  0  34   0   0 FALSE FALSE
# 71                     3367   0  0  43   0   0 FALSE FALSE
# 72                     1335   0  0  71   0   0 FALSE FALSE
# 73  Chr1 2489189   C
# 74                     1007   0  0  32   0   0 FALSE FALSE
# 75                     1012   0  0  63   0   0 FALSE FALSE
# 76                     1013   0  0  44   0   0 FALSE FALSE
# 77                     1014   0  0  26   0   0 FALSE FALSE
# 78                     1015   0  0  59   0   0 FALSE FALSE
# 79                     3367   0  0  24   0   0 FALSE FALSE
# 80                     1335   0  0 110   0   0 FALSE FALSE
# 81  Chr1 2490933   G
# 82                     1007   0 25   0   0   0 FALSE FALSE
# 83                     1012   0 57   0   0   0 FALSE FALSE
# 84                     1013   0 40   0   0   0 FALSE FALSE
# 85                     1014   0  9   0   0   0 FALSE FALSE
# 86                     1015   0 36   0   0   0 FALSE FALSE
# 87                     3367   0 37   0   1   0 FALSE FALSE
# 88                     1335   0 57   0   0   0 FALSE FALSE
# 89  Chr1 2492886   T
# 90                     1007   0  0   0  27   0 FALSE FALSE
# 91                     1012   0  0   0  61   0 FALSE FALSE
# 92                     1013   0  0   0  41   0 FALSE FALSE
# 93                     1014   0  0   0  18   0 FALSE FALSE
# 94                     1015   0  0   0  53   0 FALSE FALSE
# 95                     3367   0  0   0  48   0 FALSE FALSE
# 96                     1335   0  0   0  80   0 FALSE FALSE
# 97  Chr1 2492887   G
# 98                     1007   0 22   0   0   0 FALSE FALSE
# 99                     1012   0 61   0   0   0 FALSE FALSE
# 100                    1013   0 35   0   0   0 FALSE FALSE
# 101                    1014   0 17   0   0   0 FALSE FALSE
# 102                    1015   0 55   0   0   0 FALSE FALSE
# 103                    3367   0 50   0   0   0 FALSE FALSE
# 104                    1335   0 85   0   0   0 FALSE FALSE
# 105 Chr1 2497794   T
# 106                    1007   0  0   0  35   0  TRUE FALSE
# 107                    1012   0  0   0  60   0  TRUE FALSE
# 108                    1013   0  0   0  58   0  TRUE FALSE
# 109                    1014   0  0   0  12   0  TRUE FALSE
# 110                    1015   0  0   0  64   0  TRUE FALSE
# 111                    3367   0  0   0  43   0  TRUE FALSE
# 112                    1335   0  0   0 107   0  TRUE FALSE
# 113 Chr1 2500122   A
# 114                    1007  18  0   0   0   0 FALSE FALSE
# 115                    1012  47  0   0   0   0 FALSE FALSE
# 116                    1013  34  0   0   0   0 FALSE FALSE
# 117                    1014   6  0   0   0   0 FALSE FALSE
# 118                    1015  35  0   0   0   0 FALSE FALSE
# 119                    3367  27  0   0   0   0 FALSE FALSE
# 120                    1335  51  0   0   0   0 FALSE FALSE
# 121 Chr1 2503000   T
# 122                    1007   0  0   0  29   0 FALSE FALSE
# 123                    1012   0  0   0  35   0 FALSE FALSE
# 124                    1013   0  0   0  57   0 FALSE FALSE
# 125                    1014   0  0   0  10   0 FALSE FALSE
# 126                    1015   0  0   0  34   0 FALSE FALSE
# 127                    3367   0  0   0  41   0 FALSE FALSE
# 128                    1335   0  0   0  28   0 FALSE FALSE
# 129 Chr1 2507585   A
# 130                    1007  34  0   0   0   0  TRUE FALSE
# 131                    1012  55  0   0   0   0  TRUE FALSE
# 132                    1013  32  0   0   0   0  TRUE FALSE
# 133                    1014  13  0   0   0   0  TRUE FALSE
# 134                    1015  41  0   0   0   0  TRUE FALSE
# 135                    3367  61  0   0   0   0  TRUE FALSE
# 136                    1335 104  0   0   0   0  TRUE FALSE
# 137 Chr1 2507680   A
# 138                    1007  26  0   0   0   0 FALSE FALSE
# 139                    1012  46  0   0   0   0 FALSE FALSE
# 140                    1013  32  0   0   0   0 FALSE FALSE
# 141                    1014  15  0   0   0   0 FALSE FALSE
# 142                    1015  54  0   0   0   0 FALSE FALSE
# 143                    3367  51  0   0   0   0 FALSE FALSE
# 144                    1335  78  0   0   0   0 FALSE FALSE
# 145 Chr1 2510117   C
# 146                    1007   0  0  19   0   0  TRUE FALSE
# 147                    1012   0  0  56   1   0  TRUE FALSE
# 148                    1013   0  0  42   0   0  TRUE FALSE
# 149                    1014   0  0  13   0   0  TRUE FALSE
# 150                    1015   0  0  39   0   0  TRUE FALSE
# 151                    3367   0  0  36   0   0  TRUE FALSE
# 152                    1335   0  0  92   0   0  TRUE FALSE
# 153 Chr1 2513923   A
# 154                    1007  39  0   0   0   0 FALSE FALSE
# 155                    1012  57  0   0   0   0 FALSE FALSE
# 156                    1013  23  0   0   0   0 FALSE FALSE
# 157                    1014   4  0   0   0   0 FALSE FALSE
# 158                    1015  39  0   0   0   0 FALSE FALSE
# 159                    3367  53  0   0   0   0 FALSE FALSE
# 160                    1335  53  0   0   0   0 FALSE FALSE
```

Position 1088766, however, in a good example of the situation that motivated this analysis—one strain has a G/C SNP and 5 of the other 6 strains have nonreference reads consistent with that SNP. Although, excluding 1015, the nonreference read counts are not high enough to justify a SNP call in any strain considered in isolation, the fact that they *consistently* agree with the 1015 SNP suggests that they are real. One alternative hypothesis is that there is some sequence-dependent bias at this locus that favors misreading a G as a C. On the other hand, one could equally well posit a shared SNP, and a locus-dependant bias that *supresses* C reads, explaining the unbalanced readout that we observe. However, it is hard to reconcile either view with the significant strain-specific patterns that we see in the shared SNPs (as seen below). I think a more likely explanation is that (a) there are some number of relatively rare SNPs present in each of the sampled populations, (b) some of these SNPs happened to be present in one or two cells of the roughly 5-10 cells that we believe constituted the founding population of the culture grown for sequencing, and (c) stochastic effects during culture growth and during sequencing may have further perturbed the apparent frequency of each variant, but the bottom line is that the above-threshold presence of consistent non-reference reads is evidence for shared SNPs at the population level (and the proportions of such reads represent estimates of the population-level frequencies of the variants, albeit a noisy estimate at any specific position).

An aside: I was curious to see whether there is any consistent pattern to positions that are called consistent SNPs in all but Italy, so I repeated the above, basically. My summary is that coverage in Italy tends to be below average in these positions, but otherwise they don't stand out. For the record:

```
abit <- snp.pattern[[2]]==125
abit[is.na(abit)]<-F
sum(abit)

# [1] 13630

rabit <- rownames(non.refs[[2]])[which(abit)]
rabits <- rabit[1:20]
cabit <- as.integer(unlist(lapply(strsplit(rabits,':',fixed=TRUE),function(x){x[2]})))
cabit

#  [1]  1244  1575  6485  7181  7220  7661  8144  8208  8518  8552  8567  8670  8685 14361 15254
# [16] 15280 16103 25546 30784 33852
```

```
seecounts(cabit,snp.tables=snp.tables)

#      chr   pos Ref Strain  A  G   C  T SNP  exon indel nrf rat
# 1   Chr1  1244   G
# 2                    1007  2 25   0  0   0  TRUE FALSE
# 3                    1012  3 32   0  0   0  TRUE FALSE
# 4                    1013 10 24   0  0   1  TRUE FALSE
# 5                    1014  3 17   0  0   0  TRUE FALSE
# 6                    1015 15 43   0  0   1  TRUE FALSE
# 7                    3367  0  1   0  0   0  TRUE FALSE
# 8                    1335 82 65   0  0   1  TRUE FALSE
# 9   Chr1  1575   G
# 10                   1007 24  7   0  0   0  TRUE FALSE
# 11                   1012 42 13   0  0   0  TRUE FALSE
# 12                   1013 17 16   0  0   0  TRUE FALSE
# 13                   1014 15  4   0  0   0  TRUE FALSE
# 14                   1015 43 31   0  0   1  TRUE FALSE
# 15                   3367  0  2   0  0   0  TRUE FALSE
# 16                   1335 34 74   0  0   0  TRUE FALSE
# 17  Chr1  6485   G
# 18                   1007 24 19   0  0   0  TRUE FALSE
# 19                   1012 29 29   0  0   0  TRUE FALSE
# 20                   1013 49 33   0  0   0  TRUE FALSE
# 21                   1014  6  5   0  0   0  TRUE FALSE
# 22                   1015 31 32   0  0   1  TRUE FALSE
# 23                   3367  0 37   0  0   0  TRUE FALSE
# 24                   1335 62 52   0  0   0  TRUE FALSE
# 25  Chr1  7181   G
# 26                   1007  0 30  29  0   0  TRUE FALSE
# 27                   1012  0 52  34  0   0  TRUE FALSE
# 28                   1013  0 19  72  0   0  TRUE FALSE
# 29                   1014  0 13   7  0   0  TRUE FALSE
# 30                   1015  0 40  33  0   1  TRUE FALSE
# 31                   3367  0 29   0  0   0  TRUE FALSE
# 32                   1335  0 78  73  0   0  TRUE FALSE
# 33  Chr1  7220   C
# 34                   1007 16  0  19  6   0  TRUE FALSE
# 35                   1012 38  0  22 11   0  TRUE FALSE
# 36                   1013 82  1  30  9   0  TRUE FALSE
# 37                   1014 12  0   6  2   0  TRUE FALSE
# 38                   1015 55  0  22  5   1  TRUE FALSE
# 39                   3367  0  0   8  0   0  TRUE FALSE
# 40                   1335 55  0  32 20   0  TRUE FALSE
# 41  Chr1  7661   T
# 42                   1007  0  0   9  9   0  TRUE FALSE
# 43                   1012  0  0   5 19   0  TRUE FALSE
# 44                   1013  0  0  24 14   1  TRUE FALSE
# 45                   1014  0  0   6  3   0  TRUE FALSE
# 46                   1015  0  0   5 34   0  TRUE FALSE
# 47                   3367  0  0   0  4   0  TRUE FALSE
# 48                   1335  0  0   4 24   0  TRUE FALSE
# 49  Chr1  8144   G
# 50                   1007  8  9   0  0   0  TRUE FALSE
# 51                   1012 12 10   0  0   1  TRUE FALSE
# 52                   1013 38 29   0  0   0  TRUE FALSE
# 53                   1014  5  4   0  0   0  TRUE FALSE
# 54                   1015 15 16   0  0   0  TRUE FALSE
# 55                   3367  0  0   0  0   0  TRUE FALSE
# 56                   1335 12 15   0  0   1  TRUE FALSE
# 57  Chr1  8208   G
# 58                   1007  0  6   0  7   1  TRUE FALSE
# 59                   1012  0 19   0 11   0  TRUE FALSE
# 60                   1013  0  1   0 48   0  TRUE FALSE
# 61                   1014  0  5   0  3   0  TRUE FALSE
# 62                   1015  0 19   0 11   1  TRUE FALSE
# 63                   3367  0  1   0  0   0  TRUE FALSE
# 64                   1335  0 28   0 16   1  TRUE FALSE
# 65  Chr1  8518   T
# 66                   1007  0  0  20 15   1 FALSE FALSE
# 67                   1012  0  0  40 20   1 FALSE FALSE
# 68                   1013  0  0  45 56   1 FALSE FALSE
# 69                   1014  0  0  10 16   0 FALSE FALSE
# 70                   1015  0  0  36 13   1 FALSE FALSE
# 71                   3367  0  0   0  2   0 FALSE FALSE
# 72                   1335  0  0 113 53   1 FALSE FALSE
# 73  Chr1  8552   G
# 74                   1007  3  9   0  0   0  TRUE FALSE
# 75                   1012 20 21   0  0   0  TRUE FALSE
# 76                   1013 28 16   0  0   1  TRUE FALSE
# 77                   1014  6  2   0  0   0  TRUE FALSE
# 78                  1015 14 13   0  0   0  TRUE FALSE
# 79                  3367  0 12   0  0   0  TRUE FALSE
# 80                  1335 24 47   0  0   0  TRUE FALSE
# 81  Chr1  8567   A
# 82                  1007 14 18   0  0   1  TRUE FALSE
# 83                  1012 26 30   0  0   1  TRUE FALSE
# 84                  1013 50 66   0  0   1  TRUE FALSE
# 85                  1014  1  3   0  0   0  TRUE FALSE
# 86                  1015 12 31   0  0   1  TRUE FALSE
# 87                  3367 22  0   0  0   0  TRUE FALSE
# 88                  1335 51 40   0  0   1  TRUE FALSE
# 89  Chr1  8670   A
# 90                  1007  7  0   0  5   0  TRUE FALSE
# 91                  1012 16  0   0 10   0  TRUE FALSE
# 92                  1013 16  0   0 11   0  TRUE FALSE
# 93                  1014  2  0   0  4   0  TRUE FALSE
# 94                  1015 14  0   0 10   1  TRUE FALSE
# 95                  3367  5  0   0  0   0  TRUE FALSE
# 96                  1335  7  0   0  6   0  TRUE FALSE
# 97  Chr1  8685   G
# 98                  1007  6 15   0  0   0  TRUE FALSE
# 99                  1012 10 23   0  0   0  TRUE FALSE
# 100                 1013 18 21   0  0   1  TRUE FALSE
# 101                 1014  4  8   0  0   0  TRUE FALSE
# 102                 1015 10 24   0  0   1  TRUE FALSE
# 103                 3367  0  4   0  0   0  TRUE FALSE
# 104                 1335  5 32   0  0   0  TRUE FALSE
# 105 Chr1 14361   A
# 106                 1007 20  7   0  0   0 FALSE FALSE
# 107                 1012 35  5   0  0   0 FALSE FALSE
# 108                 1013  1 11   0  0   1 FALSE FALSE
# 109                 1014  6  2   0  0   0 FALSE FALSE
# 110                 1015 35  7   0  0   0 FALSE FALSE
# 111                 3367  2  1   0  0   0 FALSE FALSE
# 112                 1335 50  8   0  0   0 FALSE FALSE
# 113 Chr1 15254   T
# 114                 1007 11  0   0 16   1 FALSE FALSE
# 115                 1012 26  0   0 38   1 FALSE FALSE
# 116                 1013 37  0   0 48   1 FALSE FALSE
# 117                 1014  3  0   0  8   1 FALSE FALSE
# 118                 1015 18  0   0 32   1 FALSE FALSE
# 119                 3367  0  0   0 73   0 FALSE FALSE
# 120                 1335 13  0   0 32   1 FALSE FALSE
# 121 Chr1 15280   T
# 122                 1007  0 13   0 20   1 FALSE FALSE
# 123                 1012  0 27   0 28   1 FALSE FALSE
# 124                 1013  0  5   0 64   0 FALSE FALSE
# 125                 1014  0  2   0  8   0 FALSE FALSE
# 126                 1015  0 19   0 29   1 FALSE FALSE
# 127                 3367  0  0   0 42   0 FALSE FALSE
# 128                 1335  0 21   0 70   1 FALSE FALSE
# 129 Chr1 16103   A
# 130                 1007 10  0  11  0   1 FALSE FALSE
# 131                 1012 44  0  19  0   1 FALSE FALSE
# 132                 1013 21  0  13  0   1 FALSE FALSE
# 133                 1014 14  0   2  0   0 FALSE FALSE
# 134                 1015 29  0  10  0   1 FALSE FALSE
# 135                 3367 33  0   0  0   0 FALSE FALSE
# 136                 1335 47  0  11  0   0 FALSE FALSE
# 137 Chr1 25546   A
# 138                 1007 23  0   0 14   1 FALSE FALSE
# 139                 1012 46  0   0 19   1 FALSE FALSE
# 140                 1013  6  0   0 42   1 FALSE FALSE
# 141                 1014  7  0   0 15   1 FALSE FALSE
# 142                 1015 52  0   0 17   1 FALSE FALSE
# 143                 3367 60  0   0  0   0 FALSE FALSE
# 144                 1335 67  0   0  5   0 FALSE FALSE
# 145 Chr1 30784   C
# 146                 1007 16  0  13  0   1  TRUE FALSE
# 147                 1012 33  0  32  0   1  TRUE FALSE
# 148                 1013 19  0  33  0   1  TRUE FALSE
# 149                 1014  4  0  11  0   1  TRUE FALSE
# 150                 1015 39  0  29  0   1  TRUE FALSE
# 151                 3367  0  0  55  0   0  TRUE FALSE
# 152                 1335 46  0  50  0   1  TRUE FALSE
# 153 Chr1 33852   C
# 154                 1007  0 24  25  0   1 FALSE FALSE
# 155                 1012  0 18  26  0   1 FALSE FALSE
# 156                 1013  0 28  33  0   1 FALSE FALSE
# 157                 1014  0  9   4  0   1 FALSE FALSE
# 158                 1015  0 19  28  0   1 FALSE FALSE
# 159                 3367  0  0  26  0   0 FALSE FALSE
# 160                 1335  0 30  53  0   1 FALSE FALSE
```

More sanity: there are 83 sites on Chr1 shared by zero strains in the tightest condition. (I.e., SAMTOOLS called it a SNP, but the read counts/proportions fall below our 3rd threshold). Are they due to low coverage? Seemingly yes:

```
zp3 <- snp.pattern[[3]] == 0
zr3 <- rownames(non.refs[[3]])[which(zp3)]
zc3 <- as.integer(unlist(lapply(strsplit(zr3[1:min(100,length(zr3))],':',fixed=TRUE),function(x){x[2]})))
zc3

#   [1]  16115  16615  19117  25748  43500  55857  56591  65787  66879  68328  80862  81001  90622
#  [14]  90721  91284 110754 116443 116453 120183 126702 127986 129056 147698 153874 159756 160912
#  [27] 161271 170686 180314 181477 182139 196862 196864 199166 206132 206143 221888 234931 242276
#  [40] 242914 244505 268954 274655 282391 282511 283646 289363 311952 312625 314132 326217 371008
#  [53] 376784 387078 387091 389263 395153 406158 410771 431788 438958 438976 443898 447253 448223
#  [66] 452774 488812 495476 498133 501830 501975 504462 506422 515441 515595 530113 530114 532320
#  [79] 534149 541667 543095 575081 585297 586276 612732 622585 651159 652889 655373 655380 657704
#  [92] 657955 658216 685697 687653 692115 692139 700484 700845 701061

seecounts(zc3[1:5], snp.tables=snp.tables)

#     chr   pos Ref Strain  A G  C  T SNP  exon indel nrf rat
# 1  Chr1 16115   T
# 2                   1007  0 0  0  5   0 FALSE FALSE
# 3                   1012  0 0  0  9   0 FALSE FALSE
# 4                   1013  0 0  0  6   0 FALSE FALSE
# 5                   1014  0 0  0  3   0 FALSE FALSE
# 6                   1015  0 0  0 10   0 FALSE FALSE
# 7                   3367  0 0  3  3   1 FALSE FALSE
# 8                   1335  0 0  0  6   0 FALSE FALSE
# 9  Chr1 16615   C
# 10                  1007  0 0 39  0   0 FALSE FALSE
# 11                  1012  0 0 54  0   0 FALSE FALSE
# 12                  1013  0 0  4  2   1 FALSE FALSE
# 13                  1014  0 0 19  0   0 FALSE FALSE
# 14                  1015  0 0 46  0   0 FALSE FALSE
# 15                  3367  0 0 13  0   0 FALSE FALSE
# 16                  1335  0 0 40  0   0 FALSE FALSE
# 17 Chr1 19117   A
# 18                  1007 16 0  0  0   0  TRUE FALSE
# 19                  1012 21 0  0  0   0  TRUE FALSE
# 20                  1013  1 0  0  1   0  TRUE FALSE
# 21                  1014  6 0  0  0   0  TRUE FALSE
# 22                  1015 21 0  0  0   0  TRUE FALSE
# 23                  3367  0 0  0  1   1  TRUE FALSE
# 24                  1335 24 0  0  0   0  TRUE FALSE
# 25 Chr1 25748   C
# 26                  1007  0 0 17  0   0 FALSE FALSE
# 27                  1012  0 0 36  0   0 FALSE FALSE
# 28                  1013  3 0  7  0   1 FALSE FALSE
# 29                  1014  1 0  4  0   0 FALSE FALSE
# 30                  1015  0 0 32  0   0 FALSE FALSE
# 31                  3367  0 0  1  0   0 FALSE FALSE
# 32                  1335  1 0 34  0   0 FALSE FALSE
# 33 Chr1 43500   A
# 34                  1007 10 0  0  3   1 FALSE FALSE
# 35                  1012 10 0  0  3   1 FALSE FALSE
# 36                  1013 10 0  1  1   0 FALSE FALSE
# 37                  1014  5 0  0  0   0 FALSE FALSE
# 38                  1015 11 0  0  2   0 FALSE FALSE
# 39                  3367  6 0  0  3   0 FALSE FALSE
# 40                  1335 13 0  0  1   0 FALSE FALSE
```

7.3 Main Analysis

Turning to the main analysis, there is a large increase in the number of consistent positions between the loose and medium stringency levels; medium and tight are similar in most respects. The likely interpretation is that the loose criterion is including many “SNPs” induced by read errors, and that either of the tighter criteria are successfully filtering them out. In the interest of simplicity, the narrative below will focus on the shared SNPs at the medium stringency level (the “count2” column in the data frame), although the numbers for all three (sometimes all 4) are displayed. Also note that the prose and some comments in the code were based on the Chr1 analysis, and so may occasionally be off-target for the whole-genome data.

