

# Novel R tools for analysis of genome-wide population genetic data with emphasis on clonality

Zhian N. Kamvar<sup>1</sup>, Jonah C. Brooks<sup>2</sup>, Niklaus J. Grünwald<sup>1,3\*</sup>

<sup>1</sup> Botany and Plant Pathology, Oregon State University, Corvallis, OR, USA

<sup>2</sup> College of Electrical Engineering and Computer Science, Oregon State University, Corvallis, OR, USA

<sup>3</sup> Horticultural Crops Research Laboratory, USDA-Agricultural Research Service, Corvallis, OR, USA

Correspondence\*:

Niklaus J. Grünwald

Horticultural Crops Research Laboratory USDA ARS

3420 NW Orchard Ave.

Corvallis, OR, 97330, [grunwaln@science.oregonstate.edu](mailto:grunwaln@science.oregonstate.edu)

## 2 ABSTRACT

3 To gain a detailed understanding of how plant microbes evolve and adapt to hosts, pesticides,  
4 and other factors, knowledge of the population dynamics and evolutionary history of populations  
5 is crucial. Plant pathogen populations are often clonal or partially clonal which requires different  
6 analytical tools. With the advent of high throughput sequencing technologies, obtaining genome-  
7 wide population genetic data has become easier than ever before. We previously contributed  
8 the R package *poppr* specifically addressing issues with analysis of clonal populations. In  
9 this paper we provide several significant extensions to *poppr* with a focus on large, genome-  
10 wide SNP data. Specifically, we provide several new functionalities including the new function  
11 `mlg.filter` to define clone boundaries allowing for inspection and definition of what is a clonal  
12 lineage, minimum spanning networks with reticulation, a sliding-window analysis of the index of  
13 association, modular bootstrapping of any genetic distance, and analyses across any level of  
14 hierarchies.

15 **Keywords:** clonality, population genomics, bootstrap, index of association, hierarchical analysis, sliding window

## INTRODUCTION

16 To paraphrase Dobzhansky, nothing in the field of plant-microbe interactions makes sense except in  
17 the light of population genetics (Dobzhansky, 1973). Genetic forces such as selection and drift act on  
18 alleles in a population. Thus, a true understanding of how plant pathogens emerge, evolve and adapt to  
19 crops, fungicides, or other factors, can only be elucidated in the context of population level phenomena  
20 given the demographic history of populations (McDonald and Linde, 2002; Grünwald and Goss, 2011;  
21 Milgroom et al., 1989). The field of population genetics, in the era of whole genome resequencing,  
22 provides unprecedented power to describe the evolutionary history and population processes that drive  
23 coevolution between pathogens and hosts. This powerful field thus critically enables effective deployment  
24 of R genes, design of pathogen informed plant resistance breeding programs, and implementation of  
25 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 classical population genetic tools because theoretical assumptions underlying the theory are violated. Yet, many plant pathogen populations are at least partially clonal if not completely clonal (Milgroom, 1996; Anderson and Kohn, 1995). Thus, development of tools for analysis of clonal or polyploid populations is needed.

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

The research community using the R statistical and computing language (R Core Team, 2015) has developed a plethora of new resources for population genetic analysis. R is particularly appealing because all code is open source and functions can be evaluated and modified by any user. Recently, we introduced the R package *poppr* specifically developed for analysis of clonal populations (Kamvar et al., 2014b). *Poppr* previously introduced several novel features including the ability to conduct a hierarchical analysis across unlimited hierarchies, test for linkage association, graph minimum spanning networks or provide bootstrap support for Bruvo's distance in resulting trees. *Poppr* has been rapidly adopted and applied to a range of studies including for example horizontal transmission in leukemia of clams (Metzger et al., 2015), study of the vector-mediated parent-to-offspring transmission in an avian malaria-like parasite (Chakarov et al., 2015), and characterization of the emergence of the invasive forest pathogen *Hymenoscyphus pseudoalbidus* (Gross et al., 2014). It has also been used to implement real-time, online R based tools for visualizing relationships among unknown MLGs in reference databases (<http://phytophthora-id.org/>) (Grünwald et al., 2011).

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

## IMPLEMENTATIONS AND EXAMPLES

### CLONAL IDENTIFICATION

As highlighted in previous work, clone correction is an important component of population genetic analysis of organisms that are known to reproduce asexually (Kamvar et al., 2014b; Milgroom, 1996; Grünwald et al., 2003). This method is a partial correction for bias that affects metrics that rely on allele frequencies assuming panmixia and was initially designed for data with only a handful of markers. With the advent of large-scale sequencing and reduced- representation libraries, it has become easier to sequence tens of thousands of markers from hundreds of individuals (Elshire et al., 2011; Davey et al., 2011; Davey and Blaxter, 2010). With this larger number of markers, the genetic resolution is much greater, but the chance of genotyping error is also greatly increased and missing data is frequent (Mastretta-Yanes et al., 2015). Taking this fact and occasional somatic mutations into account, it would be impossible to separate true clones from independent individuals by just comparing what MLGs are different. We introduce a new method for collapsing unique multilocus genotypes determined by naive

string comparison into multilocus lineages utilizing any genetic distance given three different clustering algorithms: farthest neighbor, nearest neighbor, and UPGMA ([Unweighted Pair Group Method with Arithmetic Mean, aka](#) average neighbor) (Sokal, 1958).

These 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 genetic data such as `bruvo.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 (Fig. 1A), 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 (Fig. 1C). The UPGMA, or average neighbor clustering algorithm is the one most familiar to biologists as it is often used to generate ultra-metric trees based on genetic distance (Fig. 1B). 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 that spread to Europe in the mid 19th century (Goss et al., 2014; Yoshida et al., 2013). *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; Li et al., 2013). 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 dendrograms 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. 2). 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 to evaluate the effect of sequencing error and missing data on MLG calling. We constructed the data 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 equivalent to Provesti's distance, counting missing data as equivalent in comparison (Prevosti et al., 1975).

