

Novel tools for analyzing genome-wide data of clonal populations

Zhian N. Kamvar¹, Jonah C. Brooks², Niklaus J. Grnwald^{1,3*}

¹ Botany and Plant Pathology, Oregon State University, Corvallis, OR, USA

² College of Electrical Engineering and Computer Science, Oregon State University, Corvallis, OR, USA

³ Horticultural Crops Research Laboratory, USDA-Agricultural Research Service, Corvallis, OR, USA

Correspondence*:

Niklaus J. Grnwald
Horticultural Crops Research Laboratory USDA ARS
3420 NW Orchard Ave.
Corvallis, OR, 97330, grunwalg@science.oregonstate.edu

2 ABSTRACT

To gain a detailed understanding of how plant microbes evolve and adapt to hosts, pesticides, and other factors, knowledge of the population dynamics and evolutionary history of populations is crucial. Plant pathogen populations are often clonal or partially clonal which requires different analytical tools. With the advent of high throughput sequencing technologies, obtaining genomic sequences for representative populations has become easier than ever before. A move towards open, reproducible science has provided impetus for developing population genetic analysis tools in R. We previously contributed the R package *poppr* specifically addressing issues with analysis of clonal populations. In this paper we provide several significant extensions to *poppr* with a focus on large, genome wide SNP data. Specifically, we provide analyses across any level of hierarchies, a new function to define clone boundaries we call *mlg.filter* allowing for inspection and definition of what is a clonal lineage, the index of association for reduced representation genomic data, and modular bootstrapping of any genetic distance.

Keywords: clonality, population genetics, bootstrap, open source

INTRODUCTION

To paraphrase Dobzhansky, nothing in the field of plant-microbe interactions makes sense except in the light of population genetics (Dobzhansky, 1973). Genetic forces such as selection and drift act on alleles in a population. Thus, a true understanding of how plant pathogens evolve and adapt to crops, fungicides, or other factors, can only emerge in the context of population level phenomena given the demographic history of populations (McDonald and Linde, 2002; Grnwald and Goss, 2011; Milgroom et al., 1989). The field of population genetics, in the era of whole genome resequencing, provides unprecedented power to describe the evolutionary history and population processes that drive coevolution between pathogens and hosts. This powerful field thus critically enables effective deployment of R genes, design of pathogen informed plant resistance breeding programs, and implementation of fungicide rotations that minimize emergence of resistance.

Most computational tools for population genetics are based on concepts developed for sexual model organisms. Populations that reproduce clonally or are polyploid are thus difficult to characterize using

28 classical population genetic tools because theoretical assumptions underlying the theory are violated. Yet,
29 many plant pathogen populations are at least partially clonal if not completely clonal (Milgroom, 1996;
30 Anderson and Kohn, 1995). Thus, development of tools for analysis of clonal or polyploid populations is
31 needed.

32 Genotyping by sequencing and whole genome resequencing provide the unprecedented ability to
33 identify thousands of single nucleotide polymorphisms (SNPs) in populations (Elshire et al., 2011; Luikart
34 et al., 2003; Davey et al., 2011). With traditional marker data (e.g., SSR, AFLP) a clone was typically
35 defined as a unique multilocus genotype (MLG) (Grnwald and Hoheisel, 2006; Falush et al., 2003; Goss
36 et al., 2009; Cooke et al., 2012; Taylor and Fisher, 2003). Availability of large SNP data sets provides new
37 challenges for data analysis. These data are based on reduced representation libaries and high throughput
38 sequencing with moderate sequencing depth which invariably results in substantial missing data, error in
39 SNP calling due to sequencing error, lack of read depth or other sources of spurious allele calls (Mastretta-
40 Yanes et al., 2015). It is thus not clear what a clone is in large SNP data sets and novel tools are required
41 for definition of clone boundaries.

42 The research community using the R statistical and computing language (R Core Team, 2015) has
43 developed a plethora of new resources for population genetic analysis (Paradis, 2010; Jombart, 2008).
44 Recently, we introduced the R package *poppr* specifically developed for analysis of clonal populations
45 (Kamvar et al., 2014b). *Poppr* previously introduced several novel features including the ability to conduct
46 a hierarchical analysis across unlimited hierarchies, test for linkage association, graph minimum spanning
47 networks or provide bootstrap support for Bruvo's distance in resulting trees.

48 In version 1.1, to facilitate handling hierarchical and multilocus genotypic metadata, a new S4 data
49 object called "genclone" was defined to expand the genind object of *adegenet*. The genclone object
50 formalized the definitions of multilocus genotypes and population hierarchies by adding two slots called
51 "mlg" and "hierarchy" that carried a numeric vector and a data frame, respectively. These new slots allow
52 for increased efficiency and ease of use by allowing these metadata to travel with the genetic data. The
53 addition of the population hierarchies has proved to be advantageous enough that they have recently been
54 incorporated directly into the *adegenet* package (Jombart, 2008).

55 Here, we introduce *poppr* 2.0, which provides a major update to *poppr* (Kamvar et al., 2014b) including
56 novel tools for analysis of clonal populations specifically addressing large SNP data. Significant novel
57 tools include functions for calculating clone boundaries and collapsing individuals into user-specified
58 clones based on genetic distance, sliding window analyses, genotype accumulation curves, reticulations
59 in minimum spanning networks, and bootstrapping for any genetic distance.

