PopPsiSeq Summary

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Contents

1 Introduction

Explain motivation, overview of PsiSeq and PsiSeq2

Population-based approach, rather than ancestral

2 Materials, Methods, Data, Software

2.1 Reference Genomes

The droSim1 and droSec1 reference genomes were downloaded in FASTA format from UCSC Genome Browser. These were in the 140-170Mb range, with the droSec1 relatively unconsolidated:

Table 1: Size and Consolidation of Reference Genomes

Reference Genome:	dm6	droSec1	droSim1
number_bases	144 M	167 M	142 M
number_contigs	1.87 k	14.7 k	18

(add a by-chromosome breakdown for droSim and a histogram for droSec?)

2.2 Sequenced Reads

A backcross and introgression experiment was performed, in which simulans females were mated with sechellia males, and the hybrid offspring were selected for avoidance of morinda odorants. The offspring were sequenced after 15 rounds of backcrossing and introgression (Earley and Jones 2011). One sample was sequenced in this experiment; a follow-up experiment generated three more samples with two replicates each. As a control, several wild-type sechellia sequences were downloaded from NCBI:

Table 2: Sequenced Experimental Samples

name	paired	experimental	source
SRR6426002	TRUE	control	NCBI
SRR6426002	TRUE	control	NCBI
SRR869587	TRUE	control	NCBI
SRR869587	TRUE	control	NCBI
SRR5860570	TRUE	control	NCBI
SRR5860570	TRUE	control	NCBI
SRR303333	FALSE	selection	EarlyJones2011
SRR303333	FALSE	selection	EarlyJones2011
17B	TRUE	selection	EarlyJones2013
17B	TRUE	selection	EarlyJones2013
17A	TRUE	selection	EarlyJones2013
17A	TRUE	selection	EarlyJones2013
10B	TRUE	selection	EarlyJones2013
10B	TRUE	selection	EarlyJones2013
10A	TRUE	selection	EarlyJones2013
10A	TRUE	selection	EarlyJones2013

For population-wide data, wild D. simulans and D. sechellia flies were captured and sequenced by Daniel Matute:

Table 3: Number of Sequenced Samples per Species

species	sample_count
drosophila sechellia	10
drosophila simulans	10

2.2.1 Pre-processing

These reads were preprocessed with FASTP (S. Chen et al. 2018) for quality control and analytics.

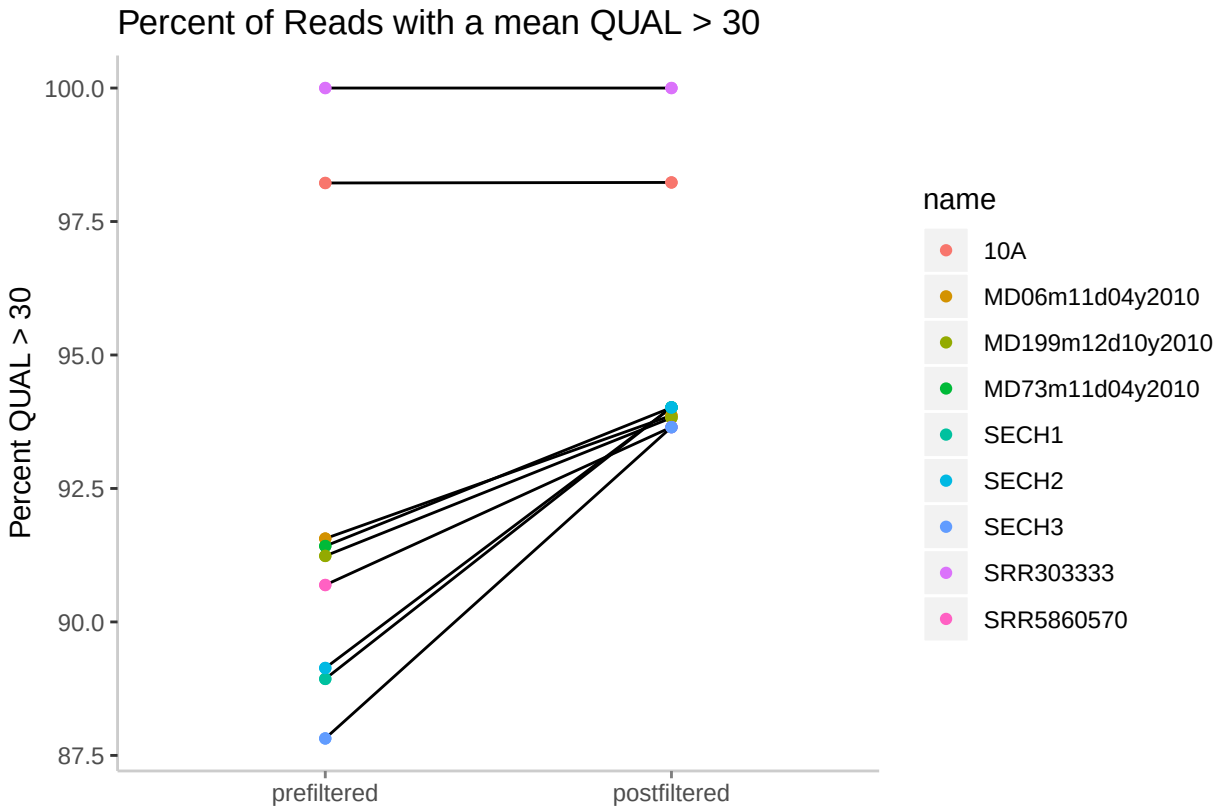
Starting FASTQ files contained a total of $800M$ reads; after QC, this dropped to $758M$.

Table 4: Read Count and Percent Retention

type	minimum	average	maximum
prefiltered	1.48 M	44.5 M	85.4 M
postfiltered	1.4 M	42.1 M	81.1 M