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.

117 Out of the 100 simulations run, we found that across all methods, detection of duplicated samples had  
 118  $\sim 98\%$  true positive fraction and  $\sim 0.8\%$  false positive fraction indicating that this method is robust to  
 119 simulated populations (supplementary materials<sup>1</sup>).

## MINIMUM SPANNING NETWORKS WITH RETICULATION

120 In its original iteration, *poppr* introduced minimum spanning networks that were based on the *igraph*  
 121 function `minimum.spanning.tree` (Csardi and Nepusz, 2006). This algorithm produces a minimum  
 122 spanning tree with no reticulations where nodes represent individual MLGs. In other minimum spanning  
 123 network programs, reticulation is obtained by calculating the minimum spanning tree several times and  
 124 returning the set of all edges included in the trees. Due to the way *igraph* has implemented Prim's  
 125 algorithm, it is not possible to utilize this strategy, thus we implemented an internal C function to walk  
 126 the space of minimum spanning trees based on genetic distance to connect groups of nodes with edges of  
 127 equal weight.

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

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

143 The infoMAP algorithm revealed 63 communities with a maximum community size of 77 and  $H = 3.56$   
 144 for the reticulate network of the H3N2 data and 117 communities with a maximum community size of  
 145 26 and  $H = 4.65$  for the minimum spanning tree. The entropy across years was greatly decreased for  
 146 all populations with the reticulate network compared to the minimum spanning tree (Fig. 3). Note that  
 147 the reticulated network (Fig. 3B) showed patterns corresponding with those resulting from a discriminant  
 148 analysis of principal components (Fig. 3D) (Jombart et al., 2010).

149 Graph walking of the reticulated minimum spanning network of *P. ramorum* by the infoMAP algorithm  
 150 revealed 16 communities with a maximum community size of 13 and  $H = 2.60$ . The un-reticulated  
 151 minimum spanning tree revealed 20 communities with a maximum community size of 7 and  $H = 2.96$ .  
 152 In the ability to predict Hunter Creek as belonging to a single community, the reticulated network was  
 153 successful whereas the minimum spanning tree separated one genotype from that community. The entropy  
 154 for the reticulated network was lower for all populations except for the coast population (supplementary  
 155 materials<sup>2</sup>).

## BOOTSTRAPPING

156 Assessing population differentiation through methods such as  $G_{st}$ , AMOVA, and Mantel tests relies on  
 157 comparing samples within and across populations (Nei, 1973; Excoffier et al., 1992; Mantel, 1967).

<sup>1</sup> Supplementary data available at <https://github.com/grunwaldlab/supplementary-poppr-2.0>; DOI: 10.5281/zenodo.17424

<sup>2</sup> Supplementary data available at <https://github.com/grunwaldlab/supplementary-poppr-2.0>; DOI: 10.5281/zenodo.17424

Confidence in distance metrics is related to the confidence in the markers to accurately represent the diversity of the data. Especially true with microsatellite markers, a single hyper-diverse locus can make a population appear to have more diversity based on genetic distance. Using a bootstrapping procedure of randomly sampling loci with replacement when calculating a distance matrix provides support for clades in hierarchical clustering.

Data in *genind* and *genpop* objects are represented as matrices with individuals in rows and alleles in columns (Jombart, 2008). This gives the advantage of being able to use R's matrix algebra capabilities to efficiently calculate genetic distance. Unfortunately, this also means that bootstrapping is a non-trivial task as all alleles at a single locus need to be sampled together. To remedy this, we have created an internal S4 class called "bootgen", which extends the internal "gen" class from *adegenet*. This class can be created from any *genind*, *genclone*, or *genpop* object, and allows loci to be sampled with replacement. To further facilitate bootstrapping, a function called *aboot*, which stands for "any boot", is introduced that will bootstrap any *genclone*, *genind*, or *genpop* object with any genetic distance that can be calculated from it.

To demonstrate calculating a dendrogram with bootstrap support, we used the *poppr* function *aboot* on population allelic frequencies derived from the data set *microbov* in the *adegenet* package with 1000 bootstrap replicates (Jombart, 2008; Laloë et al., 2007). The resulting dendrogram shows bootstrap support values > 50% (Fig. 4) and used the following code:

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

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

## GENOTYPE ACCUMULATION CURVE

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

The following code example demonstrates the genotype accumulation curve for data from Everhart and Scherm (2015) showing that these data reach a small plateau and have a greatly decreased variance with 12 markers, indicating that there are enough markers such that adding more markers to the analysis will not create very many new genotypes (Fig. 5).

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

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



## INDEX OF ASSOCIATION

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

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

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

To demonstrate the sliding window and random sampling of  $\bar{r}_d$  with respect to clonal populations, we simulated two populations containing 1,100 neutral SNPs for 100 diploid individuals under the same initial seed. One population had individuals randomly sampled with replacement, representing the clonal population. After sampling, both populations had 5% random error and 1% missing data independently propagated across all samples. On average, we obtained a higher value of  $\bar{r}_d$  for the clonal population compared to the sexual population for both methods (Fig. 6).

## DATA FORMAT UPDATES: POPULATION STRATA AND HIERARCHIES

Assessments of population structure through methods such as hierarchical  $F_{st}$  (Goudet, 2005) and AMOVA (Michalakis and Excoffier, 1996) require hierarchical sampling of populations across space or time (Linde et al., 2002; Everhart and Scherm, 2015; Grünwald and Hoheisel, 2006). With clonal organisms, basic practice has been to clone-censor data to avoid downward bias in diversity due to duplicated genotypes that may or may not represent different samples (Milgroom, 1996). This correction should be performed with respect to a population hierarchy to accurately reflect the biology of the organism. Traditional data structures for population genetic data in most analysis tools allow for only one level of hierarchical definition. The investigator thus had to provide the data set for analysis at each hierarchical level.