IMPLEMENTATIONS AND EXAMPLES

CLONAL IDENTIFICATION

60 As highlighted in previous work, clone correction is an important component of population genetic
61 analysis of organisms that have cryptic growth or are known to reproduce asexually (Kamvar et al., 2014b;
62 Milgroom, 1996; Grnwald et al., 2003). This method is a partial correction for bias that affects metrics
63 that rely on allele frequencies assuming panmixia. It was initially designed for data with only a handful
64 of markers. With the advent of large-scale sequencing and reduced-representation libraries, it has become
65 easier to sequence tens of thousands of markers from hundreds of individuals (Elshire et al., 2011; Davey
66 et al., 2011; Davey and Blaxter, 2010). With this larger number of markers, the genetic resolution is much
67 greater, but the chance of genotyping error is also greatly increased (Mastretta-Yanes et al., 2015). Taking
68 this fact and occasional somatic mutations into account, it would be impossible to separate true clones
69 from independent individuals by just comparing what multilocus genotypes are different. We introduce
70 a new method for collapsing unique multilocus genotypes determined by naive string comparison into
71 multilocus lineages utilizing any genetic distance given three different clustering algorithms: farthest
72 neighbor, nearest neighbor, and UPGMA (average neighbor) (Sokal, 1958).

The clustering algorithms act on a distance matrix that is either provided by the user or generated via a function that will calculate a distance from genclone objects such as `bruno.dist`, which in particular applies to any level of ploidy (Bruvo et al., 2004). All algorithms have been implemented in C and utilize the OpenMP framework for optional parallel processing (Dagum and Menon, 1998). Default is the conservative farthest neighbor algorithm, which will only cluster samples together if all samples in the cluster are at a distance less than the given threshold. By contrast, the nearest neighbor algorithm will have a chaining effect that will cluster samples akin to adding links on a chain where a sample can be included in a cluster if all of the samples have at least one connection below a given threshold. The UPGMA, or average neighbor clustering algorithm is the one most familiar to biologists as it is often used to generate preliminary ultra-metric trees based on genetic distance. This algorithm will cluster by creating a representative sample per cluster and joining clusters if these representative samples are closer than the given threshold.

We utilize data from the microbe *Phytophthora infestans* to show how the `mlg.filter` function collapses multilocus genotypes with Bruvo's distance assuming a genome addition model (Bruvo et al., 2004). *P. infestans* is the causal agent of potato late blight originating from Mexico and spread to Europe in the mid 19th century (Goss et al., 2014; Li et al., 2013; Lees et al., 2006). *P. infestans* reproduces both clonally and sexually. The clonal lineages of *P. infestans* have been formally defined into 18 separate clonal lineages using a combination of various molecular methods including AFLP and microsatellite markers (Lees et al., 2006). For these data, we used `mlg.filter` to detect all of the distance thresholds at which 18 multilocus lineages would be resolved. We used these thresholds to define multilocus lineages and create contingency tables and dendograms to determine how well the multilocus lineages were detected.

For the *P. infestans* population, the three algorithms were able to detect 18 multilocus lineages at different distance thresholds (Fig. 1). Contingency tables between the described multilocus genotypes and the genotypes defined by distance show that most of the 18 lineages were resolved, except for US-8, which is polytomic (Table 1).

We utilized simulated data constructed using the `glSim` function in *adegenet* (Jombart and Ahmed, 2011) to obtain a SNP data set for demonstration. Two diploid data sets were created, each with 10k SNPs (25% structured into two groups) and 200 samples with 10 ancestral populations of even sizes. Clones were created in one data set by marking each sample with a unique identifier and then randomly sampling with replacement. It is well documented that reduced-representation sequencing can introduce several erroneous calls and missing data (Mastretta-Yanes et al., 2015). To reflect this, we mutated SNPs at a rate of 10% and inserted an average of 10% missing data for each sample after clones were created, ensuring that no two sequences were alike. The number of mutations and missing data per sample were determined by sampling from a poisson distribution with $\lambda = 1000$. After pooling, 20% of the data set was randomly sampled for analysis. Genetic distance was obtained with the function `bitwise.dist`, which calculates the fraction of different sites between samples, counting missing data as equivalent in comparison.

All three filtering algorithms were run with a threshold of 1, returning a numeric vector of length $n - 1$ where each element represented a threshold at which two samples/clusters would join. Since each data set would have varying distances between samples, the clonal boundary threshold was defined as the midpoint of the largest gap between two thresholds that collapsed less than 50% of the data.

Out of the 100 simulations run, we found that across all methods, detection of duplicated samples had $\sim 98\%$ true positive fraction and $\sim 0.8\%$ false positive fraction indicating that this method is robust to simulated populations.

MINIMUM SPANNING NETWORKS WITH RETICULATION

In its original iteration, *poppr* introduced minimum spanning networks that were based on the *igraph* function `minimum.spanning.tree` (Csardi and Nepusz, 2006). This algorithm produces a minimum spanning tree with no reticulations where nodes represent individual MLGs. In other minimum spanning

120 network programs, reticulation is obtained by calculating the minimum spanning tree several times and
121 returning the set of all edges included in the trees. Due to the way *igraph* has implemented Prim's
122 algorithm, it is not possible to utilize this strategy, thus we implemented an internal C function to walk
123 the space of minimum spanning trees based on genetic distance to connect groups of nodes with edges of
124 equal weight.

125 To demonstrate the utility of minimum spanning networks with reticulation, we used two clonal data
126 sets: H3N2 flu virus data from the *aegenet* package using years of each epidemic as the population
127 factor, and *Phytophthora ramorum* data from Nurseries and Oregon forests (Jombart et al., 2010; Kamvar
128 et al., 2014a). Minimum spanning networks were created with and without reticulation using the *poppr*
129 functions *diss.dist* and *bruvo.msn* for the H3N2 and *P. ramorum* data, respectively (Kamvar et
130 al., 2014b; Bruvo et al., 2004). To detect mlg clusters, the infoMAP community detection algorithm was
131 applied with 10,000 trials as implemented in the R package *igraph* version 0.7.1 utilizing genetic distance
132 as edge weights and number of samples in each MLG as vertex weights (Csardi and Nepusz, 2006; Rosvall
133 and Bergstrom, 2008).

134 To evaluate the results, we compared the number, size, and entropy (H) of resulting communities as we
135 expect a highly clonal organism with low genetic diversity to result in a few, large communities. We also
136 created contingency tables of the community assignments with the defined populations and used those
137 to calculate entropy using Shannon's index with the function *diversity* from the R package *vegan*
138 version 2.2-1 (Oksanen et al., 2015; Shannon, 2001). A low entropy indicates presence of a few large
139 communities whereas high entropy indicates presence of many small communities.

