

# Novel tools for analyzing genome-wide data of clonal populations

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## 2 ABSTRACT

To gain a detailed understanding of how plant microbe evolve and adapt to host and other factors such as pesticides, knowledge of the population dynamics and evolutionary history of populations is crucial. 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, and 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; Grunwald 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 classical population genetic tools because theoretical assumptions underlying the theory are violated. Yet,

28 many plant pathogen populations are at least partially clonal if not completely clonal (Milgroom, 1996;  
29 Anderson and Kohn, 1995). Thus, development of tools for analysis of clonal or polyploid populations is  
30 needed.

31 Genotyping by sequencing and whole genome resequencing provide the unprecedented ability to  
32 identify >1,000 single nucleotide polymorphisms (SNPs) in populations (Elshire et al., 2011; Luikart  
33 et al., 2003; Davey et al., 2011). Availability of these large SNP data sets provides new challenges for  
34 data analysis. For example, it is not clear what a clone is in large SNP data where the chance of observing  
35 variation at a given SNP locus within independent samples of the same clone are substantial enough that  
36 novel tools for definition of clone boundaries are required. With traditional marker data (e.g., SSR, AFLP)  
37 a clone was typically defined as a unique multilocus genotype (MLG). However, with large SNP data a  
38 measure of genetic distance is required to define the boundary of an MLG (e.g., clone) or the boundaries  
39 of a clonal lineage. Definition of a clone is further complicated by the presence of missing data that is  
40 typical for reduced representation libraries used in GBS or genome re-sequencing. If two individuals are  
41 identical for all observed SNPs except for one missing allele, should they be considered different?

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. It was well received by  
48 the community, garnering 14 citations in its first year of publication. Since it's first release, however,  
49 limitations with speed, ease of use, and efficiency became more apparent as genomic data became more  
50 readily available.

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

58 In version 1, *poppr* was appropriate for traditional markers systems, but not well suited to population  
59 genomic data resulting from high throughput sequencing methods. The raw size of these data made  
60 it difficult to conduct traditional analyses. Here, we introduce *poppr* 2.0, which provides a significant  
61 update to *poppr* including novel tools for analysis of clonal populations specifically for large SNP data.  
62 Significant novel tools include functions for calculating clone boundaries and collapsing individuals into  
63 user-specified clones based on genetic distance, sliding window analyses, genotype accumulation curves,  
64 reticulations in minimum spanning networks, and bootstrapping for any genetic distance.

## POPULATION STRATA AND HIERARCHIES

65 Assessments of population structure through methods such as hierarchical  $F_{st}$  and AMOVA benefit  
66 greatly from multiple levels of population definition (Linde et al., 2002; Everhart and Scherm, 2015;  
67 Grnwald and Hoheisel, 2006). With clonal organisms, basic practice has been to clone-censor data to  
68 avoid downward bias in diversity due to duplicated genotypes that may or may not represent different  
69 samples (Milgroom, 1996). Data structures for population genetic data mostly allow for only one level of  
70 hierarchical definition. The impetus was placed on the researchers to provide the population hierarchies  
71 for every step of the analysis. In *poppr* version 1.1, the hierarchy slot was introduced to allow unlimited  
72 population hierarchies or stratifications to travel with the data. In practice, it is stored as a data frame  
73 where each column represents a separate hierarchical level. This is then used to set the population factor

74 of the data by supplying a hierarchical formula containing one or more column names of the data frame  
75 in the hierarchy slot. This functionality, developed in *poppr*, has been moved to the *adegenet* package in  
76 version 2.0 and the slot and methods have been renamed to *strata*.

## CLONAL IDENTIFICATION

77 As highlighted in previous work, clone correction is an important component of population genetic  
78 analysis of organisms that have cryptic growth or are known to reproduce asexually (Kamvar et al.,  
79 2014b; Milgroom, 1996; Grnwald et al., 2003). This method removes bias that would otherwise affect  
80 metrics that rely on allele frequencies. It was initially designed for data with only a handful of markers.  
81 With the advent of large-scale sequencing and reduced-representation libraries, it has become easier to  
82 sequence tens of thousands of markers from hundreds of individuals (Elshire et al., 2011; Davey et al.,  
83 2011; Davey and Blaxter, 2010). With this larger number of markers, the genetic resolution is much  
84 greater, but the chance of genotyping error is also greatly increased (Mastretta-Yanes et al., 2015). Taking  
85 this fact and occasional somatic mutations into account, it would be impossible to separate true clones  
86 from independent individuals by just comparing what multilocus genotypes are different. We introduce  
87 a new method for collapsing unique multilocus genotypes determined by naive string comparison into  
88 multilocus lineages utilizing any genetic distance given three different clustering algorithms: farthest  
89 neighbor, nearest neighbor, and UPGMA (average neighbor) (Sokal, 1958).