To facilitate handling hierarchical and multilocus genotypic metadata, *poppr* version 1.1 introduced a new S4 data object called "genclone", extending *adegenet*'s "genind" object (Kamvar and Grünwald, unpublished). The genclone object formalized the definitions of multilocus genotypes and population hierarchies by adding two slots called "mlg" and "hierarchy" that carried a numeric vector and a data frame, respectively. These new slots allow for increased efficiency and ease of use by allowing these metadata to travel with the genetic data. The hierarchy slot in particular contains a data frame where each column represents a separate hierarchical level. This is then used to set the population factor of the data by

supplying a hierarchical formula containing one or more column names of the data frame in the hierarchy slot.

The functionality represented by the hierarchy slot has now been migrated from the *poppr* to the *adegenet* package version 2.0 to allow hierarchical analysis in *adegenet*, *poppr*, and other dependent packages. The prior *poppr* hierarchy slot and methods have now been renamed *strata* in *adegenet*. A short example of the utility of these methods can be seen in the code segment under **Bootstrapping**, above. This migration provides end users with a broader ability to analyze data hierarchically in R across packages.

## AVAILABILITY

As of this writing, the *poppr* R package version 2.0 containing all of the features described here is located at <https://github.com/grunwaldlab/poppr/tree/2.0-rc>. It is necessary to install *adegenet* 2.0 before installing *poppr*. It can be found at <https://github.com/thibautjombart/adegenet>. Both of these can be installed via the R package *devtools* (Wickham and Chang, 2015). More information and example code can be found in the supplementary materials<sup>3</sup>.

## REQUIREMENTS

- R version 3.0 or better
- A C compiler. For windows, it can be obtained via Rtools (<http://cran.r-project.org/bin/windows/Rtools/>). On OSX, it can be obtained via Xcode. For parallel support, gcc version 4.6 or better is needed.

## INSTALLATION

From within R, *poppr* can be installed via:

```
install.packages("devtools")
library("devtools")
install_github("thibautjombart/adegenet")
install_github("grunwaldlab/poppr@2.0-rc")
```

Several population genetics packages in R are currently going through a major upgrade following the 2015 R hackathon on population genetics (<https://github.com/NESCent/r-popgen-hackathon>) and have not yet been updated in CRAN. We will upload *poppr* 2.0 to CRAN once all other reverse dependent packages have been updated.

## DISCUSSION

Given low cost and high throughput of current sequencing technologies we are entering a new era of population genetics where large SNP data sets with thousands of markers are becoming available for large populations in a genome-wide context. This data provides new possibilities and challenges for population genetic analyses. We provide novel tools that enable analysis of this data in R with a particular emphasis on clonal organisms.

Particularly useful is the implementation of  $\bar{r}_d$  in a genomic context (Agapow and Burt, 2001). Random sampling of loci across the genome can give an expected distribution of  $\bar{r}_d$ , which is expected to have

<sup>3</sup> Supplementary data available at <https://github.com/grunwaldlab/supplementary-poppr-2.0>; DOI: 10.5281/zenodo.17424

a mean of zero for panmictic populations. This metric is not affected by the number of loci sampled, is model free, and has the ability to detect population structure.  $\bar{r}_d$  is also implemented for sliding window analyses that are useful to detect candidate regions of linkage disequilibrium for further analysis.

Clustering multilocus genotypes into multilocus lineages based on genetic distances is a non-trivial task given large SNP data sets. Moreover, this has not previously been implemented for genomic data for clonal populations. Clonal assignment has previously been available in the programs GENCLONE and GENODIVE for classical markers (Arnaud-Hanod et al., 2007; Meirmans and Van Tienderen, 2004). Our method with `mlg.filter` builds upon this idea and allows the user to choose between three different approaches for clustering MLGs. The choice of clustering algorithm has an impact on the data (Fig 1, 2), where for example a genetic distance cutoff of 0.1 would be the difference between 14 multilocus lineages (MLLs) and 17 MLLs for nearest neighbor and UPGMA clustering, respectively (Fig. 2). The option to choose the clustering algorithm gives the user the ability to choose what is biologically relevant to their populations. While there is not one optimal procedure for defining boundaries in clonal lineages, our tool provides a means of exploring the potential MLG or MLL boundary space.

Minimum spanning networks are a useful tool to analyze the relationships between individuals in a population, because it reduces the complexity of a distance matrix to the connections that are strongest. By default, these networks are drawn without reticulations, but for clonal organisms where many of the connections between samples are equivalent, the minimum spanning network appears as a chain and reduces the information that can be communicated. This is problematic because the ability to detect population structure with one instance of a minimum spanning network is limited. Adding reticulation into the minimum spanning network thus presents all equivalent connections and allows population structure to be more readily detectable. As shown in Fig. 3, population structure is apparent both visually and by graph community detection algorithms such as the infoMAP algorithm (Rosvall and Bergstrom, 2008). Additionally, the current implementation in *poppr* has been successfully used in analyses such as reconstruction of the *P. ramorum* epidemic in Oregon forests (Kamvar et al., 2014a, 2015).

*Poppr* 2.0 is open source and available on GitHub. Members of the community are invited to contribute by raising issues or pull requests on our repository at <https://github.com/grunwaldlab/poppr/issues>.