140 The infoMAP algorithm revealed 63 communities with a maximum community size of 77 and $H = 3.56$
141 for the reticulate network of the H3N2 data and 117 communities with a maximum community size of
142 26 and $H = 4.65$ for the minimum spanning tree. The entropy across years was greatly decreased for all
143 populations with the reticulate network compared to the minimum spanning tree (Fig. 2).

144 Graph walking of the reticulated minimum spanning network of *P. ramorum* by the infoMAP algorithm
145 revealed 16 communities with a maximum community size of 13 and $H = 2.60$. The un-reticulated
146 minimum spanning tree revealed 20 communities with a maximum community size of 7 and $H = 2.96$.
147 In the ability to predict Hunter Creek as belonging to a single community, the reticulated network was
148 successful whereas the minimum spanning tree separated one genotype from that community. The entropy
149 for the reticulated network was lower for all populations except for the Coast population (supplementary
150 information).

BOOTSTRAPPING

151 Calculating genetic distance for among samples and populations is very important method for assessing
152 population differentiation through methods such as G_{st} , AMOVA, and Mantel tests (Nei, 1973; Excoffier
153 et al., 1992; Mantel, 1967). Confidence in distance metrics is related to the confidence in the markers to
154 accurately represent the diversity of the data. Especially true with microsatellite markers, a single hyper-
155 diverse locus can make a population appear to have more diversity based on genetic distance. Using a
156 bootstrapping procedure of randomly sampling loci with replacement when calculating a distance matrix
157 gives confidence in hierarchical clustering. Because genetic data in a genind object is represented as a
158 matrix with samples in rows and alleles in columns, bootstrapping is a non-trivial task as all alleles in
159 a single locus need to be sampled together. To remedy this, we have created an internal S4 class called
160 "bootgen", which extends the internal "gen" class from *aegenet*. This class can be created from any
161 genind, genclone, or genpop object, and allows loci to be sampled with replacement. To further facilitate
162 bootstrapping, a function called *aboot*, which stands for "any boot", is introduced that will bootstrap
163 any genclone, genind, or genpop object with any genetic distance that can be calculated from it.

164 To demonstrate calculating a dendrogram with bootstrap support, we used the *poppr* function *aboot*
165 on population allelic frequencies derived from the data set *microbov* in the *aegenet* package with 1000

166 bootstrap replicates (Jombart, 2008; Lalo et al., 2007). The resulting dendrogram shows bootstrap support
 167 values > 50% (Fig. 3).

```
library("poppr")
data("microbov", package = "adegenet")
strata(microbov) <- data.frame(other(microbov))
setPop(microbov) <- ~coun/spe/breed
bov_pop <- genind2genpop(microbov, quiet = TRUE)

set.seed(20150428)
pop_tree <- aboot(bov_pop, sample = 1000, cutoff = 50, quiet = TRUE)
```

GENOTYPE ACCUMULATION CURVE

168 Analysis of population genetics of clonal organisms often borrows from ecological methods such as
 169 analysis of diversity within populations (Milgroom, 1996; Arnaud-Hanod et al., 2007; Grnwald et al.,
 170 2003). When choosing markers for analysis, it is important to make sure that the observed diversity in your
 171 sample will not appreciably increase if an additional marker is added (Arnaud-Hanod et al., 2007). This
 172 concept is analogous to a species accumulation curve, obtained by rarefaction. The genotype accumulation
 173 curve in *poppr* is implemented in the function `genotype_curve`. The curve is constructed by randomly
 174 sampling x loci and counting the number of observed MLGs. This repeated r times for 1 locus up to $n - 1$
 175 loci, creating $n - 1$ distributions of observed MLGs.

176 The following code example demonstrates the genotype accumulation curve for data from Everhart and
 177 Scherm (2015) showing that these data reach a small plateau and have a greatly decreased variance with
 178 12 markers, indicating that there are enough markers such that adding more markers to the analysis will
 179 not create very many new genotypes (Fig. 4).

```
library("poppr")
library("ggplot2")
data("monpop", package = "poppr")

set.seed(20150428)
genotype_curve(monpop, sample = 1000, quiet = TRUE)
p <- last_plot() + theme_bw() # get the last plot
p + geom_smooth(aes(group = 1)) # plot with a trendline
```

INDEX OF ASSOCIATION

180 The index of association (I_A) is a measure of multilocus linkage disequilibrium that is most often used
 181 to detect clonal reproduction within organisms that have the ability to reproduce via sexual or asexual
 182 processes (Brown et al., 1980; Smith et al., 1993; Milgroom, 1996). It was standardized in 2001 as \bar{r}_d
 183 by Agapow and Burt (2001) to address the issue of scaling with increasing number of loci. This metric is
 184 typically applied to traditional dominant and co-dominant markers such as AFLPs, SNPs, or microsatellite
 185 markers. With the advent of high throughput sequencing, SNP data is now available in a genome-wide
 186 context and in very large matrices including thousands of SNPs. Thus, the likelihood of finding mutations
 187 within two individuals of a given clone increases and tools are needed for defining clone boundaries.
 188 For this reason, we devised two approaches using the index of association for large numbers of markers
 189 typical for population genomic studies. Both functions utilize *adegenet*'s “genlight” object class, which
 190 efficiently stores 8 binary alleles in a single byte (Jombart and Ahmed, 2011). As calculation of the \bar{r}_d
 191 requires distance matrices of absolute number of differences, we utilize a function that calculates these
 192 distances directly from the compressed data called `bitwise.dist`.

193 The first approach is a sliding window approach implemented in the function `win.ia`. It utilizes the
194 position of markers in the genome to calculate \bar{r}_d among any number of SNPs found within a user-
195 specified windowed region. It is important that this calculation utilize \bar{r}_d as the number of loci will be
196 different within each window (Agapow and Burt, 2001). This approach would be suited for a quick
197 calculation of linkage disequilibrium across the genome that can detect potential hotspots of LD that
198 could be investigated further with more computationally intensive methods assuming that the number of
199 samples << the number of loci.