type	minimum	average	maximum
percent retention	93.3	95.6	100

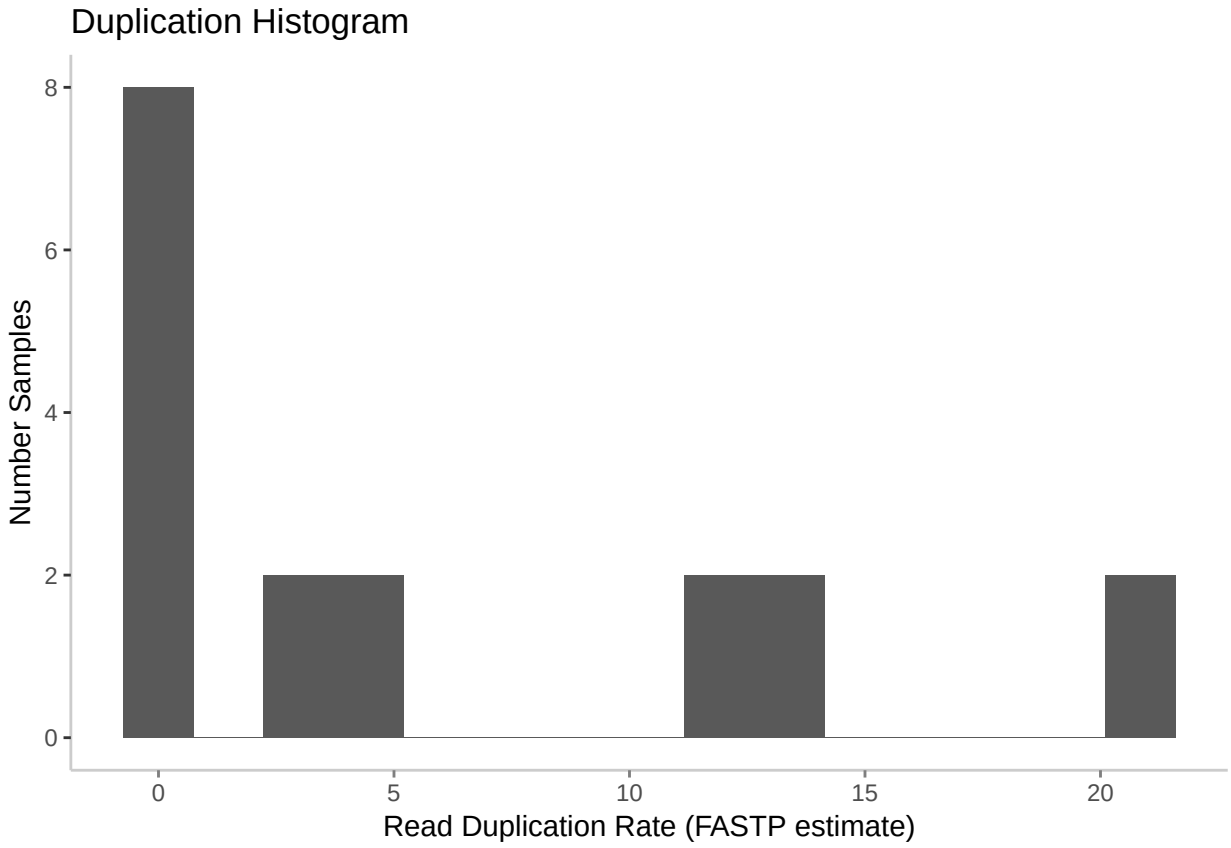
Filtration also increased the read quality, as seen in the increase in the fraction of reads with an average quality score > 30:



Duplicate reads were also detected; these will be filtered during alignment:

Table 5: Percentage Duplication

minimum	average	median	maximum
0.1	6	2.6	20.9



2.3 Mapped Reads

Reads were first mapped to a reference genome using the BWA SAMPE/SE algorithm. Then, the alignment file was filtered for uniqueness (ie, a read must be aligned optimally with no alternative or runner-up hits, "XT:A:U.*X0:i:1*.X1:i:0"), mapping/sequencing quality ("-q 20 -F 0x0100 -F 0x0200 -F 0x0300 -F 0x04"), and deduplication.

2.3.1 Read & Alignment Quality

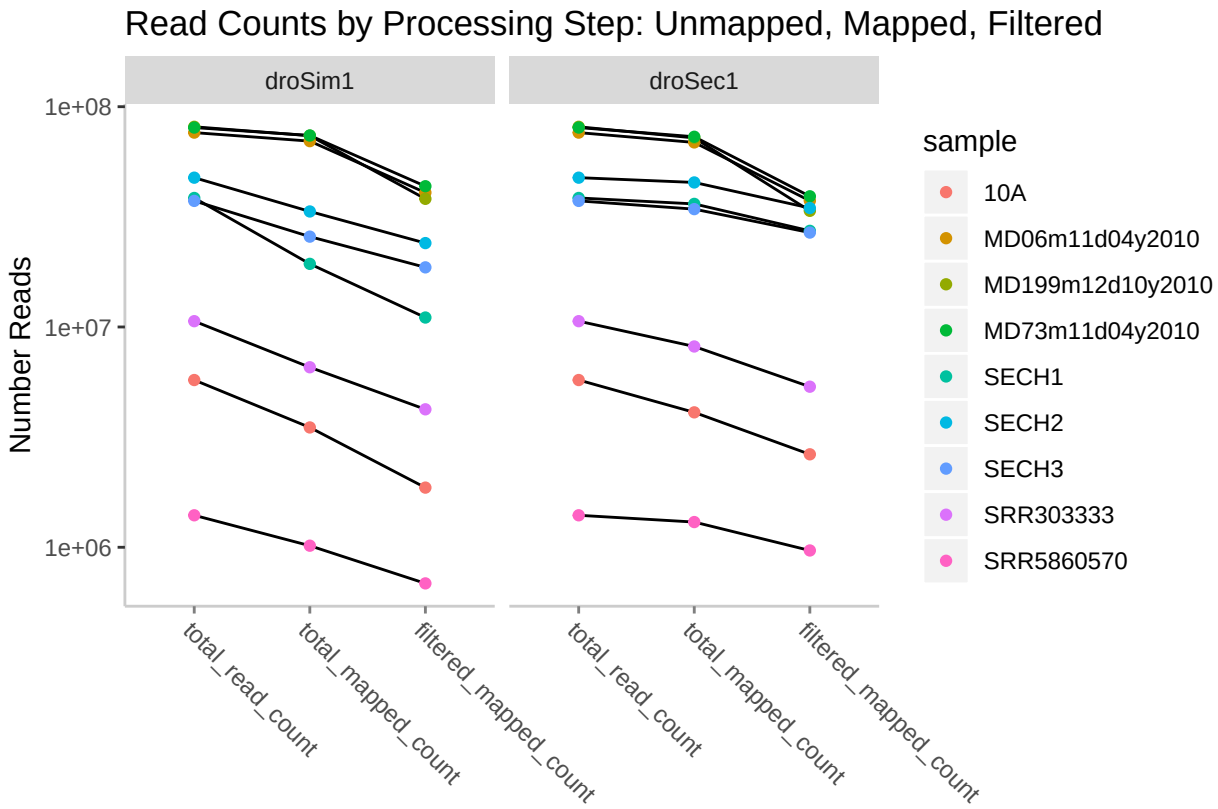


Table 6: Read Counts During Alignment & Filtration

measure	minimum	average	median	maximum
filtered_mapped_count	686 k	21.7 M	25.4 M	43.5 M
total_mapped_count	1.02 M	36.1 M	33.9 M	74 M
total_read_count	1.4 M	42.1 M	38.5 M	81.1 M