```
# Show a subset of pat.summaries, optionally with totals of count_i in last row, and optionally
# aggregating low-count rows as ``Other''
#
#   sharedBy=c(2,4) selects SNPs shared by 2 or 4 strains,
#   subset=as.octmode('35') select those with sharing pattern a subset (optionally proper) of this
#   split=as.octmode('14') additionally restricts to patterns stradling split/subset minus split
#   c2.thresh=42 suppresses printout of rows with count2 < 42
#   restrict.to=c(0,42,127) restrict to these 3 rows
showgroup <- function(p.summ=pat.summaries, sharedBy=0:7, subset=127, split=NULL, proper.subset=F,
                      total=T, c2.thresh=0, fourteenth=F, restrict.to=NULL){
  # pick just those bit patterns that are subsets of 'subset'
  pick <- bitwAnd(0:127,bitwNot(subset))==0
  if(proper.subset){
    pick[subset+1] <- F
  }
  if(!is.null(split)){ # AND that stradle left/right subtrees
    cosplit <- bitwAnd(subset,bitwNot(split))
    pick <- pick & bitwAnd(0:127,split)!=0 & bitwAnd(0:127,cosplit)!=0
  }
  # and have desired shareBy counts
  pick <- pick & (p.summ$sharedBy %in% sharedBy)
  # and are among the set of interest
  if(!is.null(restrict.to)){
    pick <- pick & (0:127 %in% restrict.to)
  }
  # find rows with low counts
  pick.low <- pick & (p.summ$count2 < c2.thresh)
  # now show them
  show <- p.summ[pick & ! pick.low,]
  # rename columns just to narrow the printouts
  colnames(show) <- c('Pat','ShrBy','1007', '1012', '1013', '1014', '1015', '3367', '1335',
                      'count1', 'count2', 'count3','count4')
  show[,1] <- format(show[,1])  # convert octal col to char so can override in last row(2)
  nlow <- sum(pick.low)
  if(nlow > 0){
    n <- nrow(show)+1
    lows <- apply(p.summ[pick.low,10:13],2,sum)
    show[n,10:13] <- lows
    show[n,1:9] <- ''
    row.names(show)[n] <- 'Other'
    if(fourteenth){
      # do this: add 14th col just to hold this comment:
      show <- cbind(show,' '='', stringsAsFactors=F)
      show[n,14] <- paste('(', nlow, 'rows w/ c2 <', c2.thresh, ')')
    } else {
      ## or this (looks a bit funky, but fits across page without line-wrap):
      show[n,1:8] <-c('(', nlow, 'rows', 'w/', 'c2', '<', c2.thresh, ')')
    }
  }
  if(total){
    n <- nrow(show)+1
    tots <- apply(show[,10:13],2,sum)
    show[n,10:13] <- tots
    show[n,1:9] <- ''
    row.names(show)[n] <- 'Total'
    if(ncol(show)==14){show[n,14]<-''}
  }
  return(show)
}
```

First, are there any SNPs that are not “consistent SNPs?” Yes, a few in c3. As noted above, they seem to be mainly low-coverage positions.

```
showgroup(pat.summaries,0,total=F)  # chr1 totals: 0 0 83

#   Pat ShrBy 1007 1012 1013 1014 1015 3367 1335 count1 count2 count3 count4
# 1   0     0                                       111    578   4755      0
```

Next, look at completely shared SNPs, those found in all 7 strains.

```
showgroup(pat.summaries,7,total=F) # Chr1 count1 = 8593, count2 = 7054, count3 = 4790 c4=1641

#     Pat ShrBy 1007 1012 1013 1014 1015 3367 1335 count1 count2 count3 count4
# 128 177     7    X    X    X    X    X    X    X  77690  62182  38744  15186
```

I.e., of the 469906 consistent positions, 62182 or 13.2% are shared by all 7 strains.

Next look at singletons, aka private SNPs—SNPs that are called in one strain and no other strain has a significant number of non-ref reads at that position. Presumably these are variants that arose in a given population after it separated from the others.

```
showgroup(pat.summaries,1)  # chr1 totals: 9669  18865  19670  23574

#       Pat ShrBy 1007 1012 1013 1014 1015 3367 1335 count1 count2 count3 count4
# 2      001     1                                  X    449    632   1129   2260
# 3      002     1                             X       73721  85117  87494 105614
# 5      004     1                        X             1720   2156   2729   4608
# 9      010     1                   X                   383    525    485   1231
# 17     020     1              X                    82364  94364  96464 113191
# 33     040     1         X                           502    655   1102   2450
# 65     100     1    X                                231    339    496   2005
# Total                                               159370 183788 189899 231359
```

The import of shared/private SNPs changes between sexual and asexual populations. Presumably asexuals slowly gain and rarely lose private SNPs; shared ones predate separation of the lineages. In sexual lineages, however, SNPs may be rather freely “gained” or “lost,” merely by recombination (converting between homo- and heterozygous in the sample we sequenced). Thus, the low private counts for the 5 L-isolates compared to the large count of het positions overall suggest that (a) they are asexual, and (b) none of them has been isolated from the others for very long (if at all). Conversely, the high counts for Italy and Wales suggest that (a) if asexual, they have been separated from each other and from the rest for a long time, but (b) if sexual, there is little surprise: we have ≈160K SNPs shared between the two (90K just in those two (below), plus 70K shared by all 7), and ≈90K additional positions that are het in one but not the other. These are close to, but not exactly equal to, the 1:2:1 ratios we would naively expect from two samples of a single HWE population. The most parsimonious explanation seems to be that the H-clade is sexual, but perhaps some het positions private to each population separates them.

Aside: counts of “consistent” SNPs minus these singletons yeilds count of shared SNPs:

```
singlets <- apply(pat.summaries[pat.summaries$sharedBy==1,10:13],2,sum)
rbind(consistent=consistent.count,singlets=singlets,shared=consistent.count-singlets)

#            count1 count2 count3 count4
# consistent 447177 469906 471171 474613
# singlets   159370 183788 189899 231359
# shared     287807 286118 281272 243254
```

The slightly higher count of shared positions in the medium case further supports this choice for subsequent analysis.

Next look at consistent SNPs shared between just a pair of isolates.

```
showgroup(pat.summaries,2) # chr 1 counts: 7641   9549   9472  6924

#       Pat ShrBy 1007 1012 1013 1014 1015 3367 1335 count1 count2 count3 count4
# 4     003     2                             X    X    994    298    532    587
# 6     005     2                        X         X    287    523   1088   1407
# 7     006     2                        X    X         624    138    282    590
# 10    011     2                   X              X    515    486    317    827
# 11    012     2                   X         X         565     49     32     93
# 13    014     2                   X    X              133    139    132    417
# 18    021     2              X                   X    998    167    337    402
# 19    022     2              X              X       82160  87499  83482  58009
# 21    024     2              X         X              686    195    410    625
# 25    030     2              X    X                   609     69     47     93
# 34    041     2         X                        X     42     92    313    368
# 35    042     2         X                   X         503    119    254    394
# 37    044     2         X              X               69    279   1001   1809
# 41    050     2         X         X                    13     24     53    105
# 49    060     2         X    X                        627    116    237    388
# 66    101     2    X                             X     29     47     73    314
# 67    102     2    X                        X         351     67     96    351
# 69    104     2    X                   X               39    122    329   1196
# 73    110     2    X              X                    12     11     29    150
# 81    120     2    X         X                        432     76     98    309
# 97    140     2    X    X                             955   1144   1235   2144
# Total                                               90643  91660  90377  70578
```

I.e., of the 91660 paired SNPs, 87499 or 95.5% are found between Italy and Wales, with comparatively few shared between any other pairs (only).

SNPs shared among exactly 3 isolates are relatively rare. (The 5 trios containing both Italy and Wales predominate in the loose set, probably because they share many pairs that become triples with the addition of a few read errors.)

```
showgroup(pat.summaries,3) # chr 1 counts: 1438    294    671   1034

#       Pat ShrBy 1007 1012 1013 1014 1015 3367 1335 count1 count2 count3 count4
# 8     007     3                        X    X    X    104    197    371    557
# 12    013     3                   X         X    X    257    226    146    338
# 14    015     3                   X    X         X   1041    984    660   1389
# 15    016     3                   X    X    X          46     60     49    152
# 20    023     3              X              X    X   1274    558   1020    533
# 22    025     3              X         X         X    135    216    466    522
# 23    026     3              X         X    X         793    431    763    789
# 26    031     3              X    X              X    268    233    151    361
# 27    032     3              X    X         X         698    131     74     86
# 29    034     3              X    X    X              103    119     91    219
# 36    043     3         X                   X    X     56     86    151    133
# 38    045     3         X              X         X    202    593   1970   1656
# 39    046     3         X              X    X          58    154    425    604
# 42    051     3         X         X              X     52     57     74    126
# 43    052     3         X         X         X           8     13     17     22
# 45    054     3         X         X    X               20     78    133    292
# 50    061     3         X    X                   X     52     80    131    115
# 51    062     3         X    X              X         703    269    454    469
# 53    064     3         X    X         X               53    184    458    601
# 57    070     3         X    X    X                    22      9     14     24
# 68    103     3    X                        X    X     24     34     46    143
# 70    105     3    X                   X         X     78    181    396    805
# 71    106     3    X                   X    X          32     66    109    377
# 74    111     3    X              X              X      6     11      8    139
# 75    112     3    X              X         X          10     11      8     26
# 77    114     3    X              X    X               12     36     56    365
# 82    121     3    X         X                   X     22     22     34     73
# 83    122     3    X         X              X         501    162    165    354
# 85    124     3    X         X         X               43     88    152    400
# 89    130     3    X         X    X                     9      9      9     27
# 98    141     3    X    X                        X     78    149    258    519
# 99    142     3    X    X                   X         386    409    463    755
# 101   144     3    X    X              X              383   1176   2395   4432
# 105   150     3    X    X         X                    28     51     55    238
# 113   160     3    X    X    X                         337    375    399    712
# Total                                                7894   7458  12171  18353
```

Four-way sharing is more common, but dominated by the coastal (i.e., non-Gyre) L-clade isolates. This is likely a reflection of the strong 5-way sharing among the L-clade, from which the Gyre commonly drops out due to the lower coverage/higher error rate in that sequencing run.

```
showgroup(pat.summaries,4) # chr 1 counts: 564   1346   2552  3479

#       Pat ShrBy 1007 1012 1013 1014 1015 3367 1335 count1 count2 count3 count4
# 16    017     4                   X    X    X    X    390    329    211    564
# 24    027     4              X         X    X    X    461    759   1423    771
# 28    033     4              X    X         X    X   1139    973    574    306
# 30    035     4              X    X    X         X    578    509    329    503
# 31    036     4              X    X    X    X         345    320    227    211
# 40    047     4         X              X    X    X    127    256    708    708
# 44    053     4         X         X         X    X     35     34     26     56
# 46    055     4         X         X    X         X    606    696    668    971
# 47    056     4         X         X    X    X          26     44     50     88
# 52    063     4         X    X              X    X    151    184    332    194
# 54    065     4         X    X         X         X    122    284    731    582
# 55    066     4         X    X         X    X         217    489   1025    851
# 58    071     4         X    X    X              X      9     20      7     28
# 59    072     4         X    X    X         X          41     36     21     31
# 61    074     4         X    X    X    X               20     46     51    116
# 72    107     4    X                   X    X    X     58     84    129    330
# 76    113     4    X              X         X    X      7      9      5     66
# 78    115     4    X              X    X         X    141    139    122    604
# 79    116     4    X              X    X    X           8      8     11    101
# 84    123     4    X         X              X    X     63     98     91    124
# 86    125     4    X         X         X         X     67    113    223    283
# 87    126     4    X         X         X    X          99    198    268    425
# 90    131     4    X         X    X              X      6      3      0     52
# 91    132     4    X         X    X         X          19     21     10     38
# 93    134     4    X         X    X    X               18     17     22    143
# 100   143     4    X    X                   X    X     37     58    103    190
# 102   145     4    X    X              X         X   5992  12644  22332  23189
# 103   146     4    X    X              X    X         196    510    969   1795
# 106   151     4    X    X         X              X     43     69     54    220
# 107   152     4    X    X         X         X          18     27     15     67
# 109   154     4    X    X         X    X             1227   1390   1065   1738
# 114   161     4    X    X    X                   X     74     96    113    207
# 115   162     4    X    X    X              X        1848   1932   1828   1014
# 117   164     4    X    X    X         X              237    627   1053   1752
# 121   170     4    X    X    X    X                    13     18     15     69
# Total                                               14438  23040  34811  38387
```

Five-way sharing is much more common, and is strongly dominated by the 5 L-clade isolates.

```
showgroup(pat.summaries,5) # chr 1 counts: 3969   5047   4624  6125

#       Pat ShrBy 1007 1012 1013 1014 1015 3367 1335 count1 count2 count3 count4
# 32    037     5              X    X    X    X    X   2247   1877   1193    620
# 48    057     5         X         X    X    X    X    221    219    189    324
# 56    067     5         X    X         X    X    X    556   1015   2544   1151
# 60    073     5         X    X    X         X    X     94     72     62     38
# 62    075     5         X    X    X    X         X    195    187    210    328
# 63    076     5         X    X    X    X    X         106    130    124    128
# 80    117     5    X              X    X    X    X     48     32     25    241
# 88    127     5    X         X         X    X    X    225    321    501    482
# 92    133     5    X         X    X         X    X     31     28     15     52
# 94    135     5    X         X    X    X         X    126    117     88    235
# 95    136     5    X         X    X    X    X          34     56     26    106
# 104   147     5    X    X              X    X    X   2073   4042   6741  10001
# 108   153      5    X    X         X         X    X     39     27     26     96
# 110   155      5    X    X         X    X         X  40157  35344  22417  30602
# 111   156      5    X    X         X    X    X        565    575    410    735
# 116   163      5    X    X    X              X    X    255    271    328    316
# 118   165      5    X    X    X         X         X   2726   5022   8440   9715
# 119   166      5    X    X    X         X    X        902   1976   3172   2688
# 122   171      5    X    X    X    X              X     41     19     13     70
# 123   172      5    X    X    X    X         X          58     71     45     86
# 125   174      5    X    X    X    X    X              659    682    468    782
# Total                                                51358  52083  47037  58796
```

Six-way sharing is also common, with the sets *ex*cluding Gyre, Italy, or Wales having the most mutually-shared SNPs.

```
showgroup(pat.summaries,6) # chr 1 counts:  4166   4741   5312  4722

#       Pat ShrBy 1007 1012 1013 1014 1015 3367 1335 count1 count2 count3 count4
# 64    077      6         X    X    X    X    X    X    850    847    827    485
# 96    137      6    X         X    X    X    X    X    405    324    240    333
# 112   157      6    X    X         X    X    X    X  13239  10814   6862  12202
# 120   167      6    X    X    X         X    X    X  11742  21003  35227  15091
# 124   173      6    X    X    X    X         X    X    131     87     47    114
# 126   175      6    X    X    X    X    X         X  16884  13630   8608  12697
# 127   176      6    X    X    X    X    X    X       2422   2412   1566   1032
# Total                                                45673  49117  53377  41954
```

8 Trees

So, overall, the picture looks like a long shared history (62182 7-way shared positions), followed by a split of the 5 L-isolates from the 2 H-isolates, then a long shared history in the 5 (35344 quintuples), in parallel with a long shared history in H- (87499 pairs), then separate histories in Italy and Wales (>85117 "private" SNPs in each, although again if they are sexual, many of these just reflect HWE), and very limited differentiation among the 5 L-isolates.

Branch lengths of course depend on filtering criteria used (and, of course, full vs Chr1 differ by about a factor of 10), but the tree *topology* appears to be fairly stable. Various versions are drawn below, exactly to explore how robust this story is. I think we should go with "medium stringency" SNP filtering (based on un-qfiltered reads).

NOTE: Much of this analysis make less sense for q-filtered read data, since (a) the point of the SNP filtering was to try to correct for noise in the raw reads, which may (or may not; haven't looked closely, yet) be largely fixed by qfiltering (e.g., "loose" or no SNP filtering may be more appropriate, post-q-filtering, esp. if we had re-run SAMTools to call SNPs based on the q-filtered reads), and (b) tree topology *does* appear to change, in that Gyre's coverage has been so sharply reduced by qfiltering that it clearly stands aside from the others (and that's confirmed by bootstrap), but this also seems to be clearly a technical rather than a biological artifact. SO, code below will run on q-filtered data, but *is not tuned to it*. Likewise, most comments in the prose below were made to describe the un-q-filtered data, and *are misleading and in some cases flatly wrong* for qfiltered data, but it doesn't seem worthwhile to bother with a rewrite...

Trees are coded in newick format, which doesn't seem to tolerate line-breaks; print with line-wrap:.

```r
# wrap a long char string across multiple lines in printout
cat.hardwrap <- function(str,width=80){
  while(nchar(str)>width){
    cat(substr(str,1,width),'\n')
    str <- substr(str,width+1,nchar(str))
  }
  cat(str,'\n')
}
```

Trees are built as follows. Code for drawing, especially, is specific to the topology of the medium tree, and placement of some of the figure elements have been hand-optimized for this case; drawings for the other variants will not be as pretty.