200 As it would necessarily focus on loci within a short section of the genome that may or may not
201 be recombining, a sliding window approach would not be good for utilizing \bar{r}_d as a test for clonal
202 reproduction. A remedy for this is implemented in the function `samp.ia`, which will randomly sample
203 m loci, calculate \bar{r}_d , and repeat r times, thus creating a distribution of expected values of \bar{r}_d .

204 To demonstrate the sliding window and random sampling of \bar{r}_d with respect to clonal populations, we
205 simulated two populations containing 1,100 neutral SNPs for 100 diploid individuals under the same
206 initial seed. One population had individuals randomly sampled with replacement, representing the clonal
207 population. After sampling, both populations had 5% random error and 1% missing data independently
208 propagated across all samples. On average, we obtained a higher value of \bar{r}_d for the clonal population
209 compared to the sexual population for both methods (Fig. 5).

CHANGE IN DEFINITION OF POPULATION STRATA AND HIERARCHIES

210 Assessments of population structure through methods such as hierarchical F_{st} (Goudet, 2005) and
211 AMOVA (Michalakis and Excoffier, 1996) require hierarchical sampling of populations across space
212 or time (Linde et al., 2002; Everhart and Scherm, 2015; Grnwald and Hoheisel, 2006). With clonal
213 organisms, basic practice has been to clone-censor data to avoid downward bias in diversity due to
214 duplicated genotypes that may or may not represent different samples (Milgroom, 1996). This correction
215 should be performed with respect to a population hierarchy to accurately reflect the biology of the
216 organism. Traditional data structures for population genetic data in most analysis tools allow for only
217 one level of hierarchical definition. The investigator thus had to provide the data set for analysis at each
218 hierarchical level. In `poppr` version 1.1, the hierarchy slot was introduced to allow unlimited population
219 hierarchies or stratifications to travel with the data (Kamvar et al., 2014b). In R, it is stored as a data
220 frame where each column represents a separate hierarchical level. This is then used to set the population
221 factor of the data by supplying a hierarchical formula containing one or more column names of the data
222 frame in the hierarchy slot. This functionality, has now been migrated to the `aedeagenet` package, version
223 2.0 to allow hierarchical analysis in `aedeagenet`, `poppr`, and other dependent packages. The prior `poppr`
224 hierarchy slot and methods have now been renamed `strata` in `aedeagenet`. This migration provides
225 endusers with a broader ability to analyze data hierarchically in R across packages.

AVAILABILITY

226 As of this writing, the `poppr` R package version 2.0 containing all of the features described here is located
227 at <https://github.com/grunwaldlab/poppr>. It is necessary to install `aedeagenet` 2.0 before
228 installing `poppr`. It can be found at <https://github.com/thibautjombart/aedeagenet>. Both
229 of these can be installed via the R package `devtools` (Wickham and Chang, 2015):

```
library("devtools")
install_github("thibautjombart/aedeagenet")
install_github("grunwaldlab/poppr")
```

DISCUSSION

230 Genomic data has become more readily accessible due to advances in low-cost sequencing technology.
231 Many tools have been developed or adapted to these data, but most of them were designed with sexual
232 populations in mind. Particularly important is the implementation of \bar{r}_d for genomic data (Agapow and
233 Burt, 2001). Random sampling of loci across the genome can give an expected distribution of \bar{r}_d , which
234 is expected to have a mean of zero for panmictic populations. Additionally, due to the fact that it acts
235 on multiple loci, is not affected by the number of loci sampled, and has the ability to detect population
236 structure, \bar{r}_d is well suited to sliding window analyses and has the potential to be applied to non-clonal
237 populations.

238 Clustering multilocus genotypes into multilocus lineages based on genetic distances is a non-trivial task.
239 Moreover, this has not previously been implemented for genomic data for clonal populations. Perhaps
240 highlighting the fact that many of the features presented in this paper are not exclusive to genomic
241 data is the fact that this method of clonal assignment has been available in the programs GENCLONE
242 and GENODIVE (Arnaud-Hanod et al., 2007; Meirmans and Van Tienderen, 2004). Our method with
243 `mlg.filter` builds upon this idea and allows the user to choose between three different approaches for
244 clustering MLGs. As shown in Fig. 1, it is clear that the choice of clustering algorithm has an impact on
245 the data, where a genetic distance cutoff of 0.1 would be the difference between 14 MLLs and 17 MLLs
246 for nearest neighbor and UPGMA clustering, respectively (Fig. 1). The option to choose the clustering
247 algorithm gives the user the ability to choose what is biologically relevant to their populations.

248 Multilocus genotypes that have been clustered can then be visualized in minimum spanning networks.
249 Reticulate minimum spanning networks are very important for clonal organisms where a minimum
250 spanning tree would become a chain, implying that the clones were derived in a progressive and linear
251 fashion. This presents but one potential scenario for clonal organisms, but does not account for any other
252 biologically relevant process. Reticulations in the minimum spanning networks allow for a representation
253 of uncertainty that goes along with clonal organisms. The current implementation in `poppr` has been
254 successfully used in analyses such as reconstruction of the *P. ramorum* epidemic in Curry County, OR
255 (Kamvar et al., 2014a, 2015). Reticulated networks also allow for the application of graph community
256 detection algorithms such as the infoMAP algorithm (Rosvall and Bergstrom, 2008). As shown in the *P.*
257 *ramorum* and H3N2 data, while it is possible to utilize these graph walking algorithms on non-reticulate
258 minimum spanning trees, the results derived from these are limited to explain populations derived from
259 serial cloning events.

260 Implementing these methods in R and hosting the code free and open on GitHub has allowed us
261 the ability to tailor our tools for the needs of the researchers who use them.