The fraction of reads retained at each point:

Table 7: Percentage of Reads Retained at Each Step

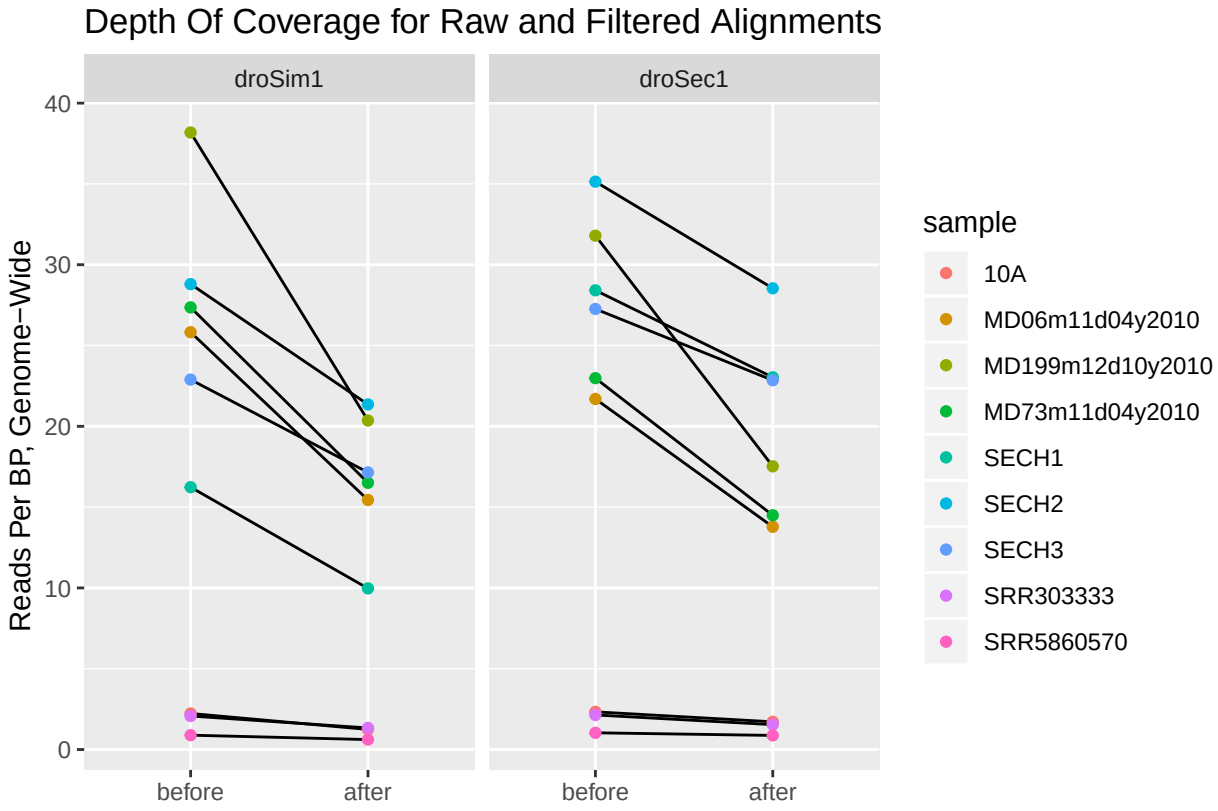
measure	minimum	average	median	maximum
filter_retention	46.7	63.6	64.5	78.3
mapping_retention	50.2	80.6	89.6	95.1

2.3.2 Depth & Breadth of Coverage

Depth of coverage, ie, the genome-wide average number of mapped reads per base pair:

Table 8: Depth of Coverage Statistics for Raw and Filtered Alignments

step	minimum	average	median	maximum
pre-filtration depth	0.9	18.7	22.9	38.2
post-filtration depth	0.6	12.7	15.0	28.5
depth retention percent	53.3	68.4	66.9	84.4



Breadth of coverage, ie, the percentage of the genome covered by at least one read:

2.4 Called Variants

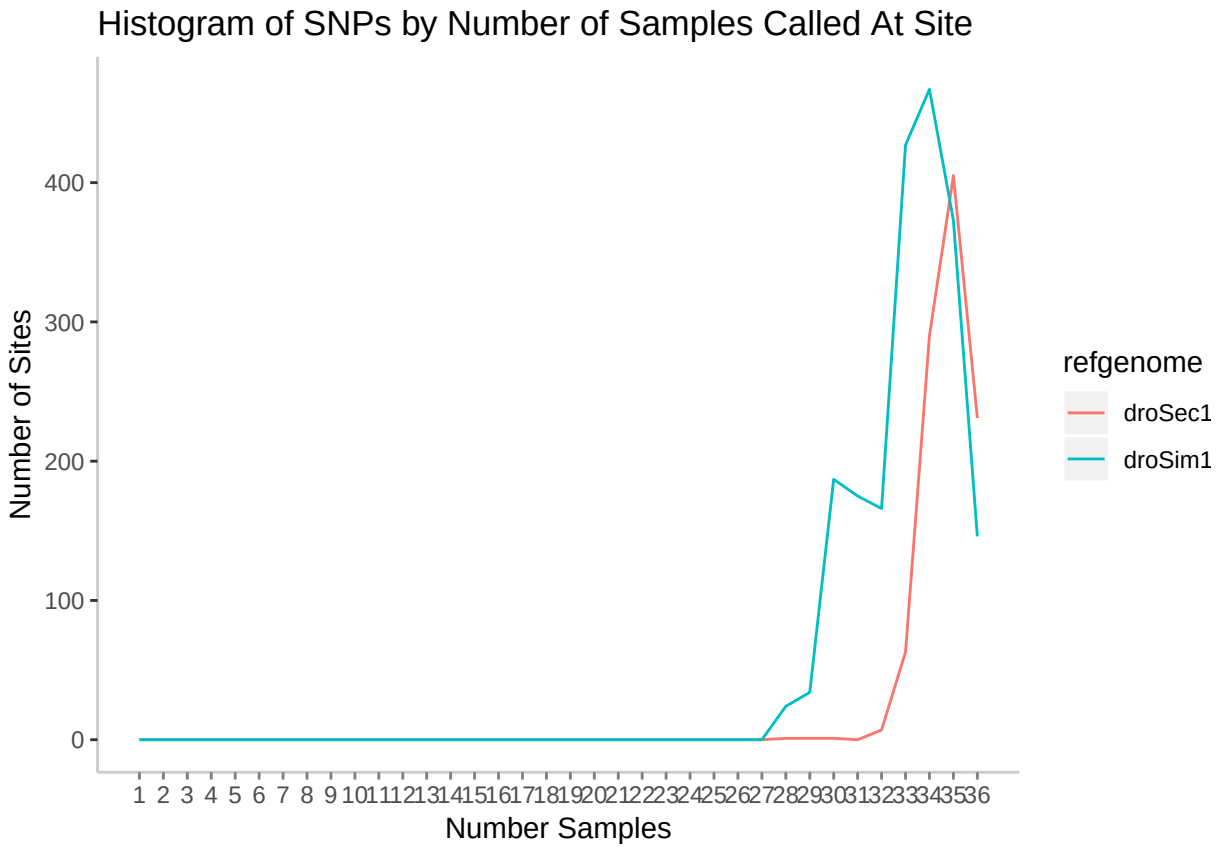
BWAUniq mappings were used to jointly call variants in VCF format via Freebayes (Garrison and Marth 2012) using standard filters.