## ACKNOWLEDGEMENTS

We thank Ignazio Carbone for discussions on the index of association; David Cooke, Sanmohan Baby, and Jens Hansen for beta testing; and Thibaut Jombart for allowing us to incorporate the `strata` slot and related methods in *adegenet*. We also thank all the members of the 2015 R hackathon on population genetics in Durham, NC for their advice and input (<https://github.com/NESCent/r-popgen-hackathon>). This work was supported in part by US Department of Agriculture (USDA) Agricultural Research Service Grant 5358-22000-039-00D, USDA National Institute of Food and Agriculture Grant 2011-68004-30154, USDA APHIS, the USDA- ARS Floriculture Nursery Initiative, and the USDA-Forest Service Forest Health Monitoring Program (to NJG).

## CONFLICT OF INTEREST STATEMENT

The authors declare no observable conflict of interest.

## AUTHOR CONTRIBUTIONS

ZNK and JCB wrote and tested the code. ZNK maintains the code. ZNK and NJG conceived, discussed implications, and wrote the manuscript. NJG coordinated the collaborative effort.



## REFERENCES

- 300 Agapow, P.-M., and Burt, A. (2001). Indices of multilocus linkage disequilibrium. *Molecular Ecology*  
 301 *Notes* 1, 101–102. doi:10.1046/j.1471-8278.2000.00014.x.
- 302 Anderson, J. B., and Kohn, L. M. (1995). Clonality in soilborne, plant-pathogenic fungi. *Annual review*  
 303 *of phytopathology* 33, 369–391.
- 304 Arnaud-Hanod, S., Duarte, C. M., Alberto, F., and Serrão, E. A. (2007). Standardizing methods to  
 305 address clonality in population studies. *Molecular Ecology* 16, 5115–5139.
- 306 Brown, A., Feldman, M., and Nevo, E. (1980). Multilocus structure of natural populations of *Hordeum*  
 307 *spontaneum*. *Genetics* 96, 523–536. Available at: [http://www.genetics.org/content/96/2/](http://www.genetics.org/content/96/2/523.abstract)  
 308 523.abstract.
- 309 Bruvo, R., Michiels, N. K., D'Souza, T. G., and Schulenburg, H. (2004). A simple method for the  
 310 calculation of microsatellite genotype distances irrespective of ploidy level. *Molecular Ecology* 13, 2101–  
 311 2106.
- 312 Chakarov, N., Linke, B., Boerner, M., Goesmann, A., Krüger, O., and Hoffman, J. I. (2015). Apparent  
 313 vector-mediated parent-to-offspring transmission in an avian malaria-like parasite. *Molecular ecology* 24,  
 314 1355–1363.
- 315 Cooke, D. E., Cano, L. M., Raffaele, S., Bain, R. A., Cooke, L. R., Etherington, G. J., Deahl, K. L.,  
 316 Farrer, R. A., Gilroy, E. M., Goss, E. M., et al. (2012). Genome analyses of an aggressive and invasive  
 317 lineage of the Irish potato famine pathogen. *PLoS pathogens* 8, e1002940.
- 318 Csardi, G., and Nepusz, T. (2006). The igraph software package for complex network research.  
 319 *InterJournal Complex Systems*, 1695. Available at: <http://igraph.org>.
- 320 Dagum, L., and Menon, R. (1998). OpenMP: An industry standard API for shared-memory  
 321 programming. *Computational Science & Engineering, IEEE* 5, 46–55.
- 322 Davey, J. W., and Blaxter, M. L. (2010). RADSeq: Next-generation population genetics. *Briefings in*  
 323 *Functional Genomics* 9, 416–423. doi:10.1093/bfpg/eq031.
- 324 Davey, J. W., Hohenlohe, P. A., Etter, P. D., Boone, J. Q., Catchen, J. M., and Blaxter, M. L.  
 325 (2011). Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nature*  
 326 *Reviews Genetics* 12, 499–510.
- 327 Dobzhansky, T. (1973). Nothing in biology makes sense except in the light of evolution. *The American*  
 328 *Biology Teacher* 75, 87–91.
- 329 Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., and Mitchell, S. E.  
 330 (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PloS one*  
 331 6, e19379.
- 332 Everhart, S., and Scherm, H. (2015). Fine-scale genetic structure of *Monilinia fructicola* during brown  
 333 rot epidemics within individual peach tree canopies. *Phytopathology* 105, 542–549.
- 334 Excoffier, L., Smouse, P. E., and Quattro, J. M. (1992). Analysis of molecular variance inferred from  
 335 metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data.  
 336 *Genetics* 131, 479–491.
- 337 Falush, D., Stephens, M., and Pritchard, J. K. (2003). Inference of population structure using multilocus  
 338 genotype data: Linked loci and correlated allele frequencies. *Genetics* 164, 1567–1587. Available at:  
 339 <http://www.genetics.org/content/164/4/1567.abstract>.
- 340 Goss, E. M., Larsen, M., Chastagner, G. A., Givens, D. R., and Grünwald, N. J. (2009). Population  
 341 genetic analysis infers migration pathways of *Phytophthora ramorum* in US nurseries. *PLoS pathogens* 5,  
 342 e1000583.