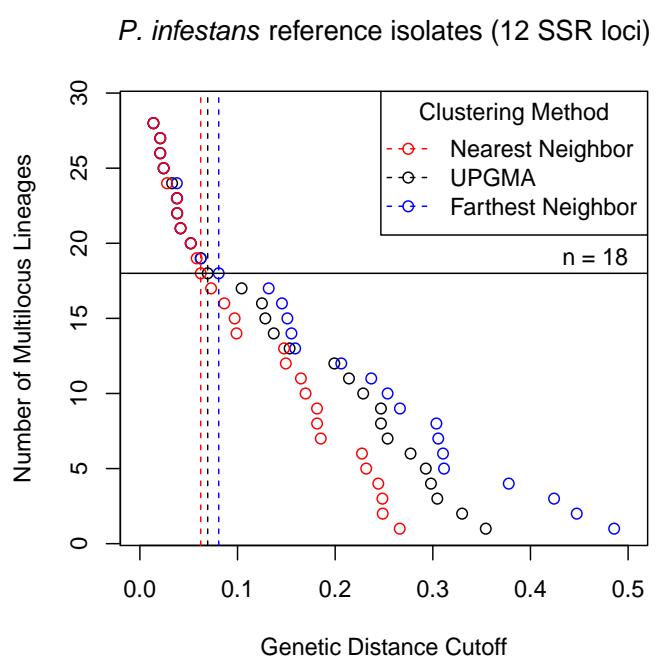
FIGURES AND TABLES**FIGURE 1**

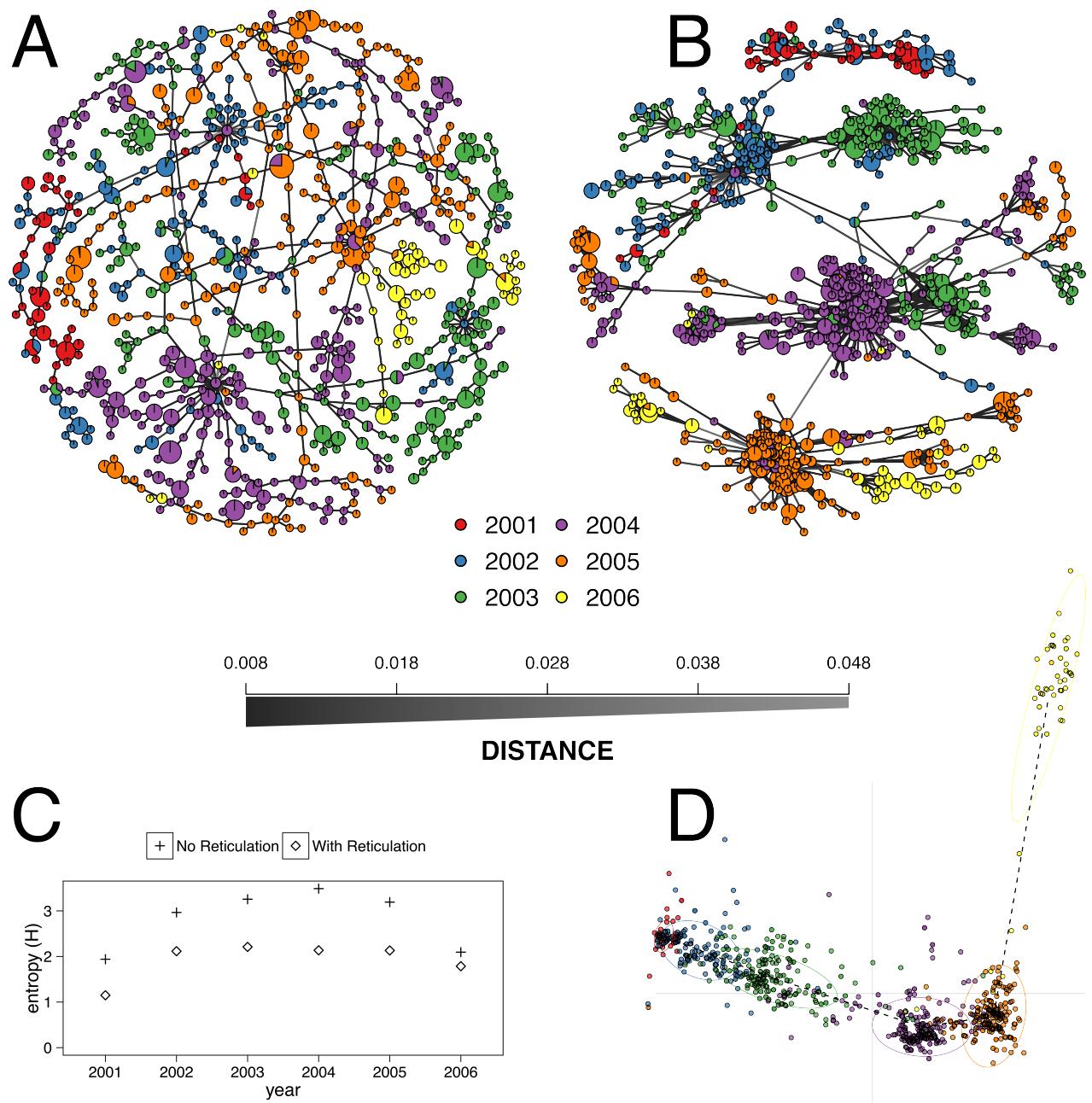
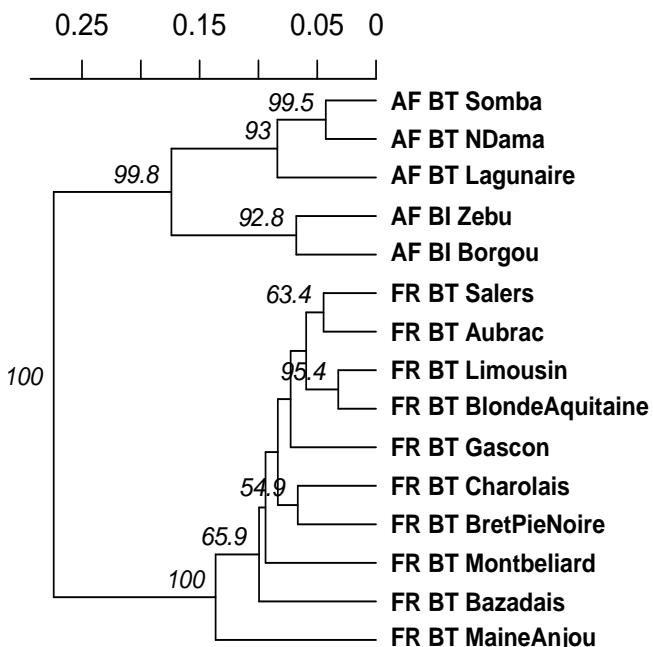
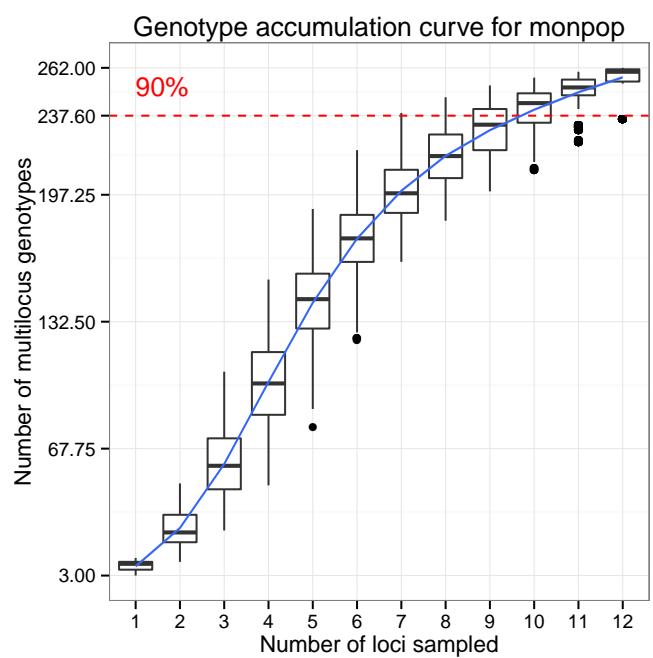
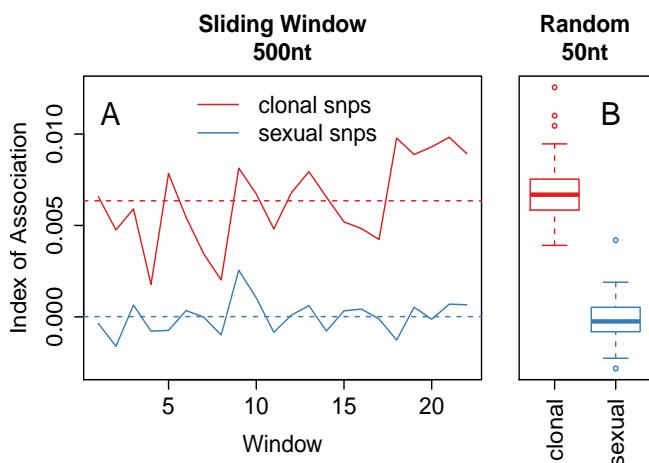
FIGURE 2