```
# set up for tree figs

# the newick parser in ape seems to be confused by commas and parens in
# tip names, and blanks are not allowed, so replace by *, <, >, _, resp.
newick.name <- function(name){
  name <- gsub(' ', '_', name, fixed=TRUE)
  name <- gsub(',', '*', name, fixed=TRUE)
  name <- gsub('(', '<', name, fixed=TRUE)
  name <- gsub(')', '>', name, fixed=TRUE)
  return(name)
}
# undo above changes
newick.name.undo <- function(name){
 #name <- gsub('_', ' ', name, fixed=TRUE) # unnecessary; ape plot routine handles this one
  name <- gsub('*', ',', name, fixed=TRUE)
  name <- gsub('<', '(', name, fixed=TRUE)
  name <- gsub('>', ')', name, fixed=TRUE)
  return(name)
}

# make a newick string from tree;  see it below
# 'pre' is prefixed to ccmpid; 'nb' optionally included;
# 'alt' can be used instead of pre/ccmp/nb/where for less formal labeling
# 'newstyle'==T => new node label: [nb_]where[(pre-less-id)]
# 'newstyle'==F => old node label: [nb_][pre id]where
newickize <- function(tree,pre='CCMP',nb=TRUE,alt=F,newstyle=TRUE){
  if(is.null(tree$where)){
    # not a leaf; paste together newick from subtrees
    sub1 <- newickize(tree$sub1,pre=pre,nb=nb,alt=alt,newstyle=newstyle)
    sub2 <- newickize(tree$sub2,pre=pre,nb=nb,alt=alt,newstyle=newstyle)
    new <- paste( '(', sub1, ',', sub2, ')', sep='')
    if(!is.null(tree$length)){
      # internal node, add length
      return(paste(new, ':', tree$length, sep=''))
    } else {
      # top level; escape blanks and add trailing ';'
      return(paste(gsub(' ', '_', new), ';', sep=''))
    }
  } else {
    # a leaf; build label and branch length
    if(alt){
      # label is just alt; if alt omitted, default to where
      new <- newick.name(ifelse( is.null(tree$alt), tree$where, tree$alt ))
    } else {
      if(newstyle){
        # new node label = [nb_]where[(pre-less-id)]
        new <- ifelse( nb && !is.null(tree$nb), paste(tree$nb, '_', sep =''), '' )
        new <- newick.name(paste(new, tree$where, sep=''))
        new <- ifelse( is.null(tree$id), new, paste(new, '_(', tree$id, ')', sep='') )
        new <- newick.name(new)
      } else {
        # old style node label = [nb_][pre id]where
        new <- ifelse( nb && !is.null(tree$nb), paste(tree$nb, '_', sep =''), '' )
        new <- ifelse( is.null(tree$id), new, paste(new, pre, tree$id, '_', sep='') )
        new <- newick.name(paste(new, tree$where, sep=''))
      }
    }
    #add length to either
    new <- paste(new, ':', tree$length, sep='')
  }
  return(new)
}

# Make a tree as nested lists, **based on the chr1, count2 topology**, but using any of the counts.
#   Internal nodes have subtrees sub1/2 and length
#   Root has sub1/2, but no length
#   Leaves have where, length, optionally, id, alt, nb.  (Omit id for 'outgroup'. Use 'alt' for less formal
#     labeling in cartoon version; it defaults to 'where'. Use 'nb' to add abcde annotations for legend.)
# The single parameter v is any of the 4 count vectors contained in pat.summaries (most conveniently
# indexed in octal).  E.g., make.tree(pat.summaries[,'count2']) reproduces the count2 tree.
# (This was previously built by hand-pasting the edge lengths; tree.by.hand is retained in appendix
# for comparison, & its counts are in comments below).
#
make.tree <- function(v){
  pat.count <- function(pat, pat.counts=v){return(pat.counts[1+strtoi(pat,8)])}
  thetree <-
    list(
      sub1 = list(
        sub1 = list(
          sub1 = list(id=3367, length=pat.count('002'), where='Venice, Italy', alt='Venice'), #8813
          sub2 = list(id=1013, length=pat.count('020'), where='Wales, UK'),                   #9652
          length=pat.count('022')),                                                           #9365
        sub2 =  list(
          sub1 = list(
            sub1 = list(
              sub1 = list(id=1007, length=pat.count('100'), nb='e', where='Virginia, USA'),    #30
              sub2 = list(id=1012, length=pat.count('040'), nb='d', where='Perth, W. Australia', alt='Perth'), #61
              length=pat.count('140')),                                                        #19
            sub2 = list(
              sub1 = list(id=1015, length=pat.count('004'),nb='c', where='Washington, USA', alt='Puget Sound'), #207
              sub2 = list(id=1335, length=pat.count('001'), nb='b', where='New York, USA',   alt='NY'), #41
              length=pat.count('005')),                                                        #18
            length=pat.count('145')),                                                          #1005
          sub2 = list(id=1014, length=pat.count('010'), nb='a', where='N. Pacific Gyre'),      #61
          length=pat.count('155')),                                                            #3912
        length=pat.count('177')),                                                              #7054
      sub2 = list(length=0, where='outgroup')
    )
  return(thetree)
}
```

Code to plot a tree given newick description. Again, code is somewhat general, but has some specializations tied to the medium-stringency, full-genome, un-qfiltered data.

```
# run following 2 lines after an R upgrade
# update.packages()
# install.packages("ape")
library(ape)
show.tree <- function(newick.str=newick.medium,
                      col.edge  ='darkblue', lwd.edge =2,
                      col.elabel='darkblue',                 cex.elabel=0.8, font.elabel=3,
                      col.arrow ='red',      lwd.arrow=1.5, cex.arrow =0.9, font.arrow =4,
                      col.clade ='black',    lwd.clade=1,    cex.clade =1.0, font.clade =3,
                      col.legbox='beige',                    cex.legend=0.8,
                      col.tip   ='darkblue',                                font.tip   =4,
                      plusx=FALSE, pltdebug=FALSE, total.snps=consistent.count[2],
                      straight.arrow=FALSE){

  ####
  #
  # ADJUST NEWICK & GET LENGTHS, COORDINATES
  #
  newick.str.noout <- sub('outgroup','_',newick.str) # Hide outgroup ('_' prints as blank)
  the.tree <- read.tree(text=newick.str.noout)

  ## nasty hack: ape's newick parser seems to be confused by commas, () in tip labels, so
  ## newickize replaced them by '*<>'; before plotting, I want to convert them back, and hope
  ## this doesn't break anything else...  And if a revised version of ape changes the internal
  ## representation of a tree, this may need to be redone.
  the.tree$tip.label <- newick.name.undo(the.tree$tip.label)

  # extract branch lengths as char string of comma-separated numbers via pattern matching hack:
  # lengths always preceded by colon
  lengths.ch <- strsplit(paste(newick.str,':'),'[^0-9][^:]*:')[[1]]

  # then convert to ints, dropping empty string at front
  lengths.int <- scan(what=integer(),quiet=T,sep=',',text=lengths.ch[-1])

  # then to data frame with named rows; a..g are terminal branches; others are internal.
  # a..e match legend in plot; f/g = wales/italy.  lengths appear in postfix order of
  # newick tree, and ape draws the 1st of them at the bottom of the plot.
  lmed <- data.frame(lengths=lengths.int,
                     row.names=c('g','f','fg','e','d','de','c','b','bc','bcde','a','abcde','all','out'))

  # extract counts needed for legend:
 #leg.counts <- c(      61, 41,207, 61, 30, 1005,    18, 19) #by hand, medium chr1
  leg.counts <- lmed[c('a','b','c','d','e','bcde','bc','de'),1]
  discord <- total.snps - sum(lmed$lengths)

  #tree.labels <- list( ## x,y,text; coords are all picked by eye
  #   3000, 3.62, paste(lmed['all'  ,1], 'shared by 7', sep='\n'), # 7054
  #   8900, 5.75, paste(lmed['abcde',1], 'by 5'       , sep='\n'), # 3912
  #  12000, 1.50, paste(lmed['fg'   ,1], 'shared by 2', sep='\n'), # 9365
  #  21000, 2.00, paste(lmed['f'    ,1], 'only\nin Wales'),         # 9652
  #  21000, 1.00, paste(lmed['g'    ,1], 'only\nin Italy'),         # 8813
  #  11500, 4.50, '*')
  # automating x-placement, below; retain above for comparison...
  tip <- integer(7)  # x coords of tree tips
  tip[1] <-sum(lmed[c('all','fg','g'),1])
```

```
tip[2] <-sum(lmed[c('all','fg','f'),1])
tip[3] <-sum(lmed[c('all','abcde','bcde','de','e'),1])
tip[4] <-sum(lmed[c('all','abcde','bcde','de','d'),1])
tip[5] <-sum(lmed[c('all','abcde','bcde','bc','c'),1])
tip[6] <-sum(lmed[c('all','abcde','bcde','bc','b'),1])
tip[7] <-sum(lmed[c('all','abcde','a'),1])

inode <- integer(5) # x coords of (some) internal nodes
inode[1] <- 0                                    # root
inode[2] <- lmed['all',1]                        # lca of all
inode[3] <- sum(lmed[c('all','fg'),1])           # lca H-clade
inode[4] <- sum(lmed[c('all','abcde'),1])        # lca L-clade
inode[5] <- sum(lmed[c('all','abcde','bcde'),1]) # lca L-clade, nonGyre
tree.labels <- list( ## x,y,text; y coords partially picked by eye
  sum(inode[c(1,2)])/2, 3.62, paste(lmed['all'  ,1], 'shared by 7', sep='\n'), # 7054
  sum(inode[c(2,4)])/2, 5.75, paste(lmed['abcde',1], 'by 5'       , sep='\n'), # 3912
  sum(inode[c(2,3)])/2, 1.50, paste(lmed['fg'   ,1], 'shared by 2', sep='\n'), # 9365
  (inode[3]+tip[2])/2,  2.00, paste(lmed['f'    ,1], 'only\nin 1013'),         # 9652
  (inode[3]+tip[1])/2,  1.00, paste(lmed['g'    ,1], 'only\nin 3367'),         # 8813
  sum(inode[c(4,5)])/2, 4.35, '* ')


tree.labels <- list( ## x,y,text; y coords partially picked by eye
  sum(inode[c(1,2)])/2, 3.62, paste(lmed['all'  ,1], 'in 7', sep='\n'), # 7054
  sum(inode[c(2,4)])/2, 5.75, paste(lmed['abcde',1], 'in 5', sep='\n'), # 3912
  sum(inode[c(2,3)])/2, 1.50, paste(lmed['fg'   ,1], 'in 2', sep='\n'), # 9365
  (inode[3]+tip[2])/2,  2.00, paste(lmed['f'    ,1], 'only\nin 1013'),  # 9652
  (inode[3]+tip[1])/2,  1.00, paste(lmed['g'    ,1], 'only\nin 3367'),  # 8813
  sum(inode[c(4,5)])/2, 4.35, '* ')

####
#
# BOGUS PLOT
#
# a messy bit: need string widths to set xlim; but strwidth needs x-scale so must plot first.
# M plot completely invisible, overlay 2nd plot via par(new=F...) .
#
# PROVISIONALLY set x.lim here at about 30% wider than tree; fine tune it for the real plot
# based on strwidth(tip labels) below.
#
provisional.tree.x.lim <- 1.3 * max(tip) # <== PROVISIONAL plot width
plot(0,0, type='n', bty='n', xaxt='n', yaxt='n', xlab='', ylab='', xlim=c(0,provisional.tree.x.lim), ylim=c(0,7))

tiplabel.x <- integer(7)
for(i in 1:7){
  # see warning above about internals of the.tree; labels have '_', printed as ' '.
  tiplabel.x[i] <- tip[i]+strwidth(gsub('_',' ',the.tree$tip.label[i],fixed=T), font=font.tip)
}


# visually show tip coords & max x to debug placement issues
plt.debug <- function(tree.x.lim, tip, tiplabel.x, spx=NULL, spy=NULL){
  if(pltdebug){ # F to hide/T to show debug
    cat('Tip labels:', paste(the.tree$tip.label,sep='',collapse='/'), '\n')
    axis(2) # useful only for placing labels
    for(i in 1:7){
      points(c(tip[i],tiplabel.x[i]),c(i,i)) # debug: do I have right tip coordinates?
    }
    lines(rep(tree.x.lim,2),c(0,7)) # where is right edge?
    if(!is.null(spx)){
      points(spx,spy) # show spline control points, for tweaking
    }
  }
}

plt.debug(provisional.tree.x.lim, tip, tiplabel.x)

label.end.H <- max(tiplabel.x[1:2])
label.end.L <- max(tiplabel.x[3:7])
clade.dx <- strwidth('x') # space between clade marker line and its label
xdel <- 3*clade.dx        # space between labeled clade tips and marker line

tree.x.lim <- 1.03*(max(tiplabel.x)+xdel)  # <== FINAL plot width
tree.y.lim <- 7
if(pltdebug){cat('Plot width hacking:', provisional.tree.x.lim, tree.x.lim, tree.x.lim/1.03/max(tip), clade.dx)}

par(new=T)  # I.e., NOT starting a new plot

####
#
```

```
# REAL PLOT
#
plot(the.tree,
     x.lim = c(0, tree.x.lim),
     y.lim = c(0, tree.y.lim),
     font=font.tip, label.offset=100,             # bold-italic, nudged slightly right
     tip.color=col.tip, edge.color=col.edge,
     edge.width=lwd.edge,
     edge.lty=c(1,1,1,1, 1 ,1,1,1,1,1,1,1,1,0)    # 5th is bottleneck edge; 14th is outgroup
    )
lines(00+c(0,0),c(3.5,6),col='white',lwd=6)       # Hide vertical line to outgroup
axis(1, pos=0.25, at=seq(0,25,by=5)*10^round(log10(max(tip)/25)))

if(pltdebug){text(tip[1]+100, 1.0, 'Venice, Italy (3367)', adj=0, font=font.tip)}

####
#
# BOTTLENECK ANNOTATION
#
# spline/elipse control points (spy/y) & tweaks thereto (dx/y)
dx <- 0.01 * tree.x.lim
dy <- .04
spx <- c(7400, 7400, 9900, 10500) # by eye, chr1, for comparison
spx <- c(inode[2]+dx,inode[2]+dx,inode[4]-3*dx,inode[4]-dx)
spy <- c( 3.8,  3.9,  5.6-dy,   5.6-dy)

plt.debug(tree.x.lim, tip, tiplabel.x, spx, spy)

if(T){
  #elipse version, defined by rect thru 2 middle pts of spx/y
  spf<-function(x){
    ifelse(x <= spx[2], spy[1],
           ifelse(x >= spx[3], spy[4],
                  spy[2]+(spy[3]-spy[2])*sqrt(pmax(0,1-((x-spx[3])/(spx[3]-spx[2]))^2))))
    }
} else {
  # spline version
  spf <- splinefun(spx,spy,method='hyman')
}
serx <- seq(spx[1],spx[length(spx)],length.out=50)
sery <- spf(serx)
tailx <- spx[1]
taily <- spy[1]
headx <- spx[4]
heady <- spy[4]
textx <- (headx+tailx)/2+(headx-tailx)*(-.01)
texty <- (heady+taily)/2+(heady-taily)*(-.10)
bottle.txt <- "inbreeding\nLoH / LoS"
if(!straight.arrow){
  arrows(headx,heady,headx+tree.x.lim*1e-3,heady, length=.1,col=col.arrow,lwd=lwd.arrow)
  lines(rev(serx), rev(sery), lty=c(5,1),col=col.arrow, lwd=lwd.arrow)
  textangle <- 66
  textadj <- c(0,0)
} else {
  # Tweak positioning slightly; visualize a rectangle from 7-node to base of L-clade;
  # center text, rotated, on diagonal towards L-clade; ditto the straight arrow.
  llx <- inode[2] # the aforementioned rectangle
  urx <- inode[4]
  lly <- 3.62
  ury <- 5.75
  # rect(llx,lly,urx,ury) # show rect for debug
  textx <- (llx+urx)/2    # center text
  texty <- (lly+ury)/2
  textangle <- atan(grconvertY(ury-lly,to='dev')/grconvertX(urx-llx,to='dev'))*360/(2*pi)
  textadj <- c(0.50, 0.43) #tweak position; ".5" = center in x , ".43" raises, THEN rotate.
  alpha <- .78 # fraction along diag at which arrow begins
  beta  <- .95 # ... and ends
  arrows((1-alpha)*llx + alpha*urx,
         (1-alpha)*lly + alpha*ury,
         (1-beta)*llx  + beta*urx,
         (1-beta)*lly  + beta*ury, length=.1,col=col.arrow,lwd=lwd.arrow,angle=25)
}
if(T){
  text(textx, texty, bottle.txt, srt=textangle, font=font.arrow, cex=cex.arrow,
       col=col.arrow, adj=textadj)
} else {
  # experiment at wrapping text along curved path; unpretty, but retain for now, maybe revisit
  bottlec <- strsplit(bottle,split=NULL)[[1]]
  for(i in 1:length(bottlec)){
    text(xser[i],yser[i],bottlec[i], srt=65, font=4, cex=.7, col=col.arrow)
    }
  }

  ####
  #
  # CLADE ANNOTATION
  #
  clade.L.x <- label.end.L + xdel
  clade.H.x <- label.end.H + xdel
  dy <-.33
  lines(rep(clade.L.x,2),c(3-dy,7+dy),lwd=lwd.clade,col=col.clade)
  lines(rep(clade.H.x,2),c(1-dy,2+dy),lwd=lwd.clade,col=col.clade)
  text(clade.L.x+clade.dx,5.0,'L-clade',srt=90,font=font.clade,cex=cex.clade,col=col.clade)
  text(clade.H.x+clade.dx,1.5,'H-clade',srt=90,font=font.clade,cex=cex.clade,col=col.clade)

  ####
  #
  # LEGEND
  #
  # parameter plusx controls whether we try to annotate b/c (+) and d/e (x) sharing in tree; I think
  # it looks cluttered, rather than adding clarity, so I vote no, but code is here, in case.  "Logic,"
  # if any, for my symbol choice is that + overlaid on x looks like the * at the next level; this
  # analogy is more visible if we use pch 3/4/8 rather than Courier or Helvetica chars, but probably
  # should use same in both tree & legend, which will take a modicum of additional work.
  legend.text <- c('a: only in 1014  ',
                   'b: only in 1335  ',
                   'c: only in 1015  ',
                   'd: only in 1012  ',
                   'e: only in 1007  ',
                   '*: shared by bcde',
                   paste(ifelse(plusx,'+:','  '),'shared by b/c '),
                   paste(ifelse(plusx,'x:','  '),'shared by d/e ')
  )

  legend.text <- c('a: only in 1014 ',
                   'b: only in 1335 ',
                   'c: only in 1015 ',
                   'd: only in 1012 ',
                   'e: only in 1007 ',
                   '*: in bcde      ',
                   paste(ifelse(plusx,'+:','  '),'in bc        '),
                   paste(ifelse(plusx,'x:','  '),'in de        '),
                   'Discordant SNPs '
  )
  legend.text <- paste(legend.text,format(c(leg.counts,discord),width=4),sep=' - ')
  legend.text <- paste(legend.text,' ') # add a little more right margin in box
  opar <- par(family='mono',cex=cex.legend)
  legend('topright', legend=legend.text, cex=cex.legend, inset=c(0.05,0), bg=col.legbox, box.col=col.legbox)
  par(opar)
  if(plusx){
    points(tree.labels[[16]],tree.labels[[17]]+.14,pch=8,col=col.elabel)
    points(tree.labels[[16]]+200,tree.labels[[17]]+1,pch=3,col=col.elabel)
    points(tree.labels[[16]]+200,tree.labels[[17]]-1,pch=4,col=col.elabel)
  }

  ####
  #
  # EDGE LENGTHS
  #
  for(i in seq(1,length(tree.labels)-ifelse(plusx,5,2),by=3)){
    if(F){ # T for \n in edge labels; F to remove (except "by 5")
      text(tree.labels[[i]], tree.labels[[i+1]], tree.labels[[i+2]])
    } else {
      # points(tree.labels[[i]], tree.labels[[i+1]], pch=3,col='green') # for debugging
      text(tree.labels[[i]], tree.labels[[i+1]], sub('\n([^z])',' \\1', tree.labels[[i+2]]),
           pos=3, offset=.4, font=font.elabel, col=col.elabel,cex=cex.elabel)
    }
  }
}
if(FALSE){#for debug convenience
  pdf(paperfig.path, width=8,height=5,onefile=TRUE,family='Helvetica',fonts='Courier',pointsize=10)
  show.tree(newick.medium, total.snps=consistent.count[2], pltdebug=F,straight.arrow=T)
  dev.off()
}
```

```
caption <- function(stringency,which.tables=which.snp.tables(string.val=F)){
  caption.where <- '(UNKNOWN genome subset).'
  if(which.tables[1]=='Chr1') {caption.where <- 'on Chr1.'}
  if(which.tables[1]=='full') {caption.where <- 'genome-wide.'}
  if(which.tables[1]=='trunc'){caption.where <- 'all Chrs.'}
  cap.stringency <- c(
    'loose SNP filters.',
    'medium SNP filters.',
    'strict SNP filters.',
    'unfiltered SNPs.')
  cap <- paste('Tree based on', which.tables[2], 'reads and', cap.stringency[stringency],
               ' ``Lengths\'\' are numbers of shared/private SNPs', caption.where)
  return(cap)
}
```

Trees based on all four SNP filtering criteria are shown below. Their topologies are exactly the same, although the branch lengths are different. In all four, the length of the branch labeled “*” is probably inflated by lower coverage and higher error rate in 1014, which may mask further legitimate sharing between it and the other L-isolates. The branch lengths among the other 4 are too short for their topology to be convincing without a more rigorous analysis (e.g., a bootstrap test), but detail there is irrelevant to the story.

My sense is that the “medium” version is the best for the paper, made here and shown in Fig 1. In theory, this should look exactly like Fig 3, but something is apparently different between Knitr and direct-to-pdf. (Increasing fig.width in Knitr’s chunk headers from 8 (as in the pdf call below) to 9 helps somewhat, but probably still best to make the paper fig directly rather than via Knitr.)