- Goss, E. M., Tabima, J. F., Cooke, D. E., Restrepo, S., Fry, W. E., Forbes, G. A., Fieland, V. J., Cardenas, M., and Grünwald, N. J. (2014). The Irish potato famine pathogen *Phytophthora infestans* originated in central Mexico rather than the Andes. *Proceedings of the National Academy of Sciences* 111, 8791–8796.
- Goudet, J. (2005). Hierfstat, a package for R to compute and test hierarchical F-statistics. *Molecular Ecology Notes* 5, 184–186.
- Gross, A., Hosoya, T., and Quelo, V. (2014). Population structure of the invasive forest pathogen *Hymenoscyphus pseudoalbidus*. *Molecular ecology* 23, 2943–2960.
- Grünwald, N. J., and Goss, E. M. (2011). Evolution and population genetics of exotic and re-emerging pathogens: Novel tools and approaches. *Annual Review of Phytopathology* 49, 249–267.
- Grünwald, N. J., and Hoheisel, G.-A. (2006). Hierarchical analysis of diversity, selfing, and genetic differentiation in populations of the oomycete *Aphanomyces euteiches*. *Phytopathology* 96, 1134–1141.
- Grünwald, N. J., Goodwin, S. B., Milgroom, M. G., and Fry, W. E. (2003). Analysis of genotypic 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>.
- Grünwald, N. J., Martin, F. N., Larsen, M. M., Sullivan, C. M., Press, C. M., Coffey, M. D., Hansen, E. M., and Parke, J. L. (2011). Phytophthora-ID. org: a sequence-based *Phytophthora* identification tool. *Plant Disease* 95, 337–342.
- Jombart, T. (2008). Adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* 24, 1403–1405. doi:10.1093/bioinformatics/btn129.
- Jombart, T., and Ahmed, I. (2011). Adegenet 1.3-1: New tools for the analysis of genome-wide SNP data. *Bioinformatics* 27, 3070–3071.
- Jombart, T., Devillard, S., and Balloux, F. (2010). Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC genetics* 11, 94.
- Kamvar, Z. N., Larsen, M. M., Kanaskie, A. M., Hansen, E. M., and Grünwald, N. J. (2015). Spatial and temporal analysis of populations of the sudden oak death pathogen in Oregon forests. *Phytopathology*, in press.
- Kamvar, Z. N., Larsen, M. M., Kanaskie, A. M., Hansen, E. M., and Grünwald, N. J. (2014a). Sudden\_Oak\_Death\_in\_Oregon\_Forests: Spatial and temporal population dynamics of the sudden oak death epidemic in Oregon Forests. doi:10.5281/zenodo.13007.
- Kamvar, Z. N., Tabima, J. F., and Grünwald, N. J. (2014b). Poppr: An R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* 2, e281.
- Laloë, D., Jombart, T., Dufour, A.-B., and Moazami-Goudarzi, K. (2007). Consensus genetic structuring and typological value of markers using multiple co-inertia analysis. *Genetics Selection Evolution* 39, 1–23.
- Lees, A., Wattier, R., Shaw, D., Sullivan, L., Williams, N., and Cooke, D. (2006). Novel microsatellite markers for the analysis of *Phytophthora infestans* populations. *Plant Pathology* 55, 311–319.
- Li, Y., Cooke, D. E., Jacobsen, E., and Lee, T. van der (2013). Efficient multiplex simple sequence repeat genotyping of the oomycete plant pathogen *Phytophthora infestans*. *Journal of microbiological methods* 92, 316–322.
- Linde, C., Zhan, J., and McDonald, B. (2002). Population structure of *Mycosphaerella graminicola*: From lesions to continents. *Phytopathology* 92, 946–955.
- Luikart, G., England, P. R., Tallmon, D., Jordan, S., and Taberlet, P. (2003). The power and promise of population genomics: From genotyping to genome typing. *Nature Reviews Genetics* 4, 981–994.
- Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer research* 27, 209–220.

- 387 Mastretta-Yanes, A., Arrigo, N., Alvarez, N., Jorgensen, T. H., Piñero, D., and Emerson, B.  
 388 (2015). Restriction site-associated DNA sequencing, genotyping error estimation and de novo assembly  
 389 optimization for population genetic inference. *Molecular ecology resources* 15, 28–41.
- 390 McDonald, B. A., and Linde, C. (2002). The population genetics of plant pathogens and breeding  
 391 strategies for durable resistance. *Euphytica* 124, 163–180. doi:10.1023/A:1015678432355.
- 392 Meirmans, P. G., and Van Tienderen, P. H. (2004). GENOTYPE and GENODIVE: Two programs for  
 393 the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes* 4, 792–794.
- 394 Metzger, M. J., Reinisch, C., Sherry, J., and Goff, S. P. (2015). Horizontal transmission of clonal cancer  
 395 cells causes leukemia in soft-shell clams. *Cell* 161, 255–263.
- 396 Michalakis, Y., and Excoffier, L. (1996). A generic estimation of population subdivision using distances  
 397 between alleles with special reference for microsatellite loci. *Genetics* 142, 1061–1064.
- 398 Milgroom, M. G. (1996). Recombination and the multilocus structure of fungal populations. *Annual*  
 399 *review of phytopathology* 34, 457–477.
- 400 Milgroom, M. G., Levin, S. A., and Fry, W. E. (1989). Population genetics theory and fungicide  
 401 resistance. *Plant disease epidemiology* 2, 340–367.
- 402 Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National*  
 403 *Academy of Sciences* 70, 3321–3323.
- 404 Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L.,  
 405 Solymos, P., Stevens, M. H. H., and Wagner, H. (2015). *Vegan: Community ecology package*. Available  
 406 at: <http://CRAN.R-project.org/package=vegan>.
- 407 Prevosti, A., Ocaña, J., and Alonso, G. (1975). Distances between populations of *Drosophila*  
 408 *subobscura*, based on chromosome arrangement frequencies. *Theoretical and Applied Genetics* 45,  
 409 231–241.
- 410 R Core Team (2015). *R: A language and environment for statistical computing*. Vienna, Austria: R  
 411 Foundation for Statistical Computing Available at: <http://www.R-project.org/>.
- 412 Rosvall, M., and Bergstrom, C. T. (2008). Maps of random walks on complex networks reveal  
 413 community structure. *Proceedings of the National Academy of Sciences* 105, 1118–1123.
- 414 Shannon, C. (2001). A mathematical theory of communication. *ACM SIGMOBILE Mobile Computing*  
 415 *and Communications Review* 5, 3–55. Available at: <http://cm.bell-labs.com/cm/ms/what/shannonday/shannon1948.pdf>.
- 417 Smith, J. M., Smith, N. H., O'Rourke, M., and Spratt, B. G. (1993). How clonal are bacteria?  
 418 *Proceedings of the National Academy of Sciences* 90, 4384–4388. doi:10.1073/pnas.90.10.4384.
- 419 Sokal, R. R. (1958). A statistical method for evaluating systematic relationships. *Univ Kans Sci Bull* 38,  
 420 1409–1438.
- 421 Taylor, J. W., and Fisher, M. C. (2003). Fungal multilocus sequence typing — it's not just for bacteria.  
 422 *Current opinion in microbiology* 6, 351–356.
- 423 Wickham, H., and Chang, W. (2015). *Devtools: Tools to make developing R packages easier*. Available  
 424 at: <http://CRAN.R-project.org/package=devtools>.
- 425 Yoshida, K., Schuenemann, V. J., Cano, L. M., Pais, M., Mishra, B., Sharma, R., Lanz, C., Martin,  
 426 F. N., Kamoun, S., Krause, J., et al. (2013). The rise and fall of the *Phytophthora infestans* lineage that  
 427 triggered the Irish potato famine. *Elife* 2, e00731.