FIGURE 3

264

FIGURE 4

265

FIGURE 5

266

TABLE 1

	3	4	5	6	8	10	12	15	16	17	18	20	21	22	24	25	27	28
B	1	.	.	.
C	1	.	.	.
D.1	1	.	.	.
D.2	1	.	.	.
EU-13	1
EU-4	1
EU-5	2
EU-8	1
US-11	2	.	.
US-12	.	1
US-14	1
US-17	1
US-20	2
US-21	2	.	.
US-22	2
US-23	3
US-24	.	.	.	3
US-8	.	.	1	1	.	2

FIGURE AND TABLE LEGENDS**FIGURE 1**

267 Graphical representation of three different clustering algorithms collapsing multilocus genotypes for 12
 268 SSR loci from *Phytophthora infestans* representing 18 clonal lineages. The horizontal axis is Bruvo's
 269 genetic distance assuming the genome addition model. The vertical axis represents the number of
 270 multilocus lineages observed. Each point shows the threshold at which one would observe a given number
 271 of multilocus genotypes. The horizontal black line represents 18 multilocus genotypes and vertical dashed
 272 lines mark the thresholds used to collapse the multilocus genotypes into 18 multilocus lineages.

FIGURE 2

273 (A-B) Minimum spanning networks of the hemagglutinin (HA) segment of H3N2 viral DNA from the
 274 *adegenet* package representing flu epidemics from 2001 to 2006 with (B) and without (A) reticulations
 275 (Jombart, 2008; Jombart et al., 2010). Each node represents a unique multilocus genotype, colors represent
 276 epidemic year, and edge color represents absolute genetic distance. (C) Shannon entropy values for
 277 population assignments compared with communities determined by the infoMAP algorithm on (A) and
 278 (B). (D) Graphic reproduced from Jombart et al. (2010) showing that the 2006 epidemic does not cluster
 279 neatly with the other years.

FIGURE 3

280 UPGMA dendrogram generated from Nei's genetic distance on 15 breeds of *Bos taurus* (BT) or *Bos indicus*
 281 (BI) from Africa (AF) or France (FR). These data are from Lalo et al. (2007). Node labels represent
 282 bootstrap support > 50% out of 1,000 bootstrap replicates.

FIGURE 4

283 Genotype accumulation curve for 694 isolates of the peach brown rot pathogen, *Monilinia fructicola*
 284 genotyped over 13 loci from Everhart and Scherm (2015). The horizontal axis represents the number
 285 of loci randomly sampled without replacement up to $n - 1$ loci, the vertical axis shows the number of
 286 multilocus genotypes observed, up to 262, the number of unique multilocus genotypes in the data set. The
 287 red dashed line represents 90% of the total observed multilocus genotypes. A trendline (blue) has been
 288 added using the *ggplot2* function *stat_smooth*.