Table 9: SNP count and per-KB SNP rate across all samples

reference genome	Genome size (bp)	total SNP count	SNPs per kB
droSec1	167 M	2.44 M	14.7
droSim1	142 M	2.7 M	18.9

To build this VCF, 36 samples called jointly. However, not all sites were called in all samples (eg, due to coverage differences). The sites had the following group-wide call rate:

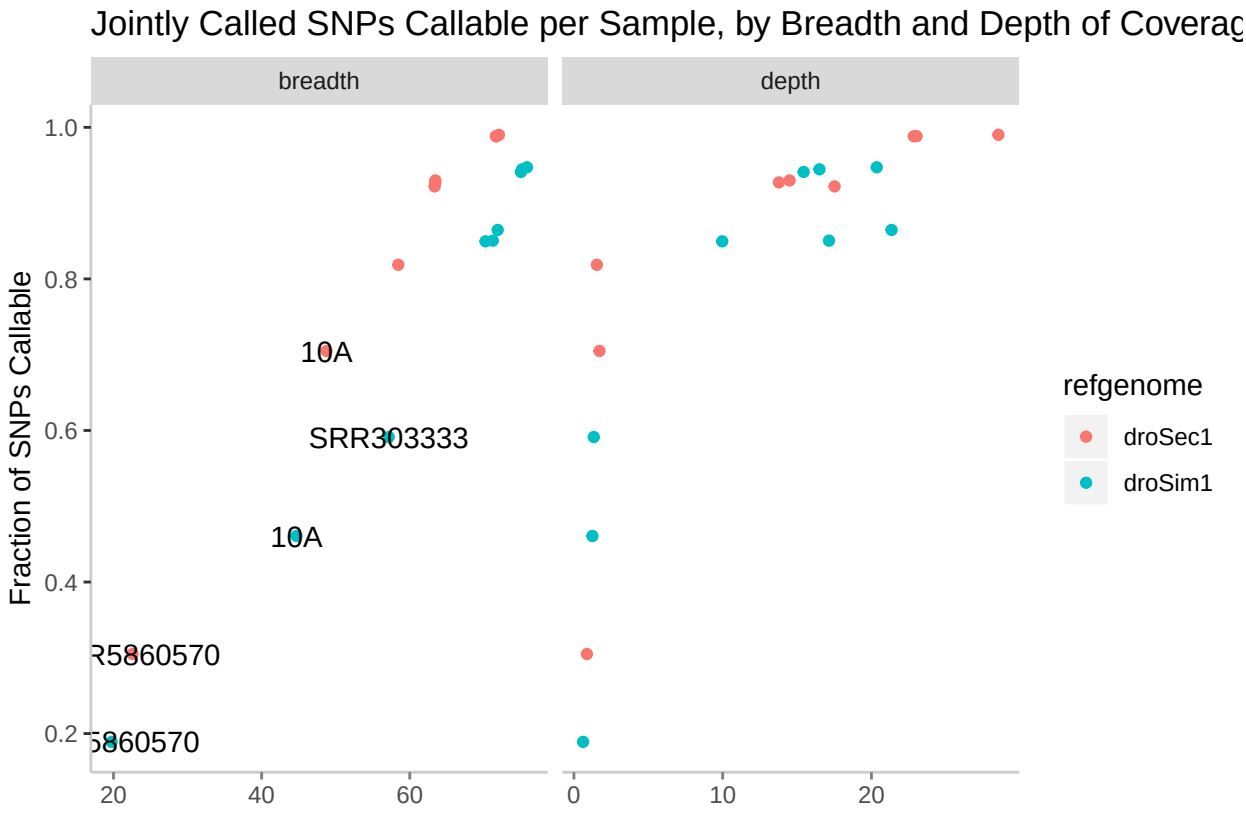
```
## Warning: Removed 4 rows containing missing values (geom_path).
```



The fraction of jointly called SNPs which are individually callable:

```
## Warning: Column `refgenome`/`reference` joining factors with different
## levels, coercing to character vector
```

```
## Warning: Column `refgenome`/`reference` joining character vector and
## factor, coercing into character vector
```



2.5 Allele Frequency Shift

Once the SNPs were called, the VCF file was split into four subsets: the wild simulans & sechellia populations, the backcross & selection lines, and pseudocontrol sechellia sequences downloaded from NCBI. For each SNP still meeting minimum requirments (biallelic, at most one missing sample), the group-wide allele frequency was calculated. The frequencies for the sechellia and simulans populations were used as reference points, and the distance to the simulans frequency and the sechellia frequency calculated for each SNP, for the selection-backcross and the pseudocontrol subsets. The per-window average shift was then calculated.

Here is a hypothetical example: suppose that at a given site in the genome, 75% of alleles in the sechellia population are T and 25% are A. Suppose in the simulans population, it's 25% T and 75% A. Now, the allele frequency is tallied in three different subgroups: In the first subgroup, 100% of alleles are T. This subgroup would have a sech-ward shift of +0.25 and a sim-ward shift of -0.75. In the second subgroup, 50% of alleles are T. This subgroup would have a sech-ward shift of -0.25 and a sim-ward shift of -0.25. In the third subgroup, 0% of alleles are T and all are A. This subgroup would have a sech-ward shift of -0.75 and a sim-ward shift of +0.25.

2.6 Windowed clustering

A distance measure on a genomic interval between two samples was defined: among sites in that interval which had a genotype called in both samples, the difference in alleles was summed and divided by SNP count to give an averaged difference. This metric was calculated for each 100kB window in the droSim1 reference genome, for each pair of samples.