```
###
#
# MAKE PROTOTYPE PDF FOR PAPER, *AND* SAVE DATA NEEDED TO BUILD IT
#
w.s.t. <- which.snp.tables()
if(w.s.t. == 'trunc-unfiltered'){
  rda.Description <- 'This .rda contains data to generate Fig 3; see shared.snps.rnw for details.'
  save(rda.Description, w.s.t., pat.summaries, consistent.count, file='Fig3-data.rda')
  paperfig.path <- paste('figs-mine/paperfig-medium-tree-', w.s.t., '--Fig3proto.pdf', sep='')
} else {
  paperfig.path <- paste('figs-mine/paperfig-medium-tree-', w.s.t., '.pdf', sep='')
}
pdf(paperfig.path, width=8,height=5,onefile=TRUE,family='Helvetica',fonts='Courier',pointsize=10)
newick.medium <- newickize(make.tree(pat.summaries[,'count2']))
show.tree(newick.medium, total.snps=consistent.count[2], pltdebug=F,straight.arrow=T)
dev.off()

# pdf
#   2
```

```
# fig.paths for knitr chunks below;  .h for "hand-made" trees; plain for automatic chr1/full versions
myfigpath   <- paste(getwd(), '/figs-knitr/newick-', which.snp.tables(), '-', sep='')
myfigpath.h <- paste(getwd(), '/figs-knitr/newick-', sep='')
```

Figure 2, i.e., criteria [[1]]:

```
newick.loose <- newickize(make.tree(pat.summaries[,'count1']))
show.tree(newick.loose, total.snps=consistent.count[1])
```

Figure 3, i.e. [[2]]:

```
# newick.medium <- newickize(tree.by.hand)
# simple.newick.medium <- newickize(tree.by.hand,alt=TRUE)
newick.medium <- newickize(make.tree(pat.summaries[,'count2']))
simple.newick.medium <- newickize(make.tree(pat.summaries[,'count2']),alt=TRUE)
show.tree(newick.medium, total.snps=consistent.count[2])
```

Figure 4, i.e. [[3]]:

```
newick.strict <- newickize(make.tree(pat.summaries[,'count3']))
show.tree(newick.strict, total.snps=consistent.count[3])
```

Figure 5, i.e. [[4]]:

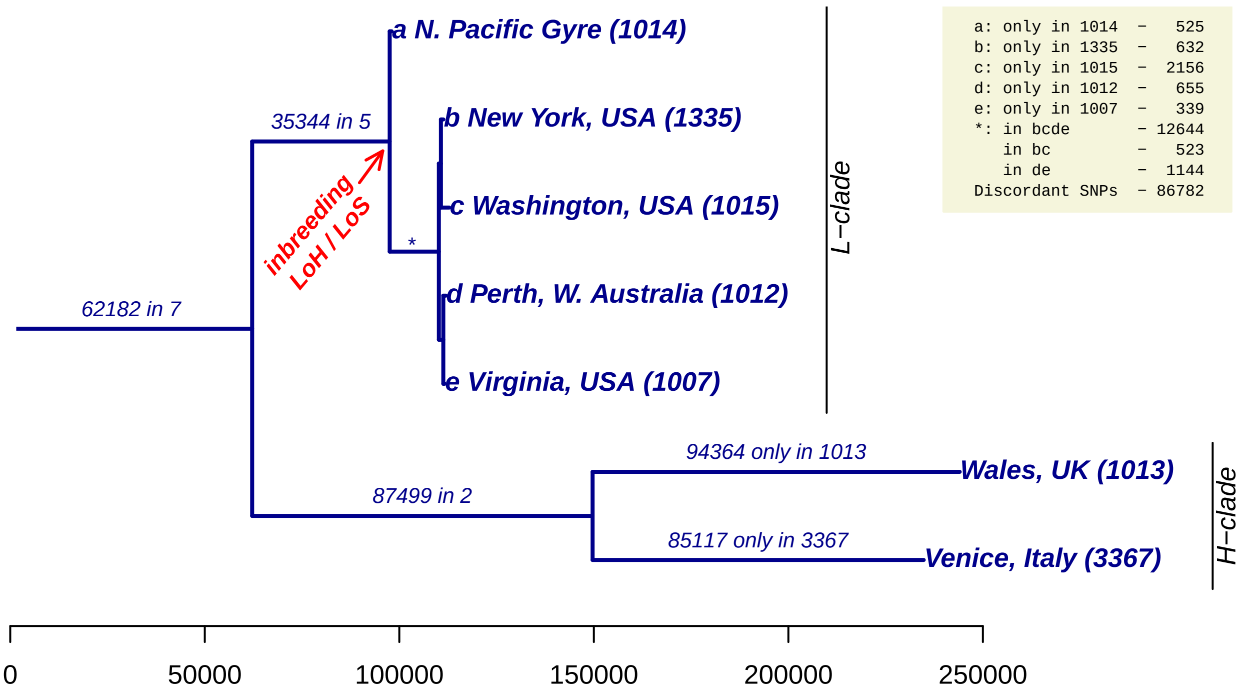


Figure 1: Proposed fig. for paper: Tree based on qfiltered reads and medium SNP filters. "Lengths" are numbers of shared/private SNPs all Chrs.

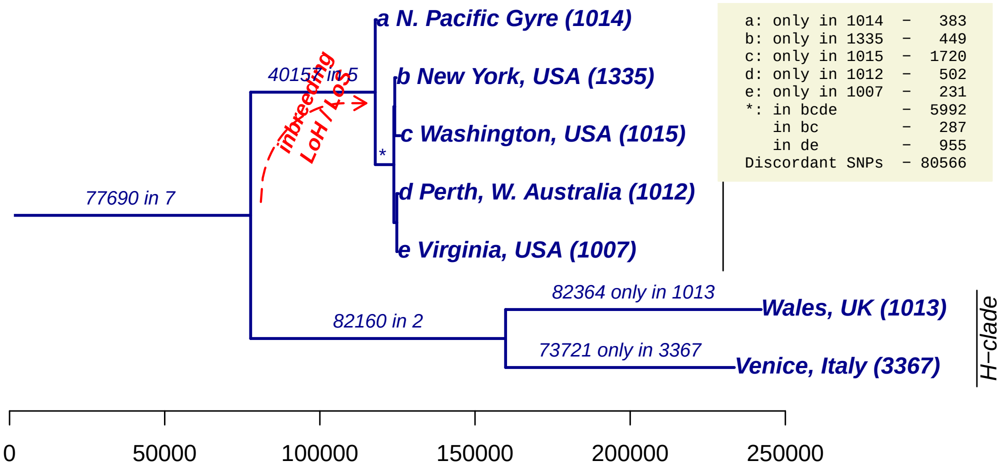


Figure 2: Tree based on qfiltered reads and loose SNP filters. “Lengths” are numbers of shared/private SNPs all Chrs.

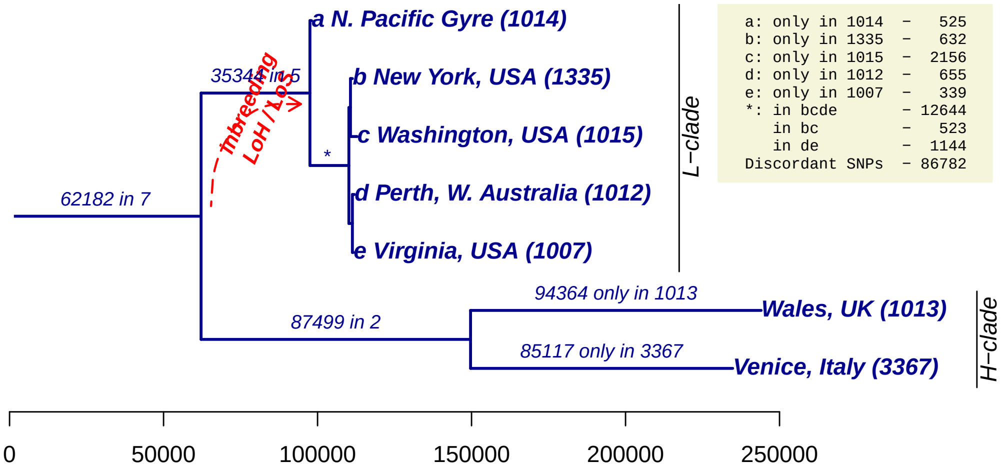


Figure 3: Tree based on qfiltered reads and medium SNP filters. “Lengths” are numbers of shared/private SNPs all Chrs.

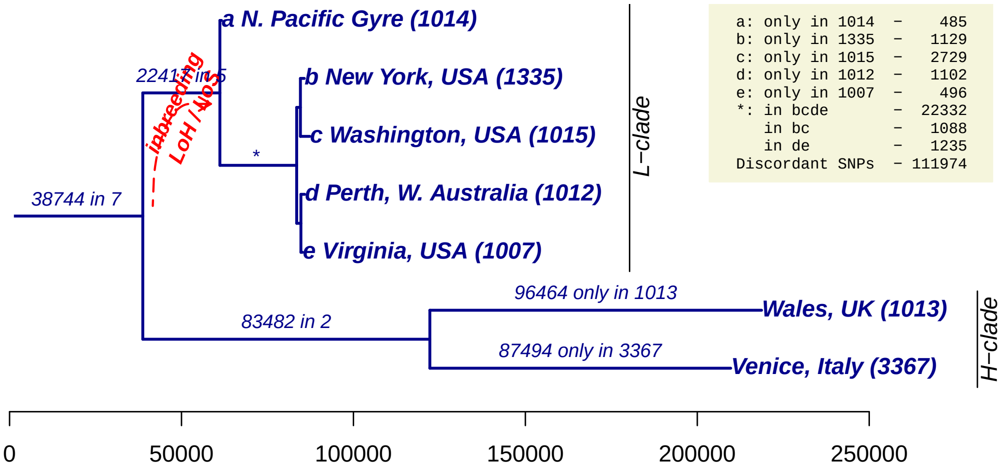


Figure 4: Tree based on qfiltered reads and strict SNP filters. “Lengths” are numbers of shared/private SNPs all Chrs.

```
newick.unfiltered <- newickize(make.tree(pat.summaries[,'count4']))
show.tree(newick.unfiltered, total.snps=consistent.count[4])
```

Some other versions of the trees are included in the appendix.

Counts for all tree edges in the medium tree:

```
#pat.summaries[c(128,110,102,6,97,19,9,2,5,33,65,17,3),]
tree.edges <- c(128,110,102,6,97,19,9,2,5,33,65,17,3)-1
non.edges <- setdiff(0:127, tree.edges)
sg.edges <- showgroup(restrict.to=tree.edges) ; sg.edges

#       Pat ShrBy 1007 1012 1013 1014 1015 3367 1335 count1 count2 count3 count4
# 2     001      1                                  X    449    632   1129   2260
# 3     002      1                             X       73721  85117  87494 105614
# 5     004      1                        X             1720   2156   2729   4608
# 6     005      2                        X         X    287    523   1088   1407
# 9     010      1                   X                   383    525    485   1231
# 17    020      1              X                      82364  94364  96464 113191
# 19    022      2              X              X       82160  87499  83482  58009
# 33    040      1         X                             502    655   1102   2450
# 65    100      1    X                                  231    339    496   2005
# 97    140      2    X    X                             955   1144   1235   2144
# 102   145      4    X    X              X         X   5992  12644  22332  23189
# 110   155      5    X    X         X    X         X  40157  35344  22417  30602
# 128   177      7    X    X    X    X    X    X    X  77690  62182  38744  15186
# Total                                              366611 383124 359197 361896
```

Counts for the top 10 discordant patterns, i.e., SNPs whose sharing pattern does not match any of the bifurcations in the tree:

```
tenth <- sort(showgroup(restrict.to=non.edges)[-(length(non.edges)+1),'count2'],decreasing=T)[10]
sg.non.edges <- showgroup(restrict.to=non.edges, c2.thresh = tenth) ; sg.non.edges

#       Pat ShrBy 1007 1012 1013 1014 1015 3367 1335 count1 count2 count3 count4
# 32    037      5              X    X    X    X    X   2247   1877   1193    620
# 104   147      5    X    X              X    X    X   2073   4042   6741  10001
# 109   154      4    X    X         X    X             1227   1390   1065   1738
# 112   157      6    X    X         X    X    X    X  13239  10814   6862  12202
```

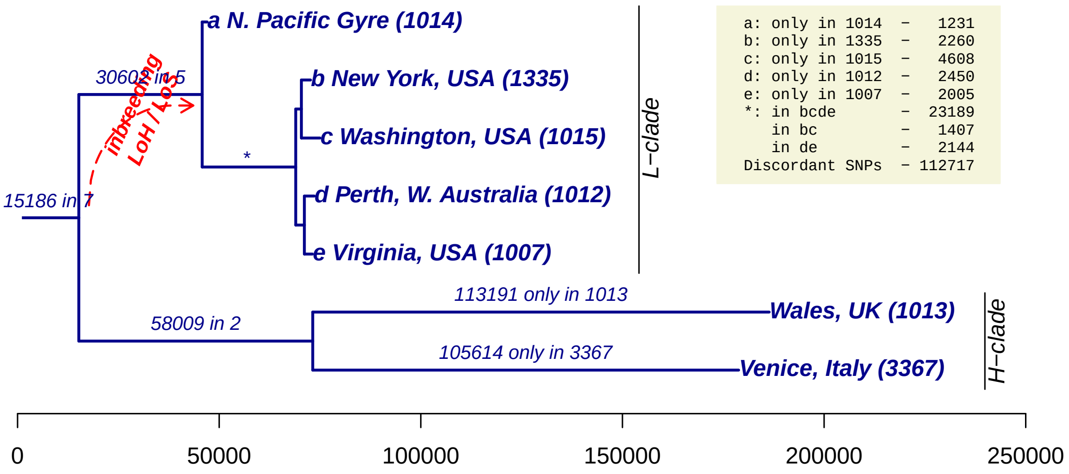


Figure 5: Tree based on qfiltered reads and unfiltered SNPs. "Lengths" are numbers of shared/private SNPs all Chrs.

```
# 115   162      4    X    X    X              X        1848   1932   1828   1014
# 118   165      5    X    X    X         X         X   2726   5022   8440   9715
# 119   166      5    X    X    X         X    X         902   1976   3172   2688
# 120   167      6    X    X    X         X    X    X  11742  21003  35227  15091
# 126   175      6    X    X    X    X    X         X  16884  13630   8608  12697
# 127   176      6    X    X    X    X    X    X        2422   2412   1566   1032
# Other   (    105  rows   w/   c2    < 1390     )       25256  22684  37272  45919
# Total                                                80566  86782 111974 112717
```

And percent of discordant SNPs:

```
nsge <- nrow(sg.edges)
discordv <- consistent.count - sg.edges[nsge,c('count1','count2','count3','count4')] ; discordv

#       count1 count2 count3 count4
# Total  80566  86782 111974 112717

discordv.pct <- round(discordv/consistent.count*100,1) ; discordv.pct

#       count1 count2 count3 count4
# Total     18   18.5   23.8   23.7
```

In short, the sharing pattern observed at 86782 or 18.5% of the 469906 medium-stringency consistent SNPs positions observed across all 7 isolates are discordant with the medium tree. (The strict tree has slightly more.)

A majority of the discordant SNPs fall into one of three patterns: 6-way sharing excluding Gyre (likely a technical artifact since the low coverage in Gyre reduces our power to detect SNPs there), or 6-way sharing excluding one of the two H-isolates (likely a reflection of sexuality in the H-clade—SNP positions in a population in Hardy-Weinberg equilibrium are fairly likely to be homozygous for the reference allele in a given individual).

```
third.biggest <- sort(showgroup(pat.summaries,6)[-8,'count2'],decreasing=T)[3]
big.three <- showgroup(pat.summaries,6,c2.thresh = third.biggest); big.three

#       Pat ShrBy 1007 1012 1013 1014  1015 3367 1335 count1 count2 count3 count4
# 112   157     6    X    X         X     X    X    X  13239  10814   6862  12202
# 120   167     6    X    X    X          X    X    X  11742  21003  35227  15091
# 126   175     6    X    X    X    X     X         X  16884  13630   8608  12697
# Other   (     4 rows   w/   c2    < 10814    )        3808   3670   2680   1964
# Total                                                45673  49117  53377  41954
```

```
big.three.frac <- sum(big.three[1:3,'count2'])/discordv$count2; big.three.frac

# [1] 0.5236915
```

I.e., 52.4% of discordant SNPs fall into one of these three categories.

Out of curiousity: what is the ratio of full genome to Chr 1 branch lengths. Except for the shortest few, generally ≈10x, as expected given the length of Chr 1:

```
# (vectors derived by editing Newick strings, and in that order)
print(
  c(Italy=86155, Wales=95697, IW=89598, Virg=330,     Aust=632,      VA=1296,
    Puget=2113,  NY=658,      PNY=480,  four=10059,   Gyre=568,      five=39517, all=69526) /
  c(Italy=8813,  Wales=9652,  IW=9365,  Virg=30,      Aust=61,       VA=19,
    Puget=207,   NY=41,       PNY=18,   four=1005,    Gyre=61,       five=3912,  all= 7054),
  digits=3)

# Italy Wales    IW  Virg  Aust    VA Puget    NY   PNY  four  Gyre  five   all
#  9.78  9.91  9.57 11.00 10.36 68.21 10.21 16.05 26.67 10.01  9.31 10.10  9.86

round(genome.length.constants()$genome.length.trunc / genome.length.constants()$chr1.length, digits=4)

# [1] 10.2879
```

9 Semi-Automated Tree-Building

Slightly formalizing the process above: Look for the bifurcation of the 7 strains that maximizes the number of shared SNPs *within* each side of the partition while minimizing the number and fraction of SNPs that are shared by subsets that include at least one strain on each side of the partition. The 2/5 split is the winner, with 6418 SNPs in confict with that partition (16% of the 39842 SNPs not shared by all 7; Chr1 data). The runner-up places the Gyre in a group by itself (7079 = 18% in conflict).

```
treepart <- function(p.summ=pat.summaries, root=127, verbose=T, stringency='count2'){
  root.shared <- p.summ[root+1,stringency]
  df<-NULL
  for(i in 1:floor(root/2)){
    if(bitwAnd(i,root)==i && i < root-i){
      l1 <- showgroup(p.summ,subset=i,split=NULL,proper.subset=F,total=T)
      l  <- l1[nrow(l1),stringency]
      r1 <- showgroup(p.summ,subset=root-i,split=NULL,proper.subset=F,total=T)
      r  <- r1[nrow(r1),stringency]
      c1 <- showgroup(p.summ,subset=root,split=i,proper.subset=T,total=T)
      c  <- c1[nrow(c1),stringency]
      df <- rbind(df, data.frame(pat=i,left=l,right=r,both=l+r,cross=c,all=l+r+c,ratio=c/(l+r+c),
                                best='',stringsAsFactors=F))
    }
  }
  df$pat<-as.octmode(df$pat)
  maxl <- which.max(df$left)
  maxr <- which.max(df$right)
  maxb <- which.max(df$both)
  minc <- which.min(df$cross)
  minr <- which.min(df$ratio)
  df$best[c(maxl,maxr,maxb,minc,minr)] <- '<'
  df$best[maxl] <- paste(df$best[maxl], 'L') # max Left
  df$best[maxr] <- paste(df$best[maxr], 'R') # max Right
  df$best[maxb] <- paste(df$best[maxb], 'B') # max Both (L+R)
  df$best[minc] <- paste(df$best[minc], 'C') # min Cross
  df$best[minr] <- paste(df$best[minr], 'O') # min ratiO (Cross/(Left+Right+Cross)
  if(verbose){
    same <- all(maxl==c(maxr,maxb,minc,minr))
    cat('root:',        format(as.octmode(root),width=3),
        '; shared:',    root.shared,
        '.  max l',     format(as.octmode(df$pat[maxl]),width=3),
        ', max r',      format(as.octmode(df$pat[maxr]),width=3),
        ', max both',  format(as.octmode(df$pat[maxb]),width=3),
        ', min cross', format(as.octmode(df$pat[minc]),width=3),
        ', min ratio', format(as.octmode(df$pat[minr]),width=3),
        '. \nAll the same?:',same,
        '\n')
    cat('\n')
  }
  return(df)
}
```

```
treepart()