FIGURE 5

289 (A) Sliding window analysis of the standardized index of association (\bar{r}_d) across a simulated 1.1×10^4 nt
 290 chromosome containing 1,100 variants among 100 individuals. Each window analyzed variants within
 291 500nt chunks. The black line indicates clonal population, the blue line indicates sexual. (B) boxplots
 292 showing 100 random samplings of 50 variants to calculate a distribution of \bar{r}_d for the clonal (black) and
 293 sexual (blue) population. Each box is centered around the mean, with whiskers extending out to 1.5 times
 294 the interquartile range. The median is indicated by the center line. (A) and (B) are plotted on the same
 295 y-axis.

TABLE 1

296 Contingency table comparing multilocus lineages assigned based on average neighbor clustering
 297 (columns) vs. multilocus lineages defined in Li et al. (2013) and Lees et al. (2006).

REFERENCES

- 298 Agapow, P.-M., and Burt, A. (2001). Indices of multilocus linkage disequilibrium. *Molecular Ecology Notes* 1, 101–102. doi:10.1046/j.1471-8278.2000.00014.x.
- 300 Anderson, J. B., and Kohn, L. M. (1995). Clonality in soilborne, plant-pathogenic fungi. *Annual review of phytopathology* 33, 369–391.
- 302 Arnaud-Hanod, S., Duarte, C. M., Alberto, F., and Serro, E. A. (2007). Standardizing methods to address
 303 clonality in population studies. *Molecular Ecology* 16, 5115–5139.

- 304 Brown, A., Feldman, M., and Nevo, E. (1980). MULTILOCUS sTRUCTURE oF nATURAL
305 pOPULATIONS oF *Hordeum spontaneum*. *Genetics* 96, 523–536. Available at: <http://www.genetics.org/content/96/2/523.abstract>.
- 307 Bruno, R., Michiels, N. K., D'Souza, T. G., and Schulenburg, H. (2004). A simple method for the
308 calculation of microsatellite genotype distances irrespective of ploidy level. *Molecular Ecology* 13, 2101–
309 2106.
- 310 Cooke, D. E., Cano, L. M., Raffaele, S., Bain, R. A., Cooke, L. R., Etherington, G. J., Deahl, K. L.,
311 Farrer, R. A., Gilroy, E. M., Goss, E. M., et al. (2012). Genome analyses of an aggressive and invasive
312 lineage of the irish potato famine pathogen. *PLoS pathogens* 8, e1002940.
- 313 Csardi, G., and Nepusz, T. (2006). The igraph software package for complex network research.
314 *InterJournal Complex Systems*, 1695. Available at: <http://igraph.org>.
- 315 Dagum, L., and Menon, R. (1998). OpenMP: An industry standard aPI for shared-memory
316 programming. *Computational Science & Engineering, IEEE* 5, 46–55.
- 317 Davey, J. W., and Blaxter, M. L. (2010). RADSeq: Next-generation population genetics. *Briefings in
318 Functional Genomics* 9, 416–423. doi:10.1093/bfgp/elq031.
- 319 Davey, J. W., Hohenlohe, P. A., Etter, P. D., Boone, J. Q., Catchen, J. M., and Blaxter, M. L.
320 (2011). Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nature Reviews Genetics* 12, 499–510.
- 322 Dobzhansky, T. (1973). Nothing in biology makes sense except in the light of evolution. *The American
323 Biology Teacher* 75, 87–91.
- 324 Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., and Mitchell, S. E.
325 (2011). A robust, simple genotyping-by-sequencing (gBS) approach for high diversity species. *Plos one*
326 6, e19379.
- 327 Everhart, S., and Scherm, H. (2015). Fine-scale genetic structure of *Monilinia fructicola* during brown
328 rot epidemics within individual peach tree canopies. *Phytopathology* 105, 542–549.
- 329 Excoffier, L., Smouse, P. E., and Quattro, J. M. (1992). Analysis of molecular variance inferred from
330 metric distances among dNA haplotypes: Application to human mitochondrial dNA restriction data.
331 *Genetics* 131, 479–491.
- 332 Falush, D., Stephens, M., and Pritchard, J. K. (2003). Inference of population structure using multilocus
333 genotype data: Linked loci and correlated allele frequencies. *Genetics* 164, 1567–1587. Available at:
334 <http://www.genetics.org/content/164/4/1567.abstract>.
- 335 Goss, E. M., Larsen, M., Chastagner, G. A., Givens, D. R., and Grnwald, N. J. (2009). Population
336 genetic analysis infers migration pathways of phytophthora ramorum in uS nurseries. *PLoS pathogens* 5,
337 e1000583.
- 338 Goss, E. M., Tabima, J. F., Cooke, D. E., Restrepo, S., Fry, W. E., Forbes, G. A., Fieland, V. J., Cardenas,
339 M., and Grnwald, N. J. (2014). The irish potato famine pathogen *Phytophthora infestans* originated in
340 central mexico rather than the andes. *Proceedings of the National Academy of Sciences* 111, 8791–8796.
- 341 Goudet, J. (2005). Hierfstat, a package for r to compute and test hierarchical f-statistics. *Molecular
342 Ecology Notes* 5, 184–186.
- 343 Grnwald, N. J., and Goss, E. M. (2011). Evolution and population genetics of exotic and re-emerging
344 pathogens: Novel tools and approaches. *Annual Review of Phytopathology* 49, 249–267.
- 345 Grnwald, N. J., and Hoheisel, G.-A. (2006). Hierarchical analysis of diversity, selfing, and genetic
346 differentiation in populations of the oomycete aphanomyces euteiches. *Phytopathology* 96, 1134–1141.