3 Results

3.1 Basic Results Summary

3.2 Allele Frequency Shift

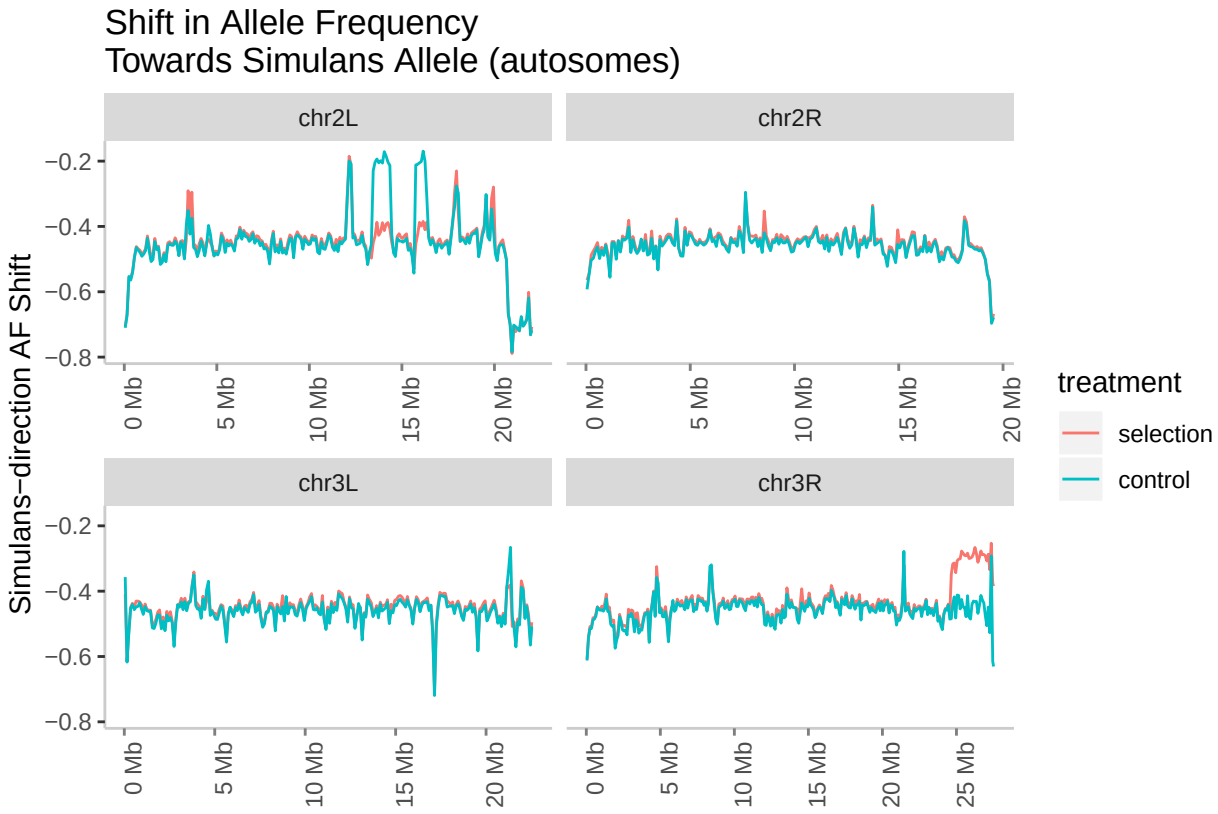
Population-wide allele frequencies were calculated for the wild-caught sechellia and simulans samples; these were taken as species-representative values. The allele frequencies in the selection and pseudocontrol groups were compared to the wild species frequencies, and the difference between treatment AF and species AF was calculated, then summed and averaged by 100kB window.

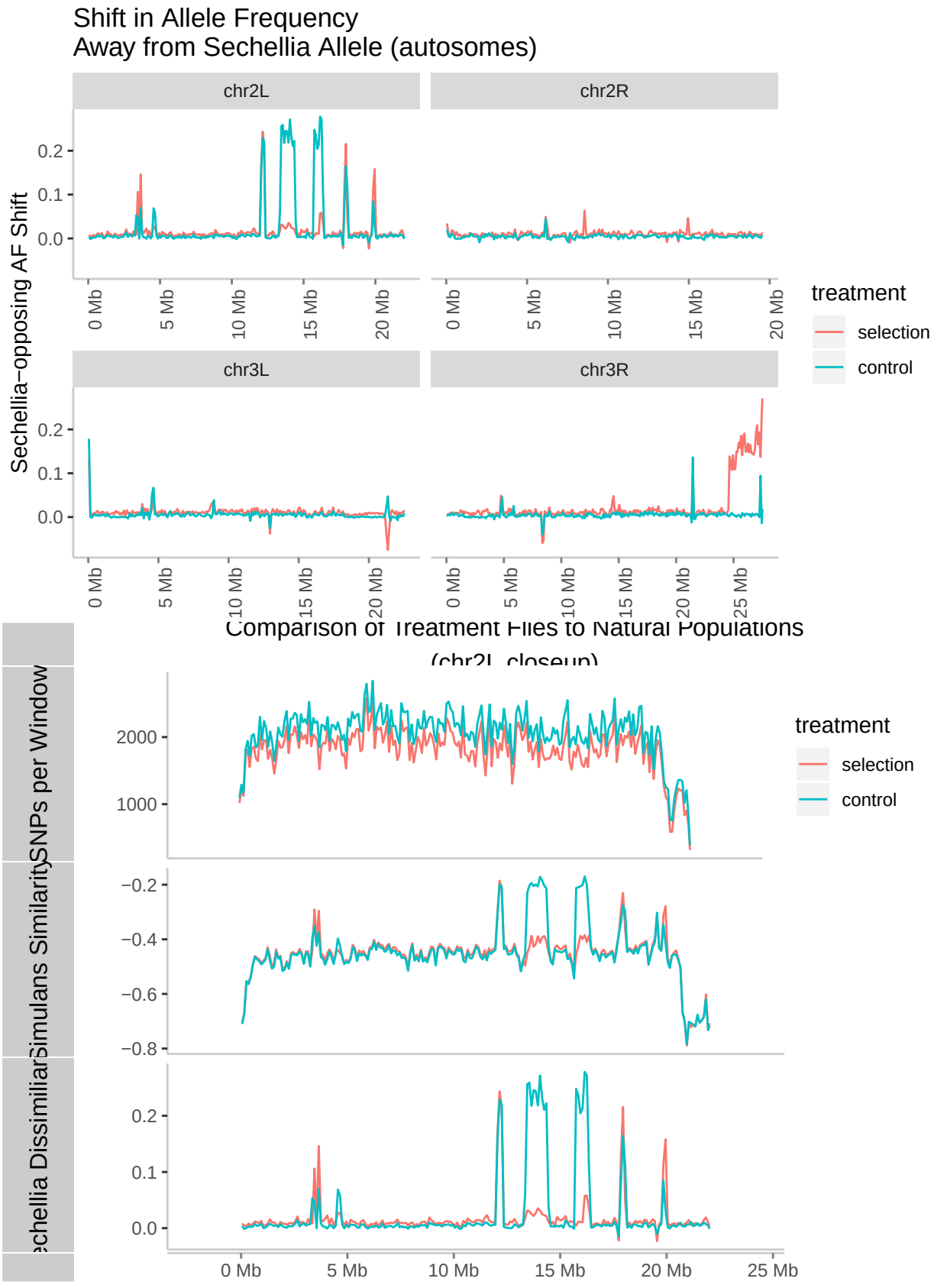
```
## Warning: NAs introduced by coercion

## Warning: NAs introduced by coercion

## Warning: NAs introduced by coercion

## Warning: NAs introduced by coercion
```





Overall, it's actually the pseudocontrol sechellia lines downloaded from NCBI which show large islands of

simulans-character (as defined by the variants called in the matute population sequences). In some cases, the backcross/selection samples show similar trends in the same regions; in other places, the pseudocontrols show clear simulans-character while the backcross/selection lines have a much more muted response. Only a region ~25+MB on chr3R shows simulans-character elevated above that of the pseudocontrols.