# root: 177 ; shared: 62182 .  max l 077 , max r 010 , max both 010 , min cross 010 , min ratio 010 .
# All the same?: FALSE
#    pat   left  right   both  cross    all     ratio      best
# 1   01   1210 289197 290407 117895 408302 0.2887446
# 2   02  85695 179062 264757 143545 408302 0.3515658
# 3   03  86625 105697 192322 215980 408302 0.5289712
# 4   04   2734 279522 282256 126046 408302 0.3087078
# 5   05   3889 274296 278185 130117 408302 0.3186783
# 6   06  87989 100546 188535 219767 408302 0.5382462
# 7   07  89639  98363 188002 220300 408302 0.5395516
# 8   10   1103 332135 333238  75064 408302 0.1838443 < R B C O
# 9   11   2221 282010 284231 124071 408302 0.3038707
# 10  12  86269 123335 209604 198698 408302 0.4866447
# 11  13  87911 102474 190385 217917 408302 0.5337152
# 12  14   3398 276093 279491 128811 408302 0.3154797
# 13  15   6023 273221 279244 129058 408302 0.3160847
# 14  16  88762  98932 187694 220608 408302 0.5403060
# 15  17  92437  97647 190084 218218 408302 0.5344524
# 16  20  94942 165462 260404 147898 408302 0.3622270
# 17  21  95741  96070 191811 216491 408302 0.5302227
# 18  22 267558  61350 328908  79394 408302 0.1944492
# 19  23 269213   8703 277916 130386 408302 0.3193372
# 20  24  97293  91454 188747 219555 408302 0.5377270
# 21  25  98831  89139 187970 220332 408302 0.5396300
# 22  26 270478   4870 275348 132954 408302 0.3256266
# 23  27 273828   3327 277155 131147 408302 0.3212010
# 24  30  95536 112945 208481 199821 408302 0.4893951
# 25  31  97054  93029 190083 218219 408302 0.5344549
# 26  32 268332  21310 289642 118660 408302 0.2906182
# 27  33 271905   6449 278354 129948 408302 0.3182644
# 28  34  98145  89824 187969 220333 408302 0.5396324
# 29  35 101895  88428 190323 217979 408302 0.5338671
# 30  36 271890   3636 275526 132776 408302 0.3251907
# 31  37 280857   2716 283573 124729 408302 0.3054822
# 32  40   1233 283707 284940 123362 408302 0.3021342
# 33  41   1957 273177 275134 133168 408302 0.3261507
# 34  42  86469 103226 189695 218607 408302 0.5354052
# 35  43  87577  98843 186420 221882 408302 0.5434262
# 36  44   3668 272853 276521 131781 408302 0.3227537
# 37  45   5508 269028 274536 133766 408302 0.3276153
# 38  46  89196  97572 186768 221534 408302 0.5425739
# 39  47  91873  95971 187844 220458 408302 0.5399386
# 40  50   1782 275846 277628 130674 408302 0.3200425
# 41  51   3049 271596 274645 133657 408302 0.3273484
# 42  52  87080  99819 186899 221403 408302 0.5422530
# 43  53  88991  97918 186909 221393 408302 0.5422285
# 44  54   4434 270058 274492 133810 408302 0.3277231
# 45  55   8497 268202 276699 131603 408302 0.3223178
# 46  56  90128  96225 186353 221949 408302 0.5435903
# 47  57  95836  95357 191193 217109 408302 0.5317363
# 48  60  95713  93634 189347 218955 408302 0.5362575
# 49  61  96684  89422 186106 222196 408302 0.5441952
# 50  62 268717   6909 275626 132676 408302 0.3249458
# 51  63 270814   3906 274720 133582 408302 0.3271647
# 52  64  98527  88440 186967 221335 408302 0.5420865
# 53  65 101114  86697 187811 220491 408302 0.5400194
# 54  66 272743   2629 275372 132930 408302 0.3255678
# 55  67 278683   1453 280136 128166 408302 0.3139000
# 56  70  96340  90579 186919 221383 408302 0.5422041
# 57  71  98107  88583 186690 221612 408302 0.5427649
# 58  72 269573   4578 274151 134151 408302 0.3285583
# 59  73 273771   3195 276966 131336 408302 0.3216639
# 60  74  99536  87112 186648 221654 408302 0.5428678
# 61  75 105295  86101 191396 216906 408302 0.5312391
# 62  76 274535   1596 276131 132171 408302 0.3237089
# 63  77 288224    917 289141 119161 408302 0.2918453       < L
```

Comparing the 5/2 split to the second-place NPG/rest split (below), the former has fewer pattern instances in conflict with the split (6418 vs 7079), as well as somewhat more random distribution of the conflicting patterns (92 vs 62 rows), whereas the 1/6 split has the majority of its conflicts (3912 of 7079, or 55%) concentrated in one pattern—the 5 NE strains. Collectively, these seem to favor the 5/2 split as the correct “history.”

```
showgroup(pat.summaries,split=strtoi('022'), subset=127, proper.subset=T, c2.thresh=100)

#       Pat ShrBy 1007 1012 1013 1014 1015 3367 1335 count1 count2 count3 count4
# 4     003     2                             X    X    994    298    532    587
# 7     006     2                        X    X         624    138    282    590
# 8     007     3                        X    X    X    104    197    371    557
# 12    013     3                   X         X    X    257    226    146    338
# 16    017     4                   X    X    X    X    390    329    211    564
# 18    021     2              X                   X    998    167    337    402
# 20    023     3              X              X    X   1274    558   1020    533
# 21    024     2              X         X              686    195    410    625
# 22    025     3              X         X         X    135    216    466    522
# 23    026     3              X         X    X         793    431    763    789
# 24    027     4              X         X    X    X    461    759   1423    771
# 26    031     3              X    X              X    268    233    151    361
# 27    032     3              X    X         X         698    131     74     86
# 28    033     4              X    X         X    X   1139    973    574    306
# 29    034     3              X    X    X              103    119     91    219
# 30    035     4              X    X    X         X    578    509    329    503
# 31    036     4              X    X    X    X         345    320    227    211
# 32    037     5              X    X    X    X    X   2247   1877   1193    620
# 35    042     2         X                   X         503    119    254    394
# 39    046     3         X              X    X          58    154    425    604
# 40    047     4         X              X    X    X    127    256    708    708
# 48    057     5         X         X    X    X    X    221    219    189    324
# 49    060     2         X    X                        627    116    237    388
# 51    062     3         X    X              X         703    269    454    469
# 52    063     4         X    X              X    X    151    184    332    194
# 53    064     3         X    X         X               53    184    458    601
# 54    065     4         X    X         X         X    122    284    731    582
# 55    066     4         X    X         X    X         217    489   1025    851
# 56    067     5         X    X         X    X    X    556   1015   2544   1151
# 62    075     5         X    X    X    X         X    195    187    210    328
# 63    076     5         X    X    X    X    X         106    130    124    128
# 64    077     6         X    X    X    X    X    X    850    847    827    485
# 83    122     3    X         X              X         501    162    165    354
# 86    125     4    X         X         X         X     67    113    223    283
# 87    126     4    X         X         X    X          99    198    268    425
# 88    127     5    X         X         X    X    X    225    321    501    482
# 94    135     5    X         X    X    X         X    126    117     88    235
# 96    137     6    X         X    X    X    X    X    405    324    240    333
# 99    142     3    X    X                   X         386    409    463    755
# 103   146     4    X    X              X    X         196    510    969   1795
# 104   147     5    X    X              X    X    X   2073   4042   6741  10001
# 111   156     5    X    X         X    X    X         565    575    410    735
# 112   157     6    X    X         X    X    X    X  13239  10814   6862  12202
# 113   160     3    X    X    X                        337    375    399    712
# 115   162     4    X    X    X              X        1848   1932   1828   1014
# 116   163     5    X    X    X              X    X    255    271    328    316
# 117   164     4    X    X    X         X              237    627   1053   1752
# 118   165     5    X    X    X         X         X   2726   5022   8440   9715
# 119   166     5    X    X    X         X    X         902   1976   3172   2688
# 120   167     6    X    X    X         X    X    X  11742  21003  35227  15091
# 125   174     5    X    X    X    X    X              659    682    468    782
# 126   175     6    X    X    X    X    X         X  16884  13630   8608  12697
# 127   176     6    X    X    X    X    X    X        2422   2412   1566   1032
# Other   (    39 rows   w/   c2    <  100    )        3209   1750   1921   4847
# Total                                               75686  79394  97058  94037

showgroup(pat.summaries,split=strtoi('010'), subset=127, proper.subset=T, c2.thresh=100)

#       Pat ShrBy 1007 1012 1013 1014 1015 3367 1335 count1 count2 count3 count4
# 10    011     2                   X              X    515    486    317    827
# 12    013     3                   X         X    X    257    226    146    338
# 13    014     2                   X    X              133    139    132    417
# 14    015     3                   X    X         X   1041    984    660   1389
# 16    017     4                   X    X    X    X    390    329    211    564
# 26    031     3              X    X              X    268    233    151    361
# 27    032     3              X    X         X         698    131     74     86
# 28    033     4              X    X         X    X   1139    973    574    306
# 29    034     3              X    X    X              103    119     91    219
# 30    035     4              X    X    X         X    578    509    329    503
# 31    036     4              X    X    X    X         345    320    227    211
# 32    037     5              X    X    X    X    X   2247   1877   1193    620
# 46    055     4         X         X    X         X    606    696    668    971
# 48    057     5         X         X    X    X    X    221    219    189    324
# 62    075     5         X    X    X    X         X    195    187    210    328
# 63    076     5         X    X    X    X    X         106    130    124    128
# 64    077     6         X    X    X    X    X    X    850    847    827    485
# 78    115     4    X              X    X         X    141    139    122    604
# 94    135     5    X         X    X    X         X    126    117     88    235
# 96    137     6    X         X    X    X    X    X    405    324    240    333
# 109   154     4    X    X         X    X             1227   1390   1065   1738
# 110   155     5    X    X         X    X         X  40157  35344  22417  30602
# 111   156     5    X    X         X    X    X         565    575    410    735
# 112   157     6    X    X         X    X    X    X  13239  10814   6862  12202
# 125   174     5    X    X    X    X    X              659    682    468    782
# 126   175     6    X    X    X    X    X         X  16884  13630   8608  12697
# 127   176     6    X    X    X    X    X    X        2422   2412   1566   1032
# Other   (    35 rows   w/   c2    <  100    )        2151   1232   1130   3730
# Total                                               87668  75064  49099  72767
```

Below is the full summary of shared SNPs that do *not* directly correspond to tree splits, e.g. deep coalescence, independent coincident mutations, false positives/false negatives in the shared SNP calls, loss of SNPs in hemizygous regions, etc. (Additionally, SAMTools' SNP calls exclude positions it judges to be homozygous, and I think it operates without regard to the reference sequence, so homozygous nonreference positions, while rare except in IT/Wales, often are not called SNPs by SAMTools, but are relevant for this analysis. Provided the position is called a SNP in some other isolate, the consistency filtering we've done above should recover it, but this is still worth keeping in mind when examining the data.)

First, here are SNPs that "coalesce" on the branch from the LCA of bcde, i.e., shared among some nonempty, proper subset of bcde other than bc or de. There are 8 such patterns: any of the 4 choose 3 trios plus any of the 4 pairs having exactly one of bc.

```
sg4 <- showgroup(pat.summaries, subset=strtoi('0145'), split=5, proper.subset = F)
sg4

#       Pat ShrBy 1007 1012 1013 1014 1015 3367 1335 count1 count2 count3 count4
# 34    041     2         X                        X     42     92    313    368
# 37    044     2         X              X               69    279   1001   1809
# 38    045     3         X              X         X    202    593   1970   1656
# 66    101     2    X                             X     29     47     73    314
# 69    104     2    X                   X               39    122    329   1196
# 70    105     3    X                   X         X     78    181    396    805
# 98    141     3    X    X                        X     78    149    258    519
# 101   144     3    X    X              X              383   1176   2395   4432
# 102   145     4    X    X              X         X   5992  12644  22332  23189
# Total                                                6912  15283  29067  34288

sg4n <- nrow(sg4)
sg4pct <- round(sg4$count2[sg4n-1]/sg4$count2[sg4n]*100,1)
sg4pct

# [1] 82.7
```

So, of the 15283 SNPs found only in bcde, 82.7% have a sharing pattern consistent with the given tree structure.

Similarly, we analyze patterns relative to the root of the L-clade (14 patterns—any nonempty proper subset of bcde together with a):

```
sg5 <- showgroup(pat.summaries,subset=strtoi('0155'), split=8, proper.subset = F)
sg5

#       Pat ShrBy 1007 1012 1013 1014 1015 3367 1335 count1 count2 count3 count4
# 10    011     2                   X              X    515    486    317    827
# 13    014     2                   X    X              133    139    132    417
# 14    015     3                   X    X         X   1041    984    660   1389
# 41    050     2         X         X                    13     24     53    105
# 42    051     3         X         X              X     52     57     74    126
# 45    054     3         X         X    X               20     78    133    292
# 46    055     4         X         X    X         X    606    696    668    971
# 73    110     2    X              X                    12     11     29    150
# 74    111     3    X              X              X      6     11      8    139
# 77    114     3    X              X    X               12     36     56    365
# 78    115     4    X              X    X         X    141    139    122    604
# 105   150     3    X    X         X                    28     51     55    238
# 106   151     4    X    X         X              X     43     69     54    220
# 109   154     4    X    X         X    X             1227   1390   1065   1738
# 110   155     5    X    X         X    X         X  40157  35344  22417  30602
# Total                                               44006  39515  25843  38183

sg5n <- nrow(sg5)
sg5pct <- round(sg5$count2[sg5n-1]/sg5$count2[sg5n]*100,1)
```

I.e., of the 39515 SNPs found only in abcde, 89.4% have a sharing pattern consistent with the given tree structure.

Finally, how many SNPs have patterns inconsistent with the 5-2 split, i.e., include at least one strain on each side of the 5-2 split, but not shared by all 7?

```
sg7 <- showgroup(pat.summaries, subset=127, split=strtoi('022'), proper.subset=F)
sg7

#       Pat ShrBy 1007 1012 1013 1014 1015 3367 1335 count1 count2 count3 count4
# 4     003     2                             X    X    994    298    532    587
# 7     006     2                        X    X         624    138    282    590
# 8     007     3                        X    X    X    104    197    371    557
# 11    012     2                   X         X         565     49     32     93
# 12    013     3                   X         X    X    257    226    146    338
# 15    016     3                   X    X    X          46     60     49    152
# 16    017     4                   X    X    X    X    390    329    211    564
# 18    021     2              X                   X    998    167    337    402
# 20    023     3              X              X    X   1274    558   1020    533
# 21    024     2              X         X              686    195    410    625
# 22    025     3              X         X         X    135    216    466    522
# 23    026     3              X         X    X         793    431    763    789
# 24    027     4              X         X    X    X    461    759   1423    771
# 25    030     2              X    X                   609     69     47     93
# 26    031     3              X    X              X    268    233    151    361
# 27    032     3              X    X         X         698    131     74     86
# 28    033     4              X    X         X    X   1139    973    574    306
# 29    034     3              X    X    X              103    119     91    219
# 30    035     4              X    X    X         X    578    509    329    503
# 31    036     4              X    X    X    X         345    320    227    211
# 32    037     5              X    X    X    X    X   2247   1877   1193    620
# 35    042     2         X                   X         503    119    254    394
# 36    043     3         X                   X    X     56     86    151    133
# 39    046     3         X              X    X          58    154    425    604
# 40    047     4         X              X    X    X    127    256    708    708
# 43    052     3         X         X         X           8     13     17     22
# 44    053     4         X         X         X    X     35     34     26     56
# 47    056     4         X         X    X    X          26     44     50     88
# 48    057     5         X         X    X    X    X    221    219    189    324
# 49    060     2         X    X                        627    116    237    388
# 50    061     3         X    X                   X     52     80    131    115
# 51    062     3         X    X              X         703    269    454    469
# 52    063     4         X    X              X    X    151    184    332    194
# 53    064     3         X    X         X               53    184    458    601
# 54    065     4         X    X         X         X    122    284    731    582
# 55    066     4         X    X         X    X         217    489   1025    851
# 56    067     5         X    X         X    X    X    556   1015   2544   1151
# 57    070     3         X    X    X                    22      9     14     24
# 58    071     4         X    X    X              X      9     20      7     28
# 59    072     4         X    X    X         X          41     36     21     31
# 60    073     5         X    X    X         X    X     94     72     62     38
# 61    074     4         X    X    X    X               20     46     51    116
# 62    075     5         X    X    X    X         X    195    187    210    328
# 63    076     5         X    X    X    X    X         106    130    124    128
# 64    077     6         X    X    X    X    X    X    850    847    827    485
# 67    102     2    X                        X         351     67     96    351
# 68    103     3    X                        X    X     24     34     46    143
# 71    106     3    X                   X    X          32     66    109    377
# 72    107     4    X                   X    X    X     58     84    129    330
# 75    112     3    X              X         X          10     11      8     26
# 76    113     4    X              X         X    X      7      9      5     66
# 79    116     4    X              X    X    X           8      8     11    101
# 80    117     5    X              X    X    X    X     48     32     25    241
# 81    120     2    X         X                        432     76     98    309
# 82    121     3    X         X                   X     22     22     34     73
# 83    122     3    X         X              X         501    162    165    354
# 84    123     4    X         X              X    X     63     98     91    124
# 85    124     3    X         X         X               43     88    152    400
# 86    125     4    X         X         X         X     67    113    223    283
# 87    126     4    X         X         X    X          99    198    268    425
# 88    127     5    X         X         X    X    X    225    321    501    482
# 89    130     3    X         X    X                     9      9      9     27
# 90    131     4    X         X    X              X      6      3      0     52
# 91    132     4    X         X    X         X          19     21     10     38
# 92    133     5    X         X    X         X    X     31     28     15     52
# 93    134     4    X         X    X    X               18     17     22    143
# 94    135     5    X         X    X    X         X    126    117     88    235
# 95    136     5    X         X    X    X    X          34     56     26    106
# 96    137     6    X         X    X    X    X    X    405    324    240    333
# 99    142     3    X    X                   X         386    409    463    755
# 100   143     4    X    X                   X    X     37     58    103    190
# 103   146     4    X    X              X    X         196    510    969   1795
# 104   147     5    X    X              X    X    X   2073   4042   6741  10001
# 107   152     4    X    X         X         X          18     27     15     67
# 108   153     5    X    X         X         X    X     39     27     26     96
# 111   156     5    X    X         X    X    X         565    575    410    735
# 112   157     6    X    X         X    X    X    X  13239  10814   6862  12202
# 113   160     3    X    X    X                        337    375    399    712
# 114   161     4    X    X    X                   X     74     96    113    207
# 115   162     4    X    X    X              X        1848   1932   1828   1014
# 116   163     5    X    X    X              X    X    255    271    328    316
# 117   164     4    X    X    X         X              237    627   1053   1752
# 118   165     5    X    X    X         X         X   2726   5022   8440   9715
# 119   166     5    X    X    X         X    X         902   1976   3172   2688
# 120   167     6    X    X    X         X    X    X  11742  21003  35227  15091
# 121   170     4    X    X    X    X                    13     18     15     69
# 122   171      5    X    X    X    X              X     41     19     13     70
# 123   172      5    X    X    X    X         X          58     71     45     86
# 124   173      6    X    X    X    X         X    X    131     87     47    114
# 125   174      5    X    X    X    X    X              659    682    468    782
# 126   175      6    X    X    X    X    X         X  16884  13630   8608  12697
# 127   176      6    X    X    X    X    X    X         2422   2412   1566   1032
# 128   177      7    X    X    X    X    X    X    X  77690  62182  38744  15186
# Total                                              153376 141576 135802 109223

sg7n <- nrow(sg7)
sg7pct <- round(sg7$count2[sg7n-1]/sg7$count2[sg7n]*100,1)
sg7pct

# [1] 43.9
```

A more compact version of that table, showing only the larger counts:

```
thresh <- signif(.02 * sg7$count2[sg7n],1)
thresh

# [1] 3000

showgroup(pat.summaries, subset=127, split=strtoi('022'), proper.subset=F, c2.thresh = thresh)

#       Pat ShrBy 1007 1012 1013 1014 1015 3367 1335 count1 count2 count3 count4
# 104   147      5    X    X              X    X    X   2073   4042   6741  10001
# 112   157      6    X    X         X    X    X    X  13239  10814   6862  12202
# 118   165      5    X    X    X         X         X   2726   5022   8440   9715
# 120   167      6    X    X    X         X    X    X  11742  21003  35227  15091
# 126   175      6    X    X    X    X    X         X  16884  13630   8608  12697
# 128   177      7    X    X    X    X    X    X    X  77690  62182  38744  15186
# Other   (     87 rows   w/   c2    < 3000    )        29022  24883  31180  34331
# Total                                              153376 141576 135802 109223
```

So, of the 141576 SNPs found both in the L- and H-clades, 43.9% have a sharing pattern consistent with the given tree structure, i.e., are found in all 7 isolates. Among the others, three patterns dominate—(i) the 6-way pattern excluding the Gyre is the largest, plausibly explained by 7-way sharing from which the Gyre drops out due to low coverage/high error rate, (ii) the 6-way excluding Italy, and (iii) ditto for Wales. Origin of the later two cases is unclear, but may partly reflect Hardy-Weinberg—some positions that are *population-level* SNPs in those isolates will be homozygous-reference in the CCMP founder cell for IT or Wales. If I take the 7-way shared SNP count (69526) as a surrogate approximating the number of population-level SNPs in either IT or Wales that are shared with the L-clade, then I might expect, based on HWE, roughly half that number to to be lost (become homozygous) in IT, and a similar number in Wales. However, the observed counts of these positions are lower by ≈20K than I might have guessed from HWE, perhaps suggesting that IT and Wales are distinct populations, each with a pool of many thousand private polymorphisms.

In aggregate:

```
untreelike <-
  sg7$count2[sg7n]-sg7$count2[sg7n-1] +
  sg5$count2[sg5n]-sg5$count2[sg5n-1] +
  sg4$count2[sg4n]-sg4$count2[sg4n-1]
untreelike

# [1] 86204

consistent.count[2]

# [1] 469906

unpct <- round(untreelike/consistent.count[2]*100,1)
unpct

# [1] 18.3
```

I.e., 86204 or 18.3% of the 469906 consistent SNPs identified (by criterion 2) across all 7 isolates are discordant with the assumed tree.

Overall, based on this data, I take the following to be obvious: (a) separation of the the H-isolates from the L-isolates (and from each other??), and (b) near-identity of the L-isolates. Due to the small counts, the exact topology among the L-isolates (esp. bcde) is uncertain, but *any* topology there is consistent with the asexual/clonal/global-expansion hypothesis, so there is little point in examining this subtree more carefuly. Again, we believe the (apparent) slight separation of the Gyre from the other L-isolates is largely driven by technical artifacts (lower coverage/higher error rates) in the sequencing rather than by biological effects. However, the discord between Gyre SNPs and others is the major substantive ambiguity in the offered tree. Nevertheless, in the next section we show by a bootstrap analysis that the offered placement of Gyre with respect to the other 4 L-isolates is strongly supported by the data.

9.1 Bootstrap

How robust is the inferred tree? Italy/Wales seem clearly related to each other but separate from the other 5. Likewise, the 4 coastal L-isolates seem to be closely related, with little data to separate them (and perhaps little sense in trying). So, the key question here is whether the top level bifurcation is 2/5 or NPG/6. Here, we do a simple bootstrap test (on c2 numbers only) to see whether the 2/5 split is consistently the most parsimonious.