## FIGURES AND TABLES

FIGURE 1

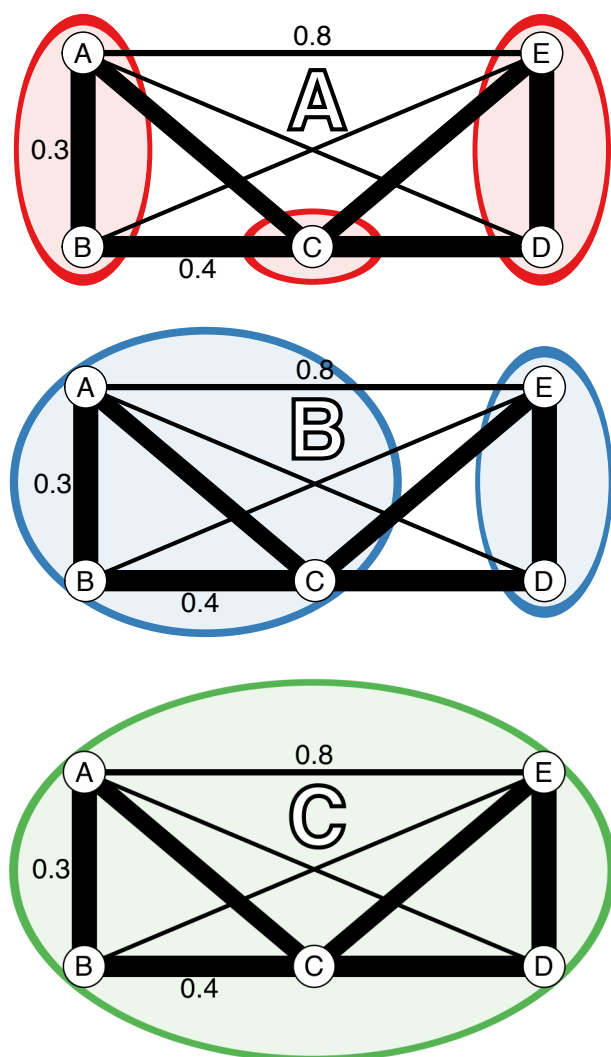


FIGURE 2

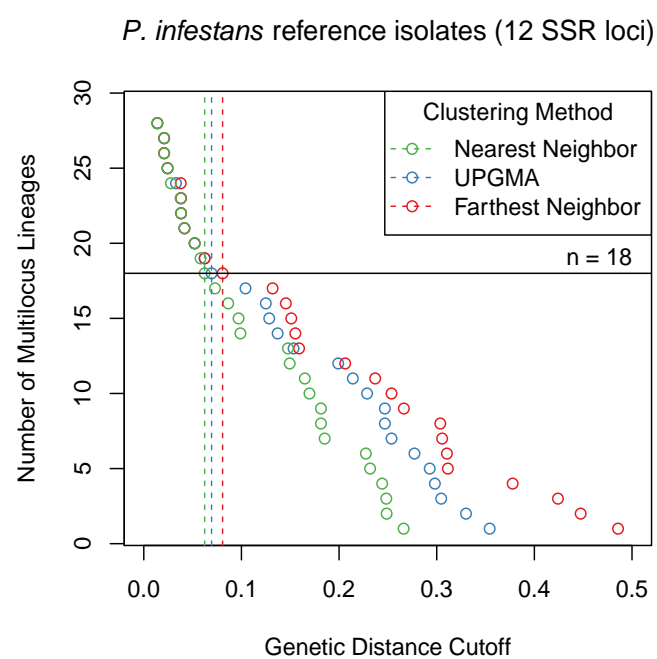
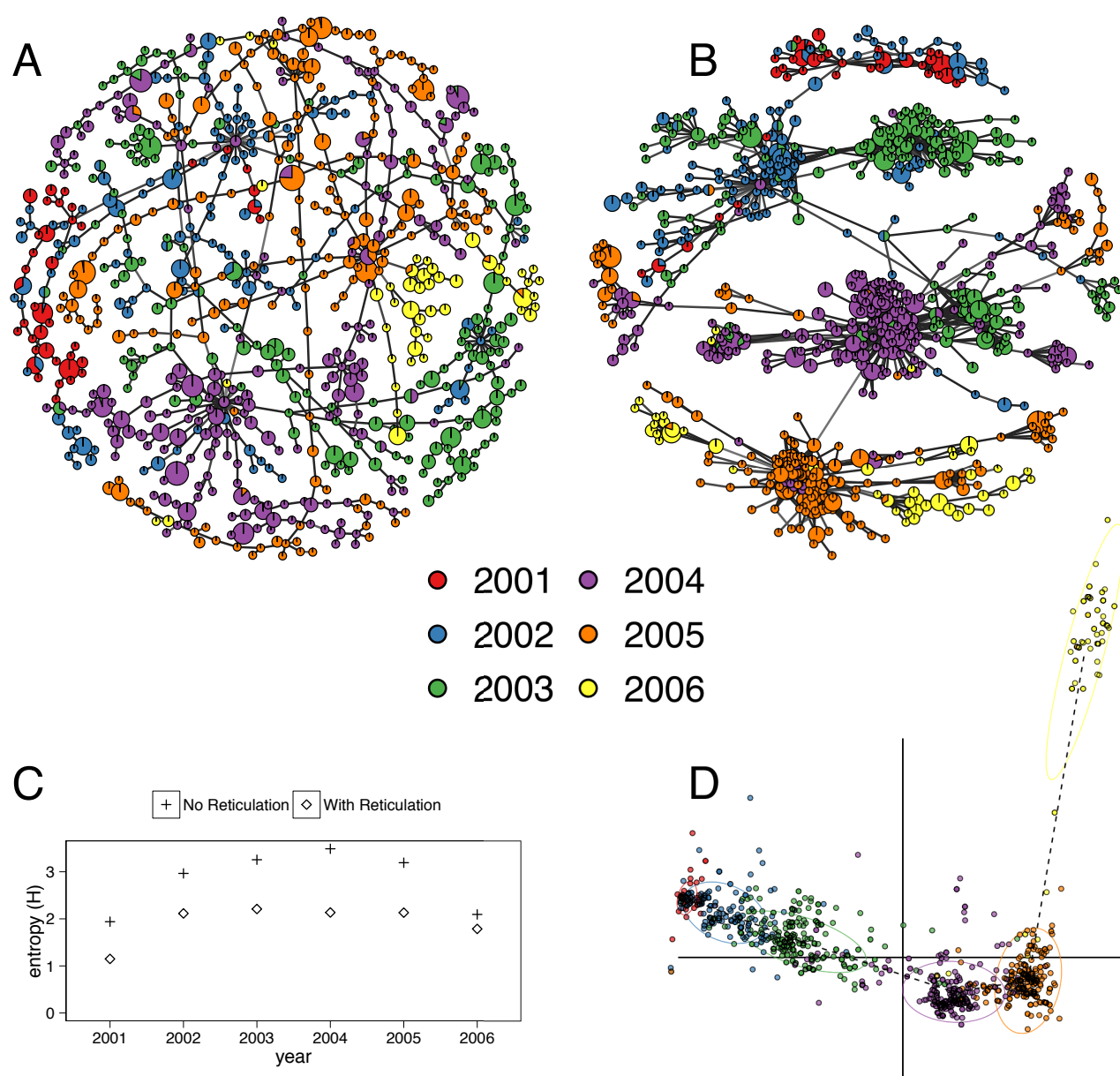