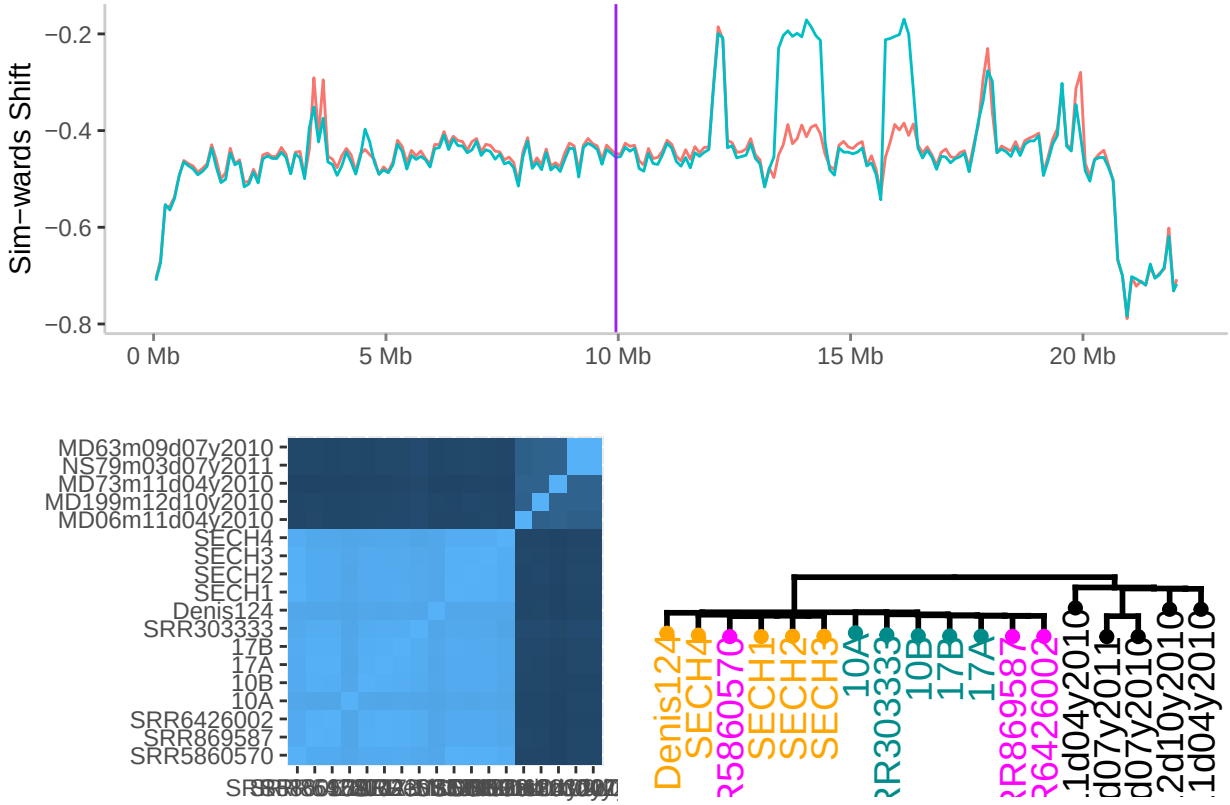
3.3 Windowed Clustering

To better understand these patterns, a sample-to-sample distance was calculated, for each pair of samples, for each 100kB window in the reference genome, by tallying the number of shared alleles at in-window variable sites. This distance function was used to construct heatmaps and dendrograms to visualize patterns of similarity and difference.

A typical baseline window is as expected: the population sechellia, backcross/selected lines, and pseudocontrols all show high similarity with one another and cluster in the dendrogram; the population simulans clusters in contrast:

```
## Warning: Using alpha for a discrete variable is not advised.
```

```
## Warning: Removed 17 rows containing missing values (geom_point).
```



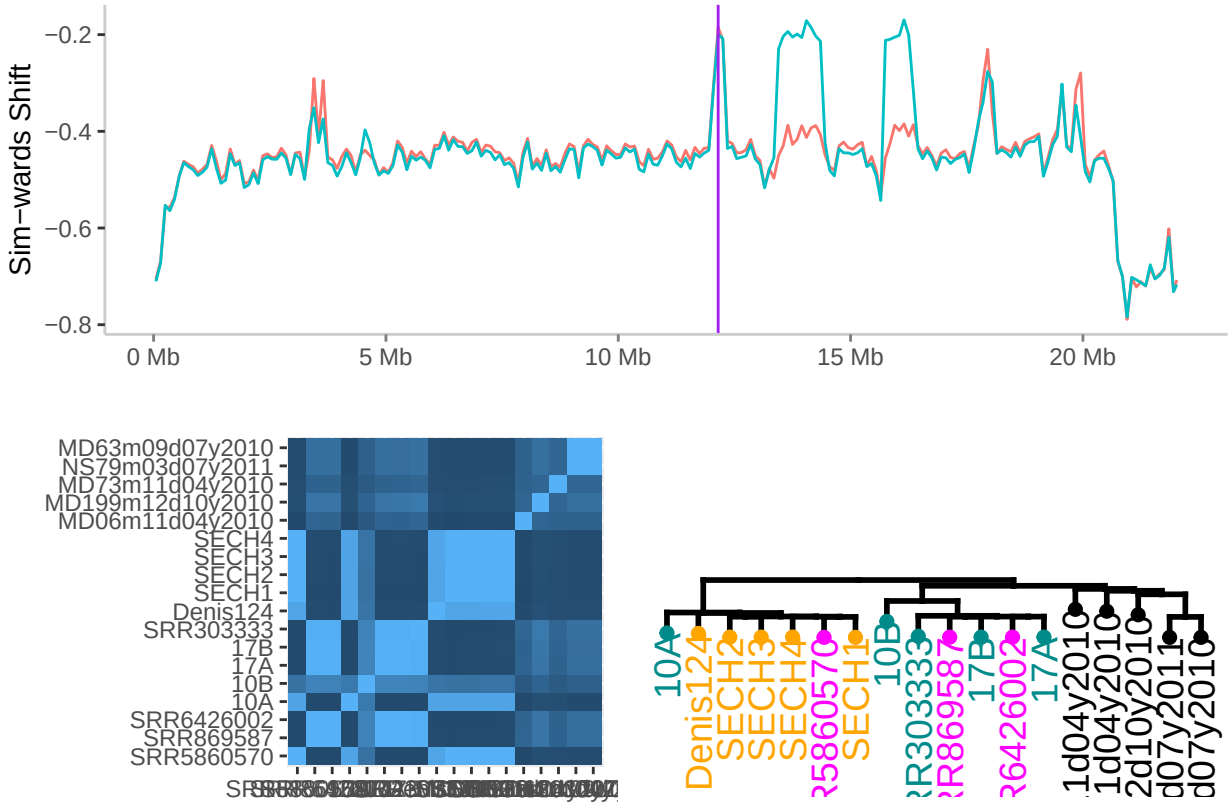

```
## TableGrob (2 x 2) "arrange": 3 grobs
##   z     cells    name           grob
## 1 1 (1-1,1-2) arrange gtable[layout]
## 2 2 (2-2,1-1) arrange gtable[layout]
## 3 3 (2-2,2-2) arrange gtable[layout]
```