```
n2 <- sum(pattern.counts[[2]][,2]); n2

# [1] 469906
```

Conceptually, we sample, with replacement, n2=469906 SNP positions from among the 469906 positions declared consisent SNPs according to criterion c2, and recalculate the statistics examined above to see whether the 2/5 split again minimizes conflicting sharing patterns. This resampling/calculation is repeated nboot times (set near front of file). Since all that matters is the sharing pattern, this procedure is expedited by actually sampling 469906 independent integers in the range 0:127 with probabilities proportional to the patttern counts given in column 2 of pattern.counts[[2]]. The sample is then tabulated in a 128 row table analogous to pattern.summaries, for analysis by showgroups/treepart, as above.

```
boot.sample <- sample(0:127,n2,replace=T,prob=pattern.counts[[2]][,2])
str(boot.sample)

#  int [1:469906] 18 2 109 18 16 2 127 18 2 25 ...

boot.count  <- mytable(boot.sample,c(0,127))
boot.count[c(1:4,125:128),] # show a few rows

#      val count
# [1,]   0   600
# [2,]   1   668
# [3,]   2 84782
# [4,]   3   317
# [5,] 124   714
# [6,] 125 13529
# [7,] 126  2444
# [8,] 127 62227

boot.counts <- list(NULL,boot.count,NULL) # dummy list with just c2 summaries
cor(pattern.counts[[2]][,2],boot.counts[[2]][,2]) # just curious - how correlated are they?

# [1] 0.9999912

boot.summaries <- pat.summary(boot.counts)
showgroup(boot.summaries,c2.thresh=400) #show a few rows

#       Pat ShrBy 1007 1012 1013 1014 1015 3367 1335 count1 count2 count3 count4
# 1     000     0                                        NA    600     NA     NA
# 2     001     1                                  X     NA    668     NA     NA
# 3     002     1                             X          NA  84782     NA     NA
# 5     004     1                        X               NA   2183     NA     NA
# 6     005     2                        X         X     NA    512     NA     NA
# 9     010     1                   X                    NA    496     NA     NA
# 10    011     2                   X              X     NA    522     NA     NA
# 14    015     3                   X    X         X     NA    962     NA     NA
# 17    020     1              X                         NA  94351     NA     NA
# 19    022     2              X              X          NA  87810     NA     NA
# 20    023     3              X              X    X     NA    622     NA     NA
# 23    026     3              X         X    X          NA    416     NA     NA
# 24    027     4              X         X    X    X     NA    756     NA     NA
# 28    033     4              X    X         X    X     NA   1024     NA     NA
# 30    035     4              X    X    X         X     NA    548     NA     NA
# 32    037     5              X    X    X    X    X     NA   1910     NA     NA
# 33    040     1         X                              NA    696     NA     NA
# 38    045     3         X              X         X     NA    590     NA     NA
# 46    055     4         X         X    X         X     NA    672     NA     NA
# 55    066     4         X    X         X    X          NA    477     NA     NA
# 56    067     5         X    X         X    X    X     NA    984     NA     NA
# 64    077     6         X    X    X    X    X    X     NA    836     NA     NA
# 97    140     2    X    X                              NA   1098     NA     NA
# 101   144     3    X    X              X               NA   1208     NA     NA
# 102   145     4    X    X              X         X     NA  12366     NA     NA
# 103   146     4    X    X              X    X          NA    512     NA     NA
# 104   147     5    X    X              X    X    X     NA   4120     NA     NA
# 109   154     4    X    X         X    X               NA   1402     NA     NA
# 110   155     5    X    X         X    X         X     NA  35560     NA     NA
# 111   156     5    X    X         X    X    X          NA    606     NA     NA
# 112   157     6    X    X         X    X    X    X     NA  10853     NA     NA
# 115   162     4    X    X    X              X          NA   1963     NA     NA
# 117   164     4    X    X    X         X               NA    624     NA     NA
# 118   165     5    X    X    X         X         X     NA   5137     NA     NA
# 119   166     5    X    X    X         X    X          NA   1934     NA     NA
# 120   167     6    X    X    X         X    X    X     NA  20694     NA     NA
# 125   174     5    X    X    X    X    X               NA    714     NA     NA
# 126   175     6    X    X    X    X    X         X     NA  13529     NA     NA
# 127   176     6    X    X    X    X    X    X          NA   2444     NA     NA
# 128   177     7    X    X    X    X    X    X    X     NA  62227     NA     NA
# Other   (    88 rows   w/   c2    <  400    )          NA  10498     NA     NA
# Total                                                  NA 469906     NA     NA
```

Tree partition analysis (and how to pluck out only the best rows based on 3 smallest cross counts and “best” criteria):

```
tp <- treepart(boot.summaries,root=127) ; tp

# root: 177 ; shared: 62227 .  max l 077 , max r 010 , max both 010 , min cross 010 , min ratio 010 .
# All the same?: FALSE
#    pat   left  right   both  cross    all     ratio      best
# 1   01   1268 289265 290533 117746 408279 0.2883959
# 2   02  85382 179137 264519 143760 408279 0.3521122
# 3   03  86367 105773 192140 216139 408279 0.5293904
# 4   04   2783 279686 282469 125810 408279 0.3081471
# 5   05   3963 274240 278203 130076 408279 0.3185959
# 6   06  87716 100567 188283 219996 408279 0.5388374
# 7   07  89418  98307 187725 220554 408279 0.5402041
# 8   10   1096 331755 332851  75428 408279 0.1847462 < R B C O
# 9   11   2286 281996 284282 123997 408279 0.3037065
# 10  12  85939 123202 209141 199138 408279 0.4877498
# 11  13  87673 102514 190187 218092 408279 0.5341739
# 12  14   3422 276174 279596 128683 408279 0.3151840
# 13  15   6086 273191 279277 129002 408279 0.3159653
# 14  16  88479  98916 187395 220884 408279 0.5410124
# 15  17  92218  97618 189836 218443 408279 0.5350336
# 16  20  94951 165296 260247 148032 408279 0.3625756
# 17  21  95790  95849 191639 216640 408279 0.5306175
# 18  22 267543  61374 328917  79362 408279 0.1943818
# 19  23 269321   8779 278100 130179 408279 0.3188481
# 20  24  97330  91176 188506 219773 408279 0.5382912
# 21  25  98899  88783 187682 220597 408279 0.5403094
# 22  26 270489   4932 275421 132858 408279 0.3254098
# 23  27 273958   3311 277269 131010 408279 0.3208835
# 24  30  95505 112516 208021 200258 408279 0.4904930
# 25  31  97115  92767 189882 218397 408279 0.5349210
# 26  32 268291  21112 289403 118876 408279 0.2911636
# 27  33 272091   6515 278606 129673 408279 0.3176088
# 28  34  98142  89531 187673 220606 408279 0.5403315
# 29  35 101992  88090 190082 218197 408279 0.5344311
# 30  36 271866   3665 275531 132748 408279 0.3251404
# 31  37 281103   2719 283822 124457 408279 0.3048332
# 32  40   1296 283851 285147 123132 408279 0.3015879
# 33  41   2061 273142 275203 133076 408279 0.3259438
# 34  42  86211 103290 189501 218778 408279 0.5358542
# 35  43  87379  98842 186221 222058 408279 0.5438879
# 36  44   3768 273016 276784 131495 408279 0.3220714
# 37  45   5635 268963 274598 133681 408279 0.3274256
# 38  46  88975  97624 186599 221680 408279 0.5429620
# 39  47  91719  95936 187655 220624 408279 0.5403756
# 40  50   1819 275928 277747 130532 408279 0.3197127
# 41  51   3179 271605 274784 133495 408279 0.3269700
# 42  52  86807  99874 186681 221598 408279 0.5427612
# 43  53  88822  97953 186775 221504 408279 0.5425310
# 44  54   4519 270157 274676 133603 408279 0.3272346
# 45  55   8615 268167 276782 131497 408279 0.3220763
# 46  56  89905  96267 186172 222107 408279 0.5440079
# 47  57  95668  95360 191028 217251 408279 0.5321141
# 48  60  95744  93371 189115 219164 408279 0.5367996
# 49  61  96761  89126 185887 222392 408279 0.5447059
# 50  62 268745   6953 275698 132581 408279 0.3247314
# 51  63 270977   3919 274896 133383 408279 0.3266957
# 52  64  98594  88179 186773 221506 408279 0.5425359
# 53  65 101214  86346 187560 220719 408279 0.5406083
# 54  66 272780   2683 275463 132816 408279 0.3253070
# 55  67 278829   1435 280264 128015 408279 0.3135478
# 56  70  96339  90329 186668 221611 408279 0.5427930
# 57  71  98221  88296 186517 221762 408279 0.5431629
# 58  72 269584   4636 274220 134059 408279 0.3283514
# 59  73 274043   3224 277267 131012 408279 0.3208884
# 60  74  99588  86839 186427 221852 408279 0.5433833
# 61  75 105462  85769 191231 217048 408279 0.5316169
# 62  76 274561   1642 276203 132076 408279 0.3234945
# 63  77 288510    925 289435 118844 408279 0.2910853       < L
```

```
otp <- order(tp[,'cross'])[1:3]     # 3 smallest 'cross' counts
btp <- which(tp[,'best'] != '')     # 'best' by Left/Right/Both/Cross/ratiO
toptp <- unique(c(otp,btp,18,8))    # above, plus 5/2, 6/1 splits
print(tp[toptp,])                   # show the winners

#    pat   left  right   both  cross    all     ratio      best
# 8   10   1096 331755 332851  75428 408279 0.1847462 < R B C O
# 18  22 267543  61374 328917  79362 408279 0.1943818
# 1   01   1268 289265 290533 117746 408279 0.2883959
# 63  77 288510    925 289435 118844 408279 0.2910853       < L
```

Now repeat the above nboot times, and summarize results:

```
nboot <- params$nboot #  default from params set in section 2
nboot <- ((nboot+2) %/% 4) * 4 + 1  # summary is cleaner if n mod 4 == 1, so int median/quartiles
cat('***\n*** Doing', nboot, 'bootstrap replicates.\n***\n')

# ***
# *** Doing 101 bootstrap replicates.
# ***

bcor <- numeric(nboot)
b52cross <- integer(nboot)
b61cross <- integer(nboot)
brev <- logical(nboot)
for(i in 1:nboot){
  boot.sample <- sample(0:127,n2,replace=T,prob=pattern.counts[[2]][,2])
  boot.count <- mytable(boot.sample,c(0,127))
  boot.counts <- list(NULL,boot.count,NULL) # dummy list with just c2 summaries
  boot.summaries <- pat.summary(boot.counts)
  tp <- treepart(boot.summaries,root=127, verbose=F)
  bcor[i] <- cor(pattern.counts[[2]][,2],boot.counts[[2]][,2]) # just curious - how correlated are they?
  b52cross[i] <- tp[18,'cross']
  b61cross[i] <- tp[ 8,'cross']
  brev[i] <- (b52cross[i] > b61cross[i])
  if(brev[i]){
    # show the unexpected ones; probably breaks w/ cache
    otp <- order(tp[,'cross'])[1:3]
    btp <- which(tp[,'best'] != '')
    toptp <- unique(c(otp,btp,18,8))
    print(tp[toptp,])
  }
}
```