FIGURE 3



430

FIGURE 4

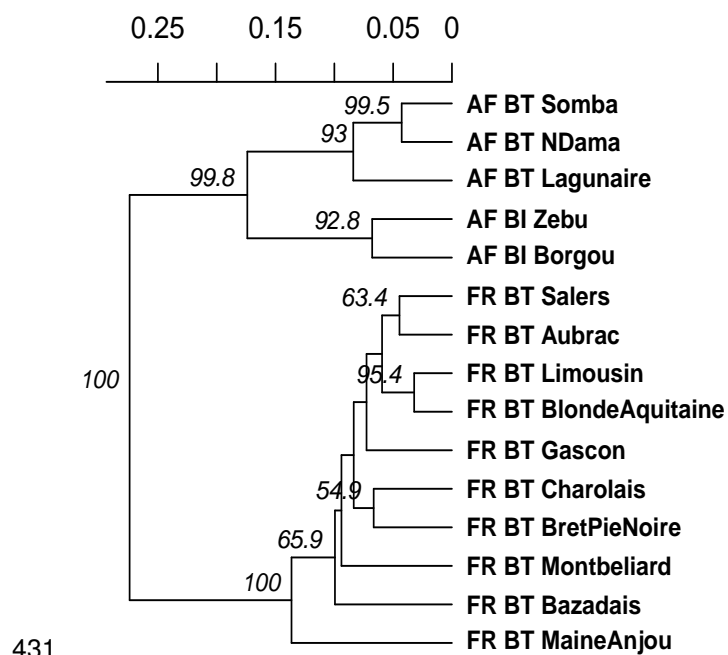


FIGURE 5

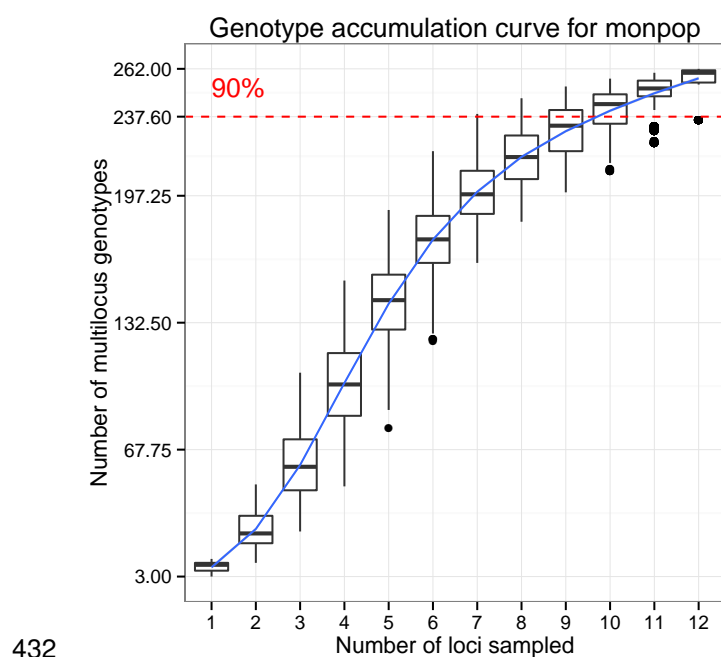
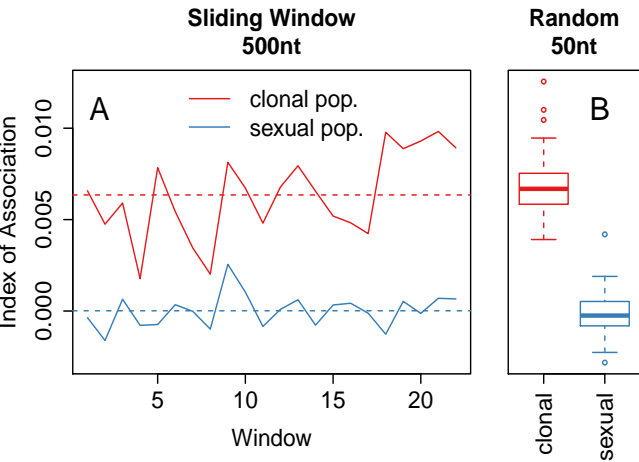