In some places, both the pseudocontrols and the selection/backcross lines show similar enhancement of simulans-character, and the patterns are more complicated: some selection lines (the 10's) retain sechellia-character, with 10A especially clustering with the wild-caught sechellia. The other selection lines cluster

with simulans, confirming their similarity. The pseudocontrols are also inconsistent: one (SRR5860570) stays clustered with the sechellia population, while the other two show greater similarity to simulans:

```
## Warning: Using alpha for a discrete variable is not advised.
```

```
## Warning: Removed 17 rows containing missing values (geom_point).
```

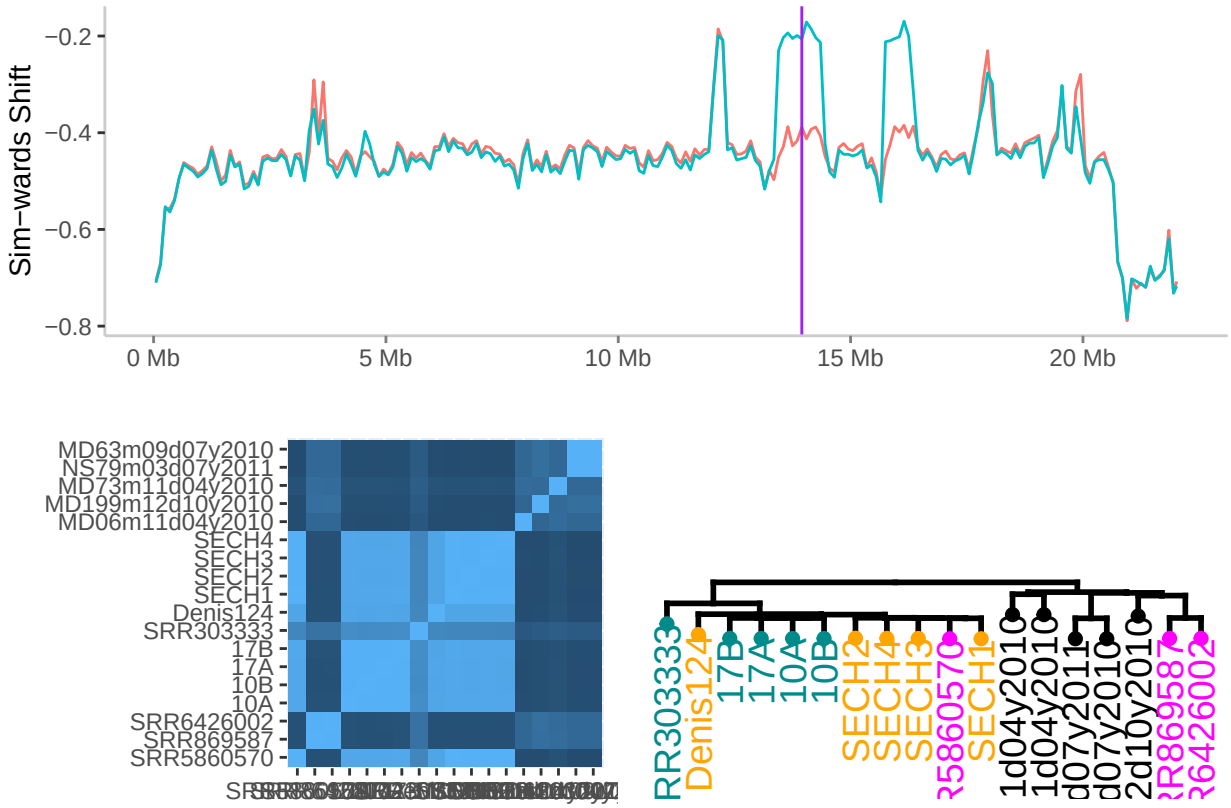



```
## TableGrob (2 x 2) "arrange": 3 grobs
##   z     cells    name           grob
## 1 1 (1-1,1-2) arrange gtable[layout]
## 2 2 (2-2,1-1) arrange gtable[layout]
## 3 3 (2-2,2-2) arrange gtable[layout]
```

In some places, the pseudocontrols actually show simulans-character which is elevated above that of the backcross/selection hybrids; again, it is SRR5860570 which is clustered with the sechellia population and the hybrids, while the other pseudocontrols cluster with the simulans population:

```
## Warning: Using alpha for a discrete variable is not advised.
```

```
## Warning: Removed 17 rows containing missing values (geom_point).
```