```
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1160 332014 333174  75109 408283 0.1839631 < R B C O
# 18  22 267271  61464 328735  79548 408283 0.1948354
# 1   01   1221 288865 290086 118197 408283 0.2894977
# 63  77 288099    927 289026 119257 408283 0.2920940       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1094 331785 332879  75081 407960 0.1840401 < R B C O
# 18  22 267351  61178 328529  79431 407960 0.1947029
# 1   01   1193 289019 290212 117748 407960 0.2886263
# 63  77 288087    923 289010 118950 407960 0.2915727       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1077 332566 333643  74877 408520 0.1832885 < R B C O
# 18  22 267919  61400 329319  79201 408520 0.1938730
# 1   01   1193 289455 290648 117872 408520 0.2885342
# 63  77 288578    897 289475 119045 408520 0.2914056       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1099 331968 333067  74994 408061 0.1837813 < R B C O
# 18  22 267149  61552 328701  79360 408061 0.1944807
# 1   01   1222 288655 289877 118184 408061 0.2896234
# 63  77 287835    915 288750 119311 408061 0.2923852       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1094 331997 333091  75318 408409 0.1844181 < R B C O
# 18  22 267448  61849 329297  79112 408409 0.1937078
# 1   01   1198 289032 290230 118179 408409 0.2893643
# 63  77 288274    936 289210 119199 408409 0.2918618       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1085 332159 333244  75184 408428 0.1840814 < R B C O
# 18  22 267430  61424 328854  79574 408428 0.1948299
# 1   01   1229 288886 290115 118313 408428 0.2896790
# 63  77 288071    913 288984 119444 408428 0.2924481       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1092 332052 333144  75376 408520 0.1845099 < R B C O
# 18  22 267464  61343 328807  79713 408520 0.1951263
# 1   01   1170 289311 290481 118039 408520 0.2889430
# 63  77 288125    932 289057 119463 408520 0.2924288       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1084 332108 333192  74966 408158 0.1836691 < R B C O
# 18  22 267710  61217 328927  79231 408158 0.1941185
# 1   01   1242 289256 290498 117660 408158 0.2882707
# 63  77 288517    886 289403 118755 408158 0.2909535       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1119 332162 333281  75485 408766 0.1846656 < R B C O
# 18  22 267660  61604 329264  79502 408766 0.1944927
# 1   01   1196 289451 290647 118119 408766 0.2889648
# 63  77 288117    983 289100 119666 408766 0.2927494       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1109 332181 333290  75171 408461 0.1840347 < R B C O
# 18  22 267410  61476 328886  79575 408461 0.1948166
# 1   01   1199 289122 290321 118140 408461 0.2892320
# 63  77 288130    942 289072 119389 408461 0.2922898       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1114 331931 333045  75374 408419 0.1845507 < R B C O
# 18  22 267525  61921 329446  78973 408419 0.1933627
# 1   01   1234 289028 290262 118157 408419 0.2893034
# 63  77 288165    971 289136 119283 408419 0.2920604       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1092 331886 332978  75297 408275 0.1844272 < R B C O
# 18  22 267181  61582 328763  79512 408275 0.1947511
# 1   01   1199 288894 290093 118182 408275 0.2894667
# 63  77 287855    898 288753 119522 408275 0.2927488       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1126 331522 332648  75408 408056 0.1847982 < R B C O
# 18  22 266928  61673 328601  79455 408056 0.1947159
# 1   01   1245 288534 289779 118277 408056 0.2898548
# 63  77 287610    949 288559 119497 408056 0.2928446       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1041 332369 333410  74817 408227 0.1832730 < R B C O
# 18  22 267763  61191 328954  79273 408227 0.1941885
# 1   01   1162 289338 290500 117727 408227 0.2883861
# 63  77 288333    898 289231 118996 408227 0.2914947       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1164 332149 333313  75134 408447 0.1839504 < R B C O
# 18  22 267737  61479 329216  79231 408447 0.1939811
# 1   01   1247 289361 290608 117839 408447 0.2885050
# 63  77 288530    923 289453 118994 408447 0.2913328       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1127 332179 333306  74791 408097 0.1832677 < R B C O
# 18  22 267615  61330 328945  79152 408097 0.1939539
# 1   01   1259 289192 290451 117646 408097 0.2882795
# 63  77 288472    923 289395 118702 408097 0.2908671       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1059 331695 332754  75177 407931 0.1842885 < R B C O
# 18  22 266826  61315 328141  79790 407931 0.1955968
# 1   01   1218 288717 289935 117996 407931 0.2892548
# 63  77 287605    891 288496 119435 407931 0.2927824       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1113 332352 333465  74951 408416 0.1835163 < R B C O
# 18  22 268249  61175 329424  78992 408416 0.1934106
# 1   01   1176 289774 290950 117466 408416 0.2876136
# 63  77 288882    889 289771 118645 408416 0.2905004       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1105 331707 332812  75289 408101 0.1844862 < R B C O
# 18  22 267191  61398 328589  79512 408101 0.1948341
# 1   01   1213 289018 290231 117870 408101 0.2888256
# 63  77 288122    968 289090 119011 408101 0.2916214       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1106 332248 333354  74938 408292 0.1835402 < R B C O
# 18  22 267667  61280 328947  79345 408292 0.1943340
# 1   01   1247 289422 290669 117623 408292 0.2880855
# 63  77 288256    935 289191 119101 408292 0.2917054       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1112 332321 333433  74941 408374 0.1835107 < R B C O
# 18  22 267698  61208 328906  79468 408374 0.1945961
# 1   01   1234 289478 290712 117662 408374 0.2881231
# 63  77 288460    886 289346 119028 408374 0.2914681       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1067 332182 333249  74826 408075 0.1833634 < R B C O
# 18  22 267830  61482 329312  78763 408075 0.1930111
# 1   01   1175 289535 290710 117365 408075 0.2876064
# 63  77 288417    888 289305 118770 408075 0.2910494       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1123 332364 333487  74821 408308 0.1832465 < R B C O
# 18  22 267954  61230 329184  79124 408308 0.1937851
# 1   01   1236 289533 290769 117539 408308 0.2878685
# 63  77 288562    949 289511 118797 408308 0.2909495       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1046 331901 332947  75232 408179 0.1843113 < R B C O
# 18  22 267330  61161 328491  79688 408179 0.1952281
# 1   01   1174 289077 290251 117928 408179 0.2889125
# 63  77 287867    921 288788 119391 408179 0.2924967       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1136 332210 333346  74916 408262 0.1834998 < R B C O
# 18  22 267418  61060 328478  79784 408262 0.1954235
# 1   01   1173 289301 290474 117788 408262 0.2885108
# 63  77 288040    870 288910 119352 408262 0.2923417       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1092 332546 333638  75071 408709 0.1836784 < R B C O
# 18  22 267576  61853 329429  79280 408709 0.1939766
# 1   01   1189 289391 290580 118129 408709 0.2890296
# 63  77 288227    893 289120 119589 408709 0.2926018       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1116 331900 333016  75085 408101 0.1839863 < R B C O
# 18  22 267508  61256 328764  79337 408101 0.1944053
# 1   01   1262 289186 290448 117653 408101 0.2882938
# 63  77 288136    924 289060 119041 408101 0.2916949       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1076 332146 333222  74983 408205 0.1836896 < R B C O
# 18  22 267828  61309 329137  79068 408205 0.1936968
# 1   01   1143 289247 290390 117815 408205 0.2886172
# 63  77 288442    890 289332 118873 408205 0.2912091       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1061 332297 333358  75054 408412 0.1837703 < R B C O
# 18  22 267597  61312 328909  79503 408412 0.1946637
# 1   01   1193 289214 290407 118005 408412 0.2889362
# 63  77 288063    885 288948 119464 408412 0.2925085       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1041 332188 333229  74801 408030 0.1833223 < R B C O
# 18  22 267732  61104 328836  79194 408030 0.1940887
# 1   01   1144 289333 290477 117553 408030 0.2880989
# 63  77 288289    919 289208 118822 408030 0.2912090       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1134 331704 332838  74970 407808 0.1838365 < R B C O
# 18  22 267111  61420 328531  79277 407808 0.1943979
# 1   01   1232 288955 290187 117621 407808 0.2884225
# 63  77 288191    927 289118 118690 407808 0.2910438       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1091 331789 332880  75217 408097 0.1843116 < R B C O
# 18  22 267288  61621 328909  79188 408097 0.1940421
# 1   01   1270 289086 290356 117741 408097 0.2885123
# 63  77 288116    939 289055 119042 408097 0.2917003       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1118 331550 332668  75272 407940 0.1845173 < R B C O
# 18  22 267262  61409 328671  79269 407940 0.1943153
# 1   01   1193 288873 290066 117874 407940 0.2889494
# 63  77 287753    918 288671 119269 407940 0.2923690       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1107 331953 333060  74953 408013 0.1837025 < R B C O
# 18  22 267920  60897 328817  79196 408013 0.1941017
# 1   01   1194 289418 290612 117401 408013 0.2877384
# 63  77 288605    894 289499 118514 408013 0.2904662       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1133 332284 333417  74786 408203 0.1832079 < R B C O
# 18  22 267548  61139 328687  79516 408203 0.1947952
# 1   01   1217 289374 290591 117612 408203 0.2881214
# 63  77 288301    924 289225 118978 408203 0.2914677       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1164 331676 332840  75409 408249 0.1847133 < R B C O
# 18  22 267218  61567 328785  79464 408249 0.1946459
# 1   01   1230 289089 290319 117930 408249 0.2888678
# 63  77 287957    991 288948 119301 408249 0.2922261       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1148 331936 333084  74919 408003 0.1836236 < R B C O
# 18  22 267492  61070 328562  79441 408003 0.1947069
# 1   01   1229 289018 290247 117756 408003 0.2886155
# 63  77 288037    905 288942 119061 408003 0.2918140       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1115 332064 333179  75078 408257 0.1838989 < R B C O
# 18  22 267679  61479 329158  79099 408257 0.1937481
# 1   01   1239 289119 290358 117899 408257 0.2887862
# 63  77 288288    933 289221 119036 408257 0.2915712       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1077 331731 332808  75096 407904 0.1841021 < R B C O
# 18  22 267283  61294 328577  79327 407904 0.1944747
# 1   01   1174 288798 289972 117932 407904 0.2891170
# 63  77 287840    891 288731 119173 407904 0.2921594       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1110 332205 333315  74724 408039 0.1831296 < R B C O
# 18  22 267699  61192 328891  79148 408039 0.1939717
# 1   01   1147 289270 290417 117622 408039 0.2882617
# 63  77 288498    896 289394 118645 408039 0.2907688       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1174 332154 333328  75231 408559 0.1841374 < R B C O
# 18  22 267486  61640 329126  79433 408559 0.1944223
# 1   01   1223 289263 290486 118073 408559 0.2889987
# 63  77 288171    916 289087 119472 408559 0.2924229       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1056 332415 333471  75010 408481 0.1836316 < R B C O
# 18  22 267522  61017 328539  79942 408481 0.1957056
# 1   01   1212 289197 290409 118072 408481 0.2890514
# 63  77 288093    881 288974 119507 408481 0.2925644       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1145 331829 332974  75131 408105 0.1840972 < R B C O
# 18  22 267139  61335 328474  79631 408105 0.1951238
# 1   01   1197 288976 290173 117932 408105 0.2889747
# 63  77 287604    993 288597 119508 408105 0.2928364       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1091 332036 333127  75511 408638 0.1847870 < R B C O
# 18  22 267495  61343 328838  79800 408638 0.1952829
# 1   01   1176 289294 290470 118168 408638 0.2891753
# 63  77 288205    883 289088 119550 408638 0.2925572       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1122 332241 333363  75029 408392 0.1837181 < R B C O
# 18  22 267707  61407 329114  79278 408392 0.1941223
# 1   01   1197 289339 290536 117856 408392 0.2885855
# 63  77 288307    944 289251 119141 408392 0.2917320       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1027 331856 332883  75051 407934 0.1839783 < R B C O
# 18  22 267542  60990 328532  79402 407934 0.1946442
# 1   01   1156 289237 290393 117541 407934 0.2881373
# 63  77 287942    890 288832 119102 407934 0.2919639       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1080 332197 333277  75099 408376 0.1838967 < R B C O
# 18  22 267559  61270 328829  79547 408376 0.1947886
# 1   01   1199 289257 290456 117920 408376 0.2887535
# 63  77 288517    868 289385 118991 408376 0.2913761       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1119 332005 333124  75131 408255 0.1840296 < R B C O
# 18  22 267285  61687 328972  79283 408255 0.1941997
# 1   01   1234 289004 290238 118017 408255 0.2890767
# 63  77 287841    971 288812 119443 408255 0.2925696       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1081 331731 332812  75434 408246 0.1847758 < R B C O
# 18  22 267354  61334 328688  79558 408246 0.1948776
# 1   01   1197 288844 290041 118205 408246 0.2895436
# 63  77 287927    885 288812 119434 408246 0.2925540       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1093 332022 333115  75225 408340 0.1842215 < R B C O
# 18  22 267666  61426 329092  79248 408340 0.1940736
# 1   01   1204 289443 290647 117693 408340 0.2882230
# 63  77 288320    927 289247 119093 408340 0.2916516       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1055 331379 332434  75436 407870 0.1849511 < R B C O
# 18  22 267115  61588 328703  79167 407870 0.1940986
# 1   01   1152 288871 290023 117847 407870 0.2889327
# 63  77 287951    902 288853 119017 407870 0.2918013       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1105 332302 333407  74890 408297 0.1834204 < R B C O
# 18  22 268082  61200 329282  79015 408297 0.1935233
# 1   01   1181 289606 290787 117510 408297 0.2878052
# 63  77 288711    904 289615 118682 408297 0.2906757       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1078 332142 333220  75209 408429 0.1841422 < R B C O
# 18  22 267283  61519 328802  79627 408429 0.1949592
# 1   01   1178 288980 290158 118271 408429 0.2895754
# 63  77 287983    903 288886 119543 408429 0.2926898       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1127 332038 333165  75031 408196 0.1838112 < R B C O
# 18  22 267354  61364 328718  79478 408196 0.1947055
# 1   01   1264 288958 290222 117974 408196 0.2890131
# 63  77 288250    925 289175 119021 408196 0.2915781       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1087 332264 333351  75074 408425 0.1838134 < R B C O
# 18  22 267382  61626 329008  79417 408425 0.1944470
# 1   01   1229 289078 290307 118118 408425 0.2892036
# 63  77 288071    917 288988 119437 408425 0.2924331       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1128 332817 333945  74557 408502 0.1825132 < R B C O
# 18  22 267873  60794 328667  79835 408502 0.1954336
# 1   01   1151 289597 290748 117754 408502 0.2882581
# 63  77 288560    941 289501 119001 408502 0.2913107       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1127 332385 333512  75240 408752 0.1840725 < R B C O
# 18  22 268226  61185 329411  79341 408752 0.1941055
# 1   01   1174 289575 290749 118003 408752 0.2886909
# 63  77 289012    917 289929 118823 408752 0.2906970       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1148 332272 333420  75027 408447 0.1836885 < R B C O
# 18  22 268023  61145 329168  79279 408447 0.1940986
# 1   01   1195 289731 290926 117521 408447 0.2877264
# 63  77 288467    923 289390 119057 408447 0.2914870       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1102 331761 332863  74937 407800 0.1837592 < R B C O
# 18  22 267268  61140 328408  79392 407800 0.1946837
# 1   01   1192 289036 290228 117572 407800 0.2883080
# 63  77 288146    909 289055 118745 407800 0.2911844       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1080 332026 333106  74915 408021 0.1836057 < R B C O
# 18  22 267540  61120 328660  79361 408021 0.1945022
# 1   01   1203 288877 290080 117941 408021 0.2890562
# 63  77 288033    934 288967 119054 408021 0.2917840       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1102 331970 333072  75098 408170 0.1839871 < R B C O
# 18  22 267329  61629 328958  79212 408170 0.1940662
# 1   01   1225 289057 290282 117888 408170 0.2888208
# 63  77 287975    914 288889 119281 408170 0.2922336       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1086 332224 333310  75231 408541 0.1841455 < R B C O
# 18  22 267226  61514 328740  79801 408541 0.1953317
# 1   01   1211 289182 290393 118148 408541 0.2891950
# 63  77 288177    924 289101 119440 408541 0.2923574       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1032 331970 333002  75095 408097 0.1840126 < R B C O
# 18  22 267177  61161 328338  79759 408097 0.1954413
# 1   01   1239 288566 289805 118292 408097 0.2898625
# 63  77 287622    870 288492 119605 408097 0.2930798       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1079 332309 333388  75123 408511 0.1838947 < R B C O
# 18  22 267445  61607 329052  79459 408511 0.1945088
# 1   01   1221 289226 290447 118064 408511 0.2890106
# 63  77 288076    948 289024 119487 408511 0.2924940       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1151 332468 333619  74727 408346 0.1829992 < R B C O
# 18  22 267745  61343 329088  79258 408346 0.1940952
# 1   01   1258 289274 290532 117814 408346 0.2885151
# 63  77 288441    959 289400 118946 408346 0.2912873       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1082 332319 333401  74760 408161 0.1831630 < R B C O
# 18  22 267397  61180 328577  79584 408161 0.1949819
# 1   01   1205 289038 290243 117918 408161 0.2889007
# 63  77 288157    908 289065 119096 408161 0.2917868       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1120 332303 333423  75026 408449 0.1836851 < R B C O
# 18  22 267792  61632 329424  79025 408449 0.1934758
# 1   01   1246 289464 290710 117739 408449 0.2882588
# 63  77 288399    929 289328 119121 408449 0.2916423       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1074 331813 332887  74955 407842 0.1837844 < R B C O
# 18  22 267249  61101 328350  79492 407842 0.1949088
# 1   01   1172 288819 289991 117851 407842 0.2889624
# 63  77 287898    910 288808 119034 407842 0.2918630       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1116 332412 333528  75202 408730 0.1839894 < R B C O
# 18  22 268008  61191 329199  79531 408730 0.1945808
# 1   01   1212 289682 290894 117836 408730 0.2882979
# 63  77 288610    889 289499 119231 408730 0.2917109       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1113 332371 333484  75068 408552 0.1837416 < R B C O
# 18  22 267733  61405 329138  79414 408552 0.1943792
# 1   01   1190 289535 290725 117827 408552 0.2884015
# 63  77 288431    915 289346 119206 408552 0.2917768       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1109 332385 333494  74803 408297 0.1832073 < R B C O
# 18  22 267603  61327 328930  79367 408297 0.1943855
# 1   01   1147 289047 290194 118103 408297 0.2892576
# 63  77 288254    971 289225 119072 408297 0.2916308       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1065 332101 333166  75043 408209 0.1838348 < R B C O
# 18  22 267045  61240 328285  79924 408209 0.1957919
# 1   01   1171 288784 289955 118254 408209 0.2896898
# 63  77 287749    885 288634 119575 408209 0.2929259       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1118 332168 333286  74889 408175 0.1834728 < R B C O
# 18  22 267943  61004 328947  79228 408175 0.1941030
# 1   01   1197 289557 290754 117421 408175 0.2876732
# 63  77 288642    884 289526 118649 408175 0.2906817       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1059 331847 332906  75171 408077 0.1842079 < R B C O
# 18  22 267922  61036 328958  79119 408077 0.1938825
# 1   01   1217 289434 290651 117426 408077 0.2877545
# 63  77 288736    873 289609 118468 408077 0.2903080       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1125 331670 332795  75154 407949 0.1842240 < R B C O
# 18  22 267221  61269 328490  79459 407949 0.1947768
# 1   01   1232 288707 289939 118010 407949 0.2892764
# 63  77 287716    924 288640 119309 407949 0.2924606       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1103 331801 332904  75038 407942 0.1839428 < R B C O
# 18  22 267190  61273 328463  79479 407942 0.1948292
# 1   01   1230 288729 289959 117983 407942 0.2892151
# 63  77 287880    926 288806 119136 407942 0.2920415       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1126 332034 333160  75395 408555 0.1845406 < R B C O
# 18  22 267653  61154 328807  79748 408555 0.1951953
# 1   01   1187 289163 290350 118205 408555 0.2893246
# 63  77 288407    957 289364 119191 408555 0.2917380       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1072 332112 333184  75332 408516 0.1844040 < R B C O
# 18  22 267519  61638 329157  79359 408516 0.1942617
# 1   01   1168 288977 290145 118371 408516 0.2897585
# 63  77 288145    913 289058 119458 408516 0.2924194       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1080 332189 333269  75366 408635 0.1844335 < R B C O
# 18  22 267485  61696 329181  79454 408635 0.1944376
# 1   01   1226 289339 290565 118070 408635 0.2889376
# 63  77 288420    901 289321 119314 408635 0.2919818       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1108 332120 333228  75153 408381 0.1840267 < R B C O
# 18  22 267504  61357 328861  79520 408381 0.1947201
# 1   01   1189 289019 290208 118173 408381 0.2893695
# 63  77 288109    905 289014 119367 408381 0.2922932       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1098 332205 333303  75119 408422 0.1839250 < R B C O
# 18  22 267400  61362 328762  79660 408422 0.1950434
# 1   01   1201 289183 290384 118038 408422 0.2890099
# 63  77 288083    909 288992 119430 408422 0.2924181       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1107 332488 333595  75053 408648 0.1836617 < R B C O
# 18  22 267864  61188 329052  79596 408648 0.1947789
# 1   01   1232 289595 290827 117821 408648 0.2883190
# 63  77 288670    933 289603 119045 408648 0.2913143       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1089 332348 333437  74991 408428 0.1836089 < R B C O
# 18  22 267691  61499 329190  79238 408428 0.1940073
# 1   01   1230 289245 290475 117953 408428 0.2887975
# 63  77 288379    915 289294 119134 408428 0.2916891       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1097 332088 333185  75191 408376 0.1841220 < R B C O
# 18  22 267788  61182 328970  79406 408376 0.1944434
# 1   01   1181 289568 290749 117627 408376 0.2880360
# 63  77 288256    923 289179 119197 408376 0.2918805       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1061 332365 333426  74856 408282 0.1833439 < R B C O
# 18  22 267729  61114 328843  79439 408282 0.1945689
# 1   01   1214 289462 290676 117606 408282 0.2880509
# 63  77 288438    891 289329 118953 408282 0.2913501       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1193 332170 333363  75205 408568 0.1840697 < R B C O
# 18  22 267575  61551 329126  79442 408568 0.1944401
# 1   01   1263 289024 290287 118281 408568 0.2895014
# 63  77 288318    984 289302 119266 408568 0.2919122       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1075 331808 332883  75602 408485 0.1850790 < R B C O
# 18  22 267350  61522 328872  79613 408485 0.1948982
# 1   01   1249 288909 290158 118327 408485 0.2896728
# 63  77 288015    935 288950 119535 408485 0.2926301       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1096 332054 333150  74968 408118 0.1836920 < R B C O
# 18  22 267575  61269 328844  79274 408118 0.1942428
# 1   01   1203 288980 290183 117935 408118 0.2889728
# 63  77 288029    893 288922 119196 408118 0.2920626       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1032 332458 333490  75104 408594 0.1838108 < R B C O
# 18  22 268134  61251 329385  79209 408594 0.1938575
# 1   01   1147 289365 290512 118082 408594 0.2889959
# 63  77 288839    826 289665 118929 408594 0.2910689       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1046 332410 333456  75054 408510 0.1837262 < R B C O
# 18  22 268428  61296 329724  78786 408510 0.1928619
# 1   01   1162 289771 290933 117577 408510 0.2878191
# 63  77 288824    884 289708 118802 408510 0.2908179       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1131 331692 332823  75027 407850 0.1839573 < R B C O
# 18  22 267152  61364 328516  79334 407850 0.1945176
# 1   01   1189 288729 289918 117932 407850 0.2891553
# 63  77 287800    959 288759 119091 407850 0.2919971       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1131 332442 333573  74636 408209 0.1828377 < R B C O
# 18  22 267639  61123 328762  79447 408209 0.1946233
# 1   01   1194 289285 290479 117730 408209 0.2884062
# 63  77 288364    934 289298 118911 408209 0.2912993       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1074 331823 332897  75058 407955 0.1839860 < R B C O
# 18  22 267257  61656 328913  79042 407955 0.1937518
# 1   01   1290 288935 290225 117730 407955 0.2885858
# 63  77 287869    921 288790 119165 407955 0.2921033       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1133 332501 333634  75107 408741 0.1837521 < R B C O
# 18  22 267785  61367 329152  79589 408741 0.1947174
# 1   01   1234 289554 290788 117953 408741 0.2885764
# 63  77 288428    944 289372 119369 408741 0.2920407       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1129 331681 332810  75431 408241 0.1847708 < R B C O
# 18  22 267425  61507 328932  79309 408241 0.1942701
# 1   01   1234 288951 290185 118056 408241 0.2891821
# 63  77 288225    916 289141 119100 408241 0.2917394       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1091 331783 332874  75234 408108 0.1843483 < R B C O
# 18  22 267568  61491 329059  79049 408108 0.1936963
# 1   01   1170 289024 290194 117914 408108 0.2889284
# 63  77 288080    875 288955 119153 408108 0.2919644       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1119 332517 333636  74829 408465 0.1831956 < R B C O
# 18  22 267585  61249 328834  79631 408465 0.1949518
# 1   01   1228 289215 290443 118022 408465 0.2889403
# 63  77 288271    931 289202 119263 408465 0.2919785       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1153 331638 332791  75567 408358 0.1850509 < R B C O
# 18  22 267163  61499 328662  79696 408358 0.1951621
# 1   01   1218 288839 290057 118301 408358 0.2896992
# 63  77 287842    953 288795 119563 408358 0.2927897       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1070 331896 332966  75345 408311 0.1845285 < R B C O
# 18  22 267415  61638 329053  79258 408311 0.1941118
# 1   01   1226 289127 290353 117958 408311 0.2888925
# 63  77 288003    924 288927 119384 408311 0.2923850       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1100 332138 333238  75067 408305 0.1838503 < R B C O
# 18  22 267449  61084 328533  79772 408305 0.1953736
# 1   01   1206 289294 290500 117805 408305 0.2885221
# 63  77 288095    938 289033 119272 408305 0.2921150       < L
#    pat   left  right   both  cross    all     ratio      best
# 8   10   1106 332217 333323  74881 408204 0.1834401 < R B C O
# 18  22 267869  60926 328795  79409 408204 0.1945326
# 1   01   1247 289350 290597 117607 408204 0.2881084
# 63  77 288314    920 289234 118970 408204 0.2914474       < L

# summarize:
corsummary <- t(as.matrix(c(summary(bcor),sd=sd(bcor))))
row.names(corsummary) <- 'bcor'
bdelta <- b61cross-b52cross
brevp <- 100*brev   # make it percent reversed instead of logical
thesummary <- rbind(summary(b52cross),summary(b61cross),summary(c(bdelta)),summary(brevp))
row.names(thesummary) <- c('b52cross', 'b61cross', 'b61-b52', '% rev')
thesummary <- cbind(thesummary, sd=c(sd(b52cross),sd(b61cross),sd(bdelta),sd(brevp)))
```

SUMMARY: In 101 bootstrap replicates, we saw 101 samples with the 6/1 split having fewer conflicts than the 5/2 split, and the minimum separation between them was ≈ -19 sigma, hence highly statistically significant.