FIGURE 6



**Table 1** Contingency table comparing multilocus lineages assigned based on average neighbor clustering (columns) vs. multilocus lineages (MLL) defined in Li et al. (2013) and Lees et al. (2006) (rows) to MLLs inferred from Bruvo’s genetic distance (columns) at a threshold of 0.07 with the average neighbor algorithm (Bruvo et al., 2004; Sokal, 1958). Values in the table represent the number of times any given inferred MLL matches with a previously defined MLL. For example, in our original data set, there were three genotypes previously defined as the US-24 MLL. All three genotypes were also determined to cluster into a single MLL by filtering. In contrast, US-8 was determined to cluster into three different MLLs by filtering.

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

FIGURE LEGENDS

**Figure 1** Diagrammatic representation of the three clustering algorithms implemented in `mlg.filter`. (A-C) Represent different clustering algorithms on the same imaginary network with a threshold of 0.451.

444 Edge weights are represented in arbitrary units noted by the line thickness and numerical values next to  
 445 the lines. All outer angles are 90 degrees, so the un-labeled edge weights can be obtained with simply  
 446 geometry. Colored circles represent clusters of genotypes. (A) Farthest neighbor clustering does not cluster  
 447 nodes B and C because nodes A and C are more than a distance of 0.451 apart. (B) UPGMA (average  
 448 neighbor) clustering clusters nodes A, B, and C together because the average distance between them and  
 449 C is  $< 0.451$ . (C) Nearest neighbor clustering clusters all nodes together because the minimum distance  
 450 between them is always  $< 0.451$ .

451 **Figure 2** Graphical representation of three different clustering algorithms collapsing multilocus  
 452 genotypes for 12 SSR loci from *Phytophthora infestans* representing 18 clonal lineages. The horizontal  
 453 axis is Bruvo's genetic distance assuming the genome addition model. The vertical axis represents the  
 454 number of multilocus lineages observed. Each point shows the threshold at which one would observe a  
 455 given number of multilocus genotypes. The horizontal black line represents 18 multilocus genotypes and  
 456 vertical dashed lines mark the thresholds used to collapse the multilocus genotypes into 18 multilocus  
 457 lineages.

458 **Figure 3 (A-B)** Minimum spanning networks of the hemagglutinin (HA) segment of H3N2 viral DNA  
 459 from the *adeget* package representing flu epidemics from 2001 to 2006 without reticulation (A) and with  
 460 reticulation (B) (Jombart, 2008; Jombart et al., 2010). Each node represents a unique multilocus genotype,  
 461 colors represent epidemic year, and edge color represents absolute genetic distance. (C) Shannon entropy  
 462 values for population assignments compared with communities determined by the infoMAP algorithm on  
 463 (A) and (B). (D) Graphic reproduced from Jombart et al. (2010) showing that the 2006 epidemic does not  
 464 cluster neatly with the other years [via Discriminant Analysis of Principal Components. Horizontal axis](#)  
 465 [represents the first discriminant component. Vertical axis represents the second discriminant component.](#)

466 **Figure 4** UPGMA dendrogram generated from Nei's genetic distance on 15 breeds of *Bos taurus* (BT)  
 467 or *Bos indicus* (BI) from Africa (AF) or France (FR). These data are from Laloë et al. (2007). Node labels  
 468 represent bootstrap support  $> 50\%$  out of 1,000 bootstrap replicates.

469 **Figure 5** Genotype accumulation curve for 694 isolates of the peach brown rot pathogen, *Monilinia*  
 470 *fructicola* genotyped over 13 loci from Everhart and Scherm (2015). The horizontal axis represents the  
 471 number of loci randomly sampled without replacement up to  $n - 1$  loci, the vertical axis shows the number  
 472 of multilocus genotypes observed, up to 262, the number of unique multilocus genotypes in the data set.  
 473 The red dashed line represents 90% of the total observed multilocus genotypes. A trendline (blue) has  
 474 been added using the *ggplot2* function `stat_smooth`.

475 **Figure 6 (A)** Sliding window analysis of the standardized index of association ( $\bar{r}_d$ ) across a simulated  
 476  $1.1 \times 10^4$  nt chromosome containing 1,100 variants among 100 individuals. Each window analyzed  
 477 variants within 500nt chunks. The black line refers to the clonal and the blue line to the sexual populations.  
 478 (B) boxplots showing 100 random samples of 50 variants to calculate a distribution of  $\bar{r}_d$  for the clonal  
 479 (red) and sexual (blue) populations. Each box is centered around the mean, with whiskers extending out  
 480 to 1.5 times the interquartile range. The median is indicated by the center line. (A) and (B) are plotted on  
 481 the same y-axis.