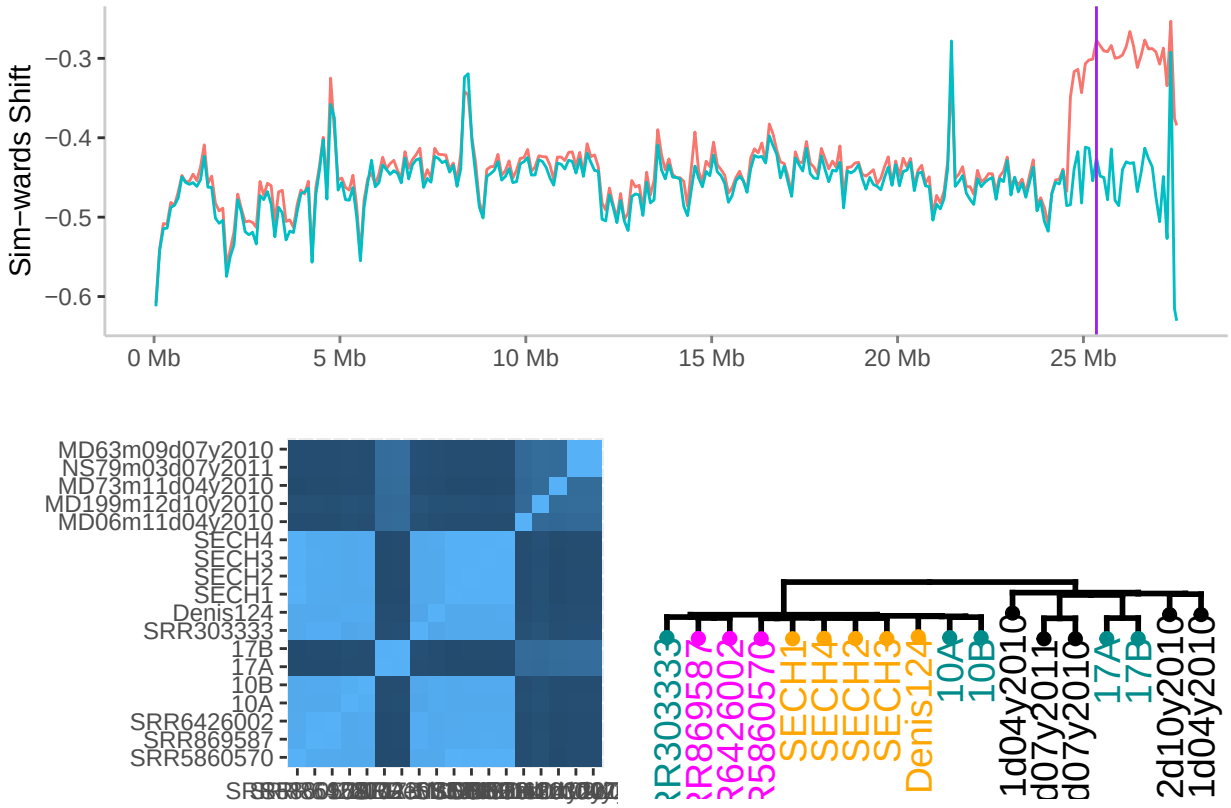



```
## TableGrob (2 x 2) "arrange": 3 grobs
##   z     cells    name           grob
## 1 1 (1-1,1-2) arrange gtable[layout]
## 2 2 (2-2,1-1) arrange gtable[layout]
## 3 3 (2-2,2-2) arrange gtable[layout]
```

Only one autosomal region appeared to have simulans-nature in backcross/selection hybrids elevated above that in pseudocontrols, in the telomeric end of chr3R. This appears to be driven mostly by samples 17A and 17B.

```
## Warning: Using alpha for a discrete variable is not advised.
```

```
## Warning: Removed 17 rows containing missing values (geom_point).
```




```
## TableGrob (2 x 2) "arrange": 3 grobs
##   z     cells    name           grob
## 1 1 (1-1,1-2) arrange gtable[layout]
## 2 2 (2-2,1-1) arrange gtable[layout]
## 3 3 (2-2,2-2) arrange gtable[layout]
```

4 Discussion

SRR5860570 is the only pseudocontrol which appears to be consistently sechellia-like, judging by its comparison to the wild-caught sechellia population. The others tend to show islands of simulans-nature, including some which don't show up in the backcross/selection lines.

SRR5860570 was wildcaught in 2012 by Dave Turisini. The other pseudocontrols are laboratory strains.

5 References

5.1 Software

```
##
## To cite package 'tidyverse' in publications use:
##
##   Hadley Wickham (2017). tidyverse: Easily Install and Load the
##   'Tidyverse'. R package version 1.2.1.
##   https://CRAN.R-project.org/package=tidyverse
##
```

```
## A BibTeX entry for LaTeX users is
##
##   @Manual{,
##     title = {tidyverse: Easily Install and Load the 'Tidyverse'},
##     author = {Hadley Wickham},
##     year = {2017},
##     note = {R package version 1.2.1},
##     url = {https://CRAN.R-project.org/package=tidyverse},
##   }
##
## To cite the 'knitr' package in publications use:
##
##   Yihui Xie (2018). knitr: A General-Purpose Package for Dynamic
##   Report Generation in R. R package version 1.21.
##
##   Yihui Xie (2015) Dynamic Documents with R and knitr. 2nd
##   edition. Chapman and Hall/CRC. ISBN 978-1498716963
##
##   Yihui Xie (2014) knitr: A Comprehensive Tool for Reproducible
##   Research in R. In Victoria Stodden, Friedrich Leisch and Roger
##   D. Peng, editors, Implementing Reproducible Computational
##   Research. Chapman and Hall/CRC. ISBN 978-1466561595
##
## To see these entries in BibTeX format, use 'print(<citation>,
## bibtex=TRUE)', 'toBibtex(.)', or set
## 'options(citation.bibtex.max=999)'.
##
## To cite package 'yaml' in publications use:
##
##   Jeremy Stephens, Kirill Simonov, Yihui Xie, Zhuoer Dong, Hadley
##   Wickham, Jeffrey Horner, reikoch, Will Beasley, Brendan O'Connor
##   and Gregory R. Warnes (2018). yaml: Methods to Convert R Data to
##   YAML and Back. R package version 2.2.0.
##   https://CRAN.R-project.org/package=yaml
##
## A BibTeX entry for LaTeX users is
##
##   @Manual{,
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##     author = {Jeremy Stephens and Kirill Simonov and Yihui Xie and Zhuoer Dong and Hadley Wickham an
##     year = {2018},
##     note = {R package version 2.2.0},
##     url = {https://CRAN.R-project.org/package=yaml},
##   }
##
## ATTENTION: This citation information has been auto-generated from
## the package DESCRIPTION file and may need manual editing, see
## 'help("citation")'.
```

Bibliography

Chen, Shifu, Yanqing Zhou, Yaru Chen, and Jia Gu. 2018. "Fastp: An ultra-fast all-in-one FASTQ preprocessor." *Bioinformatics* 34 (17): i884–i890. doi:10.1093/bioinformatics/bty560.

Earley, Eric J., and Corbin D. Jones. 2011. "Next-generation mapping of complex traits with phenotype-based selection and introgression." *Genetics* 189 (4): 1203–9. doi:10.1534/genetics.111.129445.

Garrison, Erik, and Gabor Marth. 2012. "Haplotype-based variant detection from short-read sequencing," July. http://arxiv.org/abs/1207.3907.