- 347 Grnwald, N. J., Goodwin, S. B., Milgroom, M. G., and Fry, W. E. (2003). Analysis of genotypic
348 diversity data for populations of microorganisms. *Phytopathology* 93, 738–46. Available at: <http://apsjournals.apsnet.org/doi/abs/10.1094/PHYTO.2003.93.6.738>.
- 350 Jombart, T. (2008). Adegenet: a R package for the multivariate analysis of genetic markers.
351 *Bioinformatics* 24, 1403–1405. doi:10.1093/bioinformatics/btn129.
- 352 Jombart, T., and Ahmed, I. (2011). Adegenet 1.3-1: New tools for the analysis of genome-wide sNP
353 data. *Bioinformatics* 27, 3070–3071.
- 354 Jombart, T., Devillard, S., and Balloux, F. (2010). Discriminant analysis of principal components: A
355 new method for the analysis of genetically structured populations. *BMC genetics* 11, 94.
- 356 Kamvar, Z. N., Larsen, M. M., Kanaskie, A. M., Hansen, E. M., and Grnwald, N. J. (2014a).
357 Sudden_Oak_Death_in_Oregon_Forests: Spatial and temporal population dynamics of the sudden oak
358 death epidemic in Oregon Forests. doi:10.5281/zenodo.13007.
- 359 Kamvar, Z. N., Larsen, M. M., Kanaskie, A., Hansen, E., and Grnwald, N. J. (2015). Spatial and
360 temporal analysis of populations of the sudden oak death pathogen in oregon forests. *Phytopathology*, in
361 press.
- 362 Kamvar, Z. N., Tabima, J. F., and Grnwald, N. J. (2014b). Poppr: An r package for genetic analysis of
363 populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* 2, e281.
- 364 Lalo, D., Jombart, T., Dufour, A.-B., and Moazami-Goudarzi, K. (2007). Consensus genetic structuring
365 and typological value of markers using multiple co-inertia analysis. *Genetics Selection Evolution* 39, 1–23.
- 366 Lees, A., Wattier, R., Shaw, D., Sullivan, L., Williams, N., and Cooke, D. (2006). Novel microsatellite
367 markers for the analysis of phytophthora infestans populations. *Plant Pathology* 55, 311–319.
- 368 Li, Y., Cooke, D. E., Jacobsen, E., and Lee, T. van der (2013). Efficient multiplex simple sequence repeat
369 genotyping of the oomycete plant pathogen phytophthora infestans. *Journal of microbiological methods*
370 92, 316–322.
- 371 Linde, C., Zhan, J., and McDonald, B. (2002). Population structure of mycosphaerella graminicola:
372 From lesions to continents. *Phytopathology* 92, 946–955.
- 373 Luikart, G., England, P. R., Tallmon, D., Jordan, S., and Taberlet, P. (2003). The power and promise of
374 population genomics: From genotyping to genome typing. *Nature Reviews Genetics* 4, 981–994.
- 375 Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer research*
376 27, 209–220.
- 377 Mastretta-Yanes, A., Arrigo, N., Alvarez, N., Jorgensen, T. H., Piero, D., and Emerson, B.
378 (2015). Restriction site-associated dNA sequencing, genotyping error estimation and de novo assembly
379 optimization for population genetic inference. *Molecular ecology resources* 15, 28–41.
- 380 McDonald, B. A., and Linde, C. (2002). The population genetics of plant pathogens and breeding
381 strategies for durable resistance. *Euphytica* 124, 163–180. doi:10.1023/A:1015678432355.
- 382 Meirmans, P. G., and Van Tienderen, P. H. (2004). GENOTYPE and gENODIVE: Two programs for the
383 analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes* 4, 792–794.
- 384 Michalakis, Y., and Excoffier, L. (1996). A generic estimation of population subdivision using distances
385 between alleles with special reference for microsatellite loci. *Genetics* 142, 1061–1064.
- 386 Milgroom, M. G. (1996). Recombination and the multilocus structure of fungal populations. *Annual review of phytopathology* 34, 457–477.
- 388 Milgroom, M. G., Levin, S. A., and Fry, W. E. (1989). Population genetics theory and fungicide
389 resistance. *Plant disease epidemiology* 2, 340–367.

- 390 Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National*
391 *Academy of Sciences* 70, 3321–3323.
- 392 Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L.,
393 Solymos, P., Stevens, M. H. H., and Wagner, H. (2015). *Vegan: Community ecology package*. Available
394 at: <http://CRAN.R-project.org/package=vegan>.
- 395 Paradis, E. (2010). Pegas: an R package for population genetics with an integrated–modular approach.
396 *Bioinformatics* 26, 419–420.
- 397 R Core Team (2015). *R: A language and environment for statistical computing*. Vienna, Austria: R
398 Foundation for Statistical Computing Available at: <http://www.R-project.org/>.
- 399 Rosvall, M., and Bergstrom, C. T. (2008). Maps of random walks on complex networks reveal
400 community structure. *Proceedings of the National Academy of Sciences* 105, 1118–1123.
- 401 Shannon, C. (2001). A mathematical theory of communication. *ACM SIGMOBILE Mobile Computing*
402 *and Communications Review* 5, 3–55. Available at: <http://cm.bell-labs.com/cm/ms/what/shannonday/shannon1948.pdf>.
- 403 Smith, J. M., Smith, N. H., O'Rourke, M., and Spratt, B. G. (1993). How clonal are bacteria?
404 *Proceedings of the National Academy of Sciences* 90, 4384–4388. doi:10.1073/pnas.90.10.4384.
- 405 Sokal, R. R. (1958). A statistical method for evaluating systematic relationships. *Univ Kans Sci Bull* 38,
406 1409–1438.
- 407 Taylor, J. W., and Fisher, M. C. (2003). Fungal multilocus sequence typing—it's not just for bacteria.
408 *Current opinion in microbiology* 6, 351–356.
- 409 Wickham, H., and Chang, W. (2015). *Devtools: Tools to make developing r packages easier*. Available
410 at: <http://CRAN.R-project.org/package=devtools>.