```
# 'opt' hacking is trying to force knitr to show more digits of bcor in summary, as Rstudio does, but
# it still fails...  Bottom line is the correlation seems to be  > .999 in all samples, rounds to 1.0,
# as seen in this run of 1001 samples cut/paste from Rstudio:
#          Min.        1st Qu.     Median      Mean        3rd Qu. Max.   sd
# bcor     "   0.9998" "   0.9999" "   0.9999" "   0.9999" "   1"  "   1" "   0.00003462"
# > max(bcor)
# [1] 0.9999915
o.opts <- options(digits=7,width=127)
format(rbind(corsummary,thesummary),scientific=F,digits=4,drop0trailing=T)
```

```
#           Min.              1st Qu.           Median            Mean              3rd Qu.           Max.
# bcor     "    0.999981368" "    0.999992195" "    0.999994211" "    0.999993689" "    0.999995819" "    0.99999
# b52cross "78763"           "79238"           "79409"           "79396.445544554" "79547"           "79942"
# b61cross "74557"           "74953"           "75078"           "75089.237623762" "75217"           "75602"
# b61-b52  "-5278"           "-4455"           "-4324"           "-4307.207920792" "-4105"           "-3599"
# % rev    "  100"           "  100"           "  100"           "  100"           "  100"           "  100"
#           sd
# bcor     "    0.000003173"
# b52cross "  235.554260214"
# b61cross "  207.05314045"
# b61-b52  "  282.072945063"
# % rev    "    0"

options(o.opts)
```

Based on this, it is reasonable to claim that we are confident that the tree topology is as shown in the earlier figures, with the exception of the exact order of the splits with the 4 NE coastal isolates.

10 Notes

This section is a random brain dump of limitations of the current analysis, ideas for improvements, etc. In the main, these may not be worth doing, unless we see significant holes or get pushed by reviewers, etc, but I wanted to catalog before we forget them.

Noise: Various sources of "noise" in the data:

1. Read errors, low read depth — perhaps fixed by medium/strict thresholding
2. Deep coalescence
3. Skew because 1335 is the reference. (Julie notes we could partially fix this by remapping based on discovered SNPs, tho that wouldn't fix gross misassembly in 1335, e.g. collapsed or misordered tandem duplicates, or segments missing in 1335 that are present in one or more other strains, etc.; much harder to fix those, let's just hope they are rare...)
4. Varying error rates and sequencing depth among the 7. E.g., plausibly the 1000 SNPs shared by 4 but not by Gyre are a result of lower read depth (we missed a SNP that is actually present) and/or higher error rates (causing a position to appear inconsistent in gyre) in the gyre data. I can't think of a way to correct for this effect. It might be possible, perhaps by simulation, to estimate the size of the effect and see whether it could explain ≈1000 SNPs.
5. Varying numbers of founder cells in the sequencing cultures. (Again, I made some attempts at modeling this, but nothing very satisfactory yet.)
6. Tri-allelic positions where stochastic fluctuation in sequence sampling promotes the rare allele to prominence. (Julie replies: "isn't this the same as more than one founder cell? If they are diploid there should only ever be two alleles, unless there were random and very rare, thus unlikely, trisomy events?" I agree, but it is a concrete example of an effect of multiple founders that might be important. Not sure this is the most important such effect...)
7. Gaps/indels - alignments are likely to be of lower quality in the vicinity of an indel, so, maybe lower coverage/more SNPs. We ignored them. Does this add any systematic bias? e.g. if one strain had more indels than another, would this confound other analyses? unclear. Julie suggested a paper titled "Barking up the wrong tree-length: yada yada yada gap penalties"; maybe relevant?

Other Items/Potential To Dos:

1. any spacial structure to various sub-classes?
2. after top level split, should I reanalyze halves of partition in isolation? said another way, I think the tree-building is sensible, but not sure it's optimal.

3. if we believe no sex, then I think gain of SNP should be more common than loss of SNP, since the later can only happen by (a) mutation reverting to reference, (b) second mutation matching nonreference, (c) homologous repair (look for blocks of LOH), or (d) false negative e.g. from low read depth. Does tree-building appropriately weight the gain vs loss cases? (Does it even care?)

4. should we weight coding and/or nonsynonomous SNPs more heavily? Julie says "you do not want to weight the coding or nonsynonomous/coding SNPs because for time you want the more clock-like neutral mutations." I.e., I got this backwards. Maybe should redo tree based on noncoding SNPs only.

5. We could also do an actual parsimony analysis based on 2-state model (homozygous-ref vs not), but I'm not quite sure how to handle this in a mixed sex/nosex case.

6. Might be interesting to look at sharing just within (shared?) deserts. Given tree model above and expectation that bottleneck followed split of H- from L-clades, I would expect little or no sharing of L-clade desert SNPs with H-clade; sharing between It/Wales might suggest "desert" is actually a region under strong purifying selection (e.g. a gene); sharing/non-sharing within L-clade deserts might suggest more about evo history of the 5.

11 Appendix: Old Trees, etc.

Tangents, old stuff of historical interest at best, etc..

11.1 HWE Sharing

Tangent: As a function of nonref allele freq, assuming HWE, what is probability that nonref allele will be seen in k strains, $0 \leq k \leq 4$ (Fig 6).

```
myfigpath.h <- paste(getwd(), '/figs-knitr/', sep='')
```

```
p <- (0:20)/20
q <- 1-p
r <- 2*p*q+p^2
plot(  p, 1*q^0*r^4, type='b',pch='4', ylab="share prob")
points(p, 4*q^2*r^3, type='b',pch='3')
points(p, 6*q^4*r^2, type='b',pch='2')
points(p, 4*q^6*r^1, type='b',pch='1')
points(p, 1*q^8*r^0, type='b',pch='0')
```

11.2 Old Tree Stuff

All based on un-q-filtered reads.

The first pass at the tree analysis was the Chr1 tree, *loose criteria* (c1); it is rendered via `http://iubio.bio.indiana.edu/treeapp/treeprint-form.html` as Fig 7, and in newick format is:

```
newick.chr1.loose <- '(((tp3367_Italy:4551,tp1013_Wales:4954):5920,(((tp1007_Virginia:10,tp1012_Australia:29):9,(
cat.hardwrap(newick.chr1.loose)

# (((tp3367_Italy:4551,tp1013_Wales:4954):5920,(((tp1007_Virginia:10,tp1012_Austra
# lia:29):9,(tp1015_Puget_Sound:90,tp1335_NY:13):11):320,tp1014_Gyre:22):3484):859
# 3,outgroup:0);
```

Chr 1 tree based on *medium criteria* (c2) has exactly the same topology is, although the branch lengths are different. As noted earlier, the length of the branch labeled "*" is probably inflated by lower coverage and higher error rate in 1014, which may mask further legitimate sharing between it and the other L-isloates. The branch lengths among the other 4 are too short for its topology to be convincing without a more rigorous analysis (e.g., a bootstrap test).

Chr1 tree, medium criteria, in newick format:

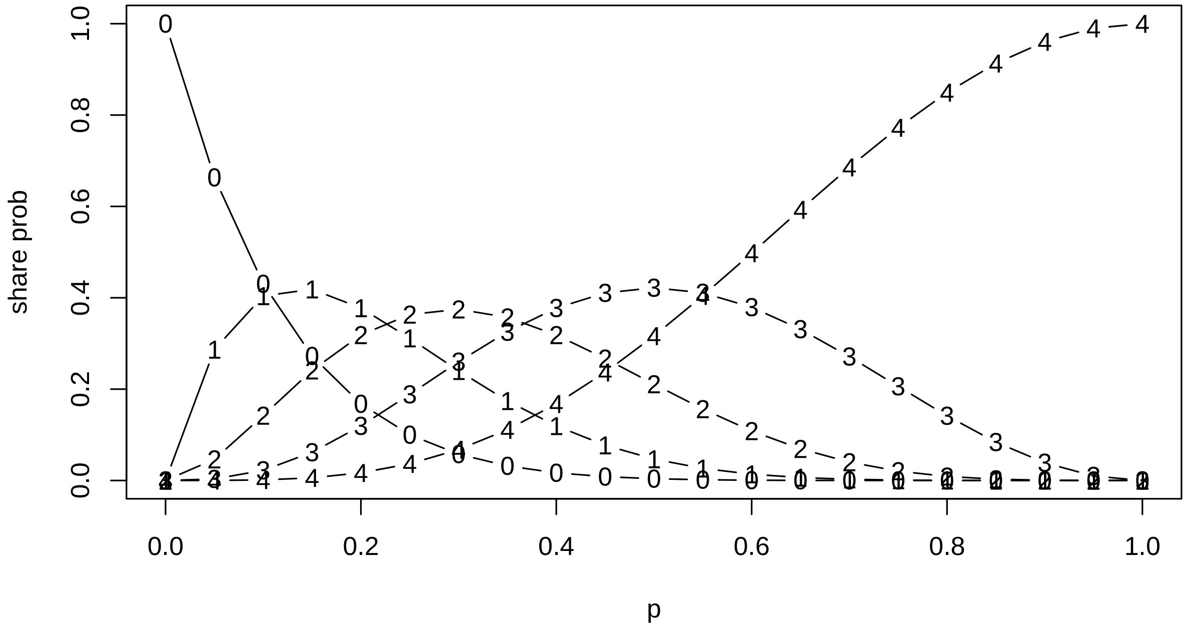


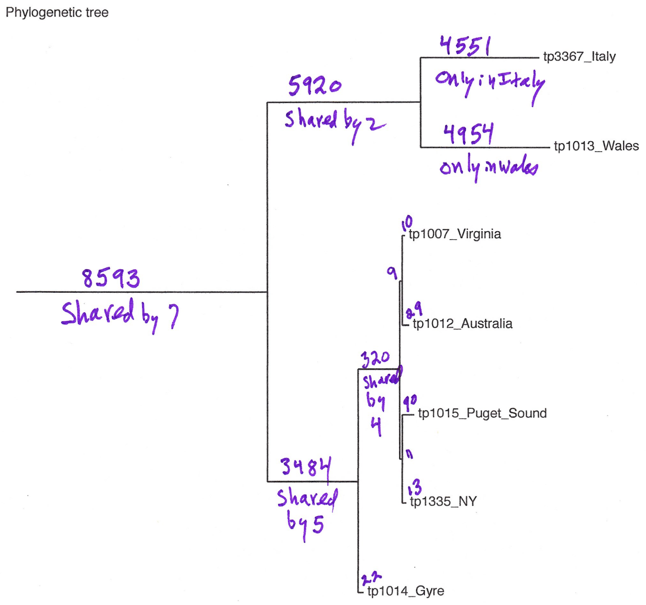
Figure 6: Sharing Probability

```
newick.chr1.med <- '(((tp3367_Italy:8813,tp1013_Wales:9652):9365,(((e_tp1007_Virginia:30,d_tp1012_Australia:61):1
cat.hardwrap(newick.chr1.med)

# (((tp3367_Italy:8813,tp1013_Wales:9652):9365,(((e_tp1007_Virginia:30,d_tp1012_Au
# stralia:61):19,(c_tp1015_Puget_Sound:207,b_tp1335_NY:41):18):1005,a_tp1014_Gyre:
# 61):3912):7054,outgroup:0);
```

NOTE: In early code, tree was not being recalculated; it was defined by constants in the following code chunk, hand-copied from the analysis above.

```
# tree parameters as nested lists
#   Internal nodes have subtrees sub1/2 and length
#   Root has sub1/2, but no length
#   Leaves have where, length, optionally, id, alt, nb.  (Omit id for 'outgroup'. Use 'alt' for less formal
#     labeling in cartoon version; it defaults to 'where'. Use 'nb' to add abcde annotations for legend.)
# This hand-made version is now subsumed by make.tree; retained for comparison
tree.by.hand <-
  list(
    sub1 = list(
      sub1 = list(
        sub1 = list(id=3367, length=8813, where='Venice, Italy', alt='Venice'),
        sub2 = list(id=1013, length=9652, where='Wales, UK'),
        length=9365),
      sub2 =  list(
        sub1 = list(
          sub1 = list(
            sub1 = list(id=1007, length=30, nb='e', where='Virginia, USA'),
            sub2 = list(id=1012, length=61, nb='d', where='Perth, W. Australia', alt='Perth'),
            length=19),
          sub2 = list(
            sub1 = list(id=1015, length=207,nb='c', where='Washington, USA', alt='Puget Sound'),
            sub2 = list(id=1335, length=41, nb='b', where='New York, USA',   alt='NY'),
            length=18),
          length=1005),
        sub2 = list(id=1014, length=61, nb='a', where='N. Pacific Gyre'),
```



This accounts for 28006 of 36040 "consistent" SNPS.

~1950 on long branches, but short branches scaled 5-10x for visibility

Figure 7: Inferred Tree, based on Chr1, un-q-filtered reads, loose criteria. (Note: to visually resolve the edges among the 5, their lengths were scaled by 5x – 10x in this figure, but not in the newick description shown in the text.)

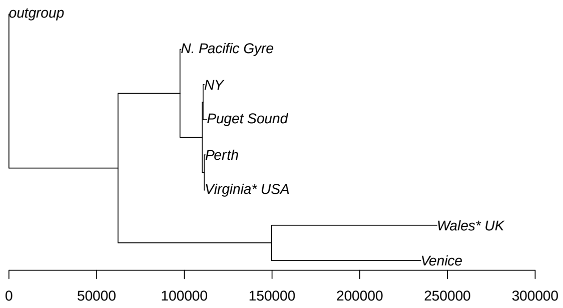


Figure 8: Tree based on qfiltered reads and medium SNP filters. "Lengths" are numbers of shared/private SNPs all Chrs. (no edge labels, nolegend)

```
        length=3912),
      length=7054),
    sub2 = list(length=0, where='outgroup')
  )

# historical, format example, and debug help:
oldwick.medium <- '(((CCMP3367_Italy:8813,CCMP1013_Wales:9652):9365,(((e_CCMP1007_Virginia:30,d_CCMP1012_Australia:61):19,(c_CCMP
# with simpler labeling for cartoon
simple.oldwick.medium <- '(((Italy:8813,Wales:9652):9365,(((Virginia:30,Australia:61):19,(Puget:207,NY:41):18):1005,Gyre:61):3912
cat.hardwrap(oldwick.medium)

# (((CCMP3367_Italy:8813,CCMP1013_Wales:9652):9365,(((e_CCMP1007_Virginia:30,d_CCM
# P1012_Australia:61):19,(c_CCMP1015_Puget_Sound:207,b_CCMP1335_NY:41):18):1005,a_
# CCMP1014_NPG:61):3912):7054,outgroup:0);

cat.hardwrap(simple.oldwick.medium)

# (((Italy:8813,Wales:9652):9365,(((Virginia:30,Australia:61):19,(Puget:207,NY:41)
# :18):1005,Gyre:61):3912):7054,outgroup:0);
```

Two other versions of the tree, for possible use in figs in the main paper.

Figure 8: [** as of 10/4/2015, this fig and next have stray stars on virginia, wales labels; probably due to hacking with commas in newick; not worth fixing unless we resurrect these trees for some purpose, but if so, see use of newick.name.undo in show.tree as probable fix. **]

```
tree.scale <- ifelse(which.snp.tables(string.val=F)[1]=='Chr1', 1, 10)
tree.x.lim <- 3e4 * tree.scale
the.simple.tree <- read.tree(text=simple.newick.medium)
plot(the.simple.tree, x.lim = tree.x.lim)
axis(1)
```

Figure 9:

```
plot(the.simple.tree, x.lim = tree.x.lim)
axis(1,(0:4)*7000*tree.scale,(0:4)*7000*tree.scale)
```

At some much earlier point, Tony ran the whole-genome version of the then-current code above, and manually entered tree branch lengths/legend for the resuting tree, shown in Fig 10. Code above can now automatically generate such a tree, but retain the following for comparison. The basic story seems clear—same topology and branch lengths scaled by about 10x, which is completely reasonable given that Chr1 is about 10% of the genome. Note that this tree is not being recalculated; it is defined by constants in the following code chunk.

```
fullgenome.newick.medium <- '(((3367_Italy:86155,1013_Wales:95697):89598,(((e_1007_VA:330,d_1012_Australia:632):1296,(c_1015_WA:2
cat.hardwrap(fullgenome.newick.medium)
```

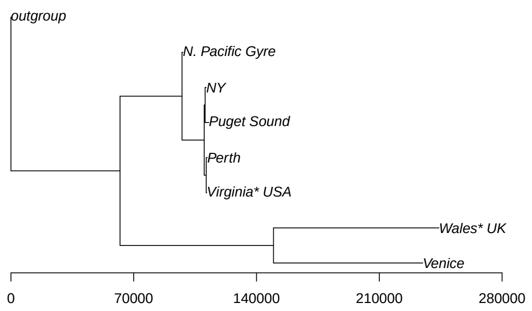


Figure 9: Tree based on qfiltered reads and medium SNP filters. “Lengths” are numbers of shared/private SNPs all Chrs. (no edge labels, no legend, short scale bar)

```
# (((3367_Italy:86155,1013_Wales:95697):89598,(((e_1007_VA:330,d_1012_Australia:63
# 2):1296,(c_1015_WA:2113,b_1335_NY:658):480):10059,a_1014_NPG:568):39517):69526,o
# utgroup:0);

legend.text <- c('a: only in 1014  ',
                 'b: only in 1335  ',
                 'c: only in 1015  ',
                 'd: only in 1012  ',
                 'e: only in 1007  ',
                 '*: shared by bcde',
                 '   shared by b/c ',
                 '   shared by d/e '
)
fullgenome.tree.x.lim <- 300000
fullgenome.counts <- c( 568, 658, 2113, 632, 330, 10059, 480, 1296 )
fullgenome.legend.text <- paste(legend.text,format(fullgenome.counts,width=5),sep=' - ')
fullgenome.tree.labels <- list( ## x,y,text
     41000,3.63,'69526\nshared by 7',
     90000,5.75,'39517\nby 5 (**)',
    115000,1.5, '89598\nshared by 2',
    210000,2.0, '95697 only\nin Wales',
    210000,1.0, '86155 only\nin Italy',
    113500,4.6, '*')
```

Figure 10:

```
library(ape)
the.fullgenome.tree <- read.tree(text=fullgenome.newick.medium)
plot(the.fullgenome.tree, x.lim = fullgenome.tree.x.lim)
axis(1) # ; axis(2) useful only for placing labels
opar <- par(family='mono',cex=.8)
legend('topright', legend=fullgenome.legend.text)
par(opar)
for(i in seq(1,length(fullgenome.tree.labels)-2,by=3)){
  text(fullgenome.tree.labels[[i]], fullgenome.tree.labels[[i+1]], fullgenome.tree.labels[[i+2]])
}
```

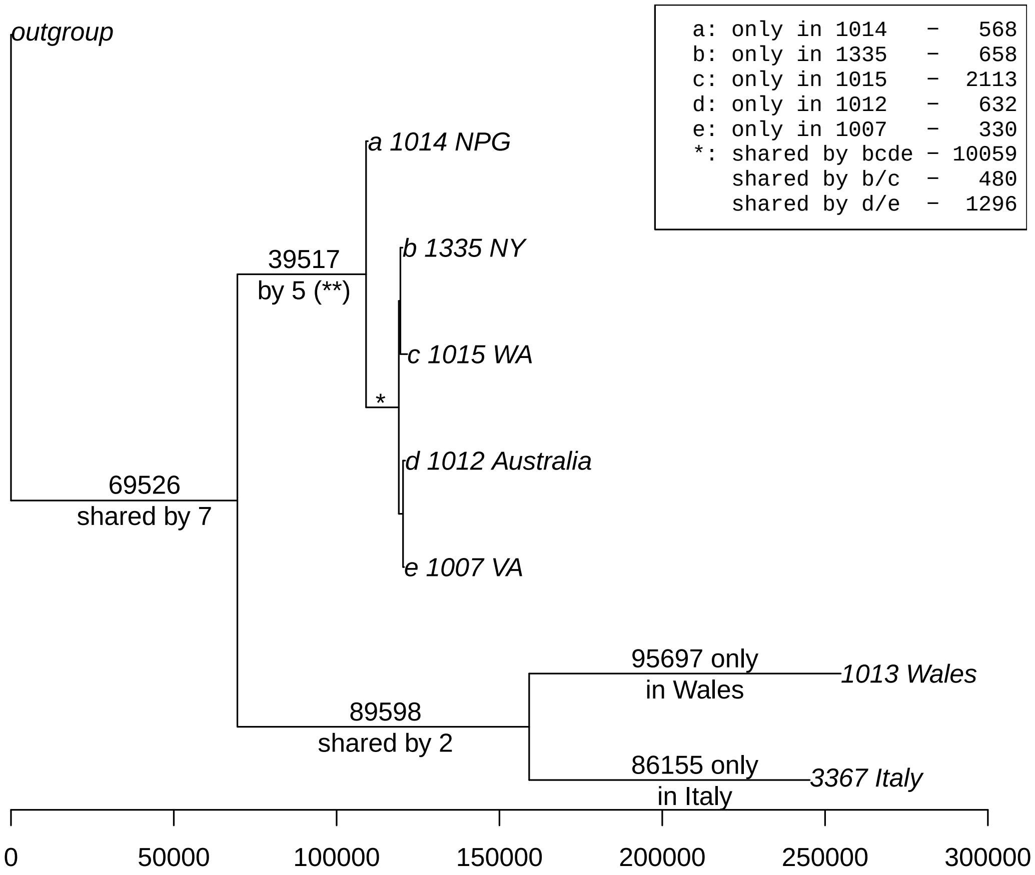


Figure 10: Tree based on unqfiltered reads and medium SNP filters. "Lengths" are numbers of shared/private SNPs genome-wide. (By-hand legacy version)