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

## DEMONSTRATION DATA: *P. INFESTANS*

102 We utilize data from the microbe *Phytophthora infestans* to show how the *mlg.filter* function  
103 collapses multilocus genotypes with Bruvo's distance assuming a genome addition model (Bruvo et al.,  
104 2004). *P. infestans* is the causal agent of potato late blight originating from Mexico and spread to Europe  
105 in the mid 19th century (Goss et al., 2014; Li et al., 2013; Lees et al., 2006). *P. infestans* reproduces  
106 both clonally and sexually. The clonal lineages of *P. infestans* have been formally defined into 18 separate  
107 clonal lineages using a combination of various molecular methods including AFLP and microsatellite  
108 markers (Lees et al., 2006). For these data, we used *mlg.filter* to detect all of the distance thresholds  
109 at which 18 multilocus lineages would be resolved. We used these thresholds to define multilocus lineages  
110 and create contingency tables and dendograms to determine how well the multilocus lineages were  
111 detected.

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

## DEMONSTRATION DATA: SIMULATED DATA

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

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

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

## MINIMUM SPANNING NETWORKS WITH RETICULATION

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

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

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

157 The infoMAP algorithm revealed 63 communities with a maximum community size of 77 and  $H = 3.56$   
158 for the reticulate network of the H3N2 data and 117 communities with a maximum community size of

159 26 and  $H = 4.65$  for the minimum spanning tree. The entropy across years was greatly decreased for all  
 160 populations with the reticulate network compared to the minimum spanning tree (Fig. 2).

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

## BOOTSTRAPPING

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

181 To demonstrate calculating a dendrogram with bootstrap support, we used the *poppr* function *aboot*  
 182 on population allelic frequencies derived from the data set *microbov* in the *aedgenet* package with 1000  
 183 bootstrap replicates (Jombart, 2008; Lalo et al., 2007). The resulting dendrogram shows bootstrap support  
 184 values > 50% (Fig. 3).

```
library("poppr")
data("microbov", package = "aedgenet")
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

185 Analysis of population genetics of clonal organisms often borrows from ecological methods such as  
 186 analysis of diversity within populations (Milgroom, 1996; Arnaud-Hanod et al., 2007; Grnwald et al.,  
 187 2003). When choosing markers for analysis, it is important to make sure that the observed diversity in your  
 188 sample will not appreciably increase if an additional marker is added (Arnaud-Hanod et al., 2007). This  
 189 concept is analogous to a species accumulation curve, obtained by rarefaction. The genotype accumulation  
 190 curve in *poppr* is implemented in the function *genotype.curve*. The curve is constructed by randomly  
 191 sampling  $x$  loci and counting the number of observed MLGs. This repeated  $r$  times for 1 locus up to  $n - 1$   
 192 loci, creating  $n - 1$  distributions of observed MLGs.

193 The following code example demonstrates the genotype accumulation curve for data from Everhart and  
 194 Scherm (2015) showing that these data reach a small plateau and have a greatly decreased variance with  
 195 12 markers, indicating that there are enough markers such that adding more markers to the analysis will  
 196 not create very many new genotypes (Fig. 4).

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

set.seed(20150428)
genotype_curve(monitor, 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

197 The index of association ( $I_A$ ) is a measure of multilocus linkage disequilibrium that is most often used  
 198 to detect clonal reproduction within organisms that have the ability to reproduce via sexual or asexual  
 199 processes (Brown et al., 1980; Smith et al., 1993; Milgroom, 1996). It was standardized in 2001 as  $\bar{r}_d$   
 200 by Agapow and Burt (2001) to address the issue of scaling with increasing number of loci. This metric is  
 201 typically applied to traditional dominant and co-dominant markers such as AFLPs, SNPs, or microsatellite  
 202 markers. With the advent of high throughput sequencing, SNP data is now available in a genome-wide  
 203 context and in very large matrices including thousands of SNPs. Thus, the likelihood of finding mutations  
 204 within two individuals of a given clone increases and tools are needed for defining clone boundaries. For  
 205 this reason, we devised two approaches using the index of association for large numbers of markers typical  
 206 for population genomic studies.

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

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

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

## AVAILABILITY

224 As of this writing, the `poppr` R package version 2.0 containing all of the features described here is located  
 225 at <https://github.com/grunwaldlab/poppr>. It is necessary to install `aedegenet` 2.0 before

226 installing *poppr*. It can be found at <https://github.com/thibautjombart/adegenet>. Both  
227 of these can be installed via the R package *devtools* (Wickham and Chang, 2015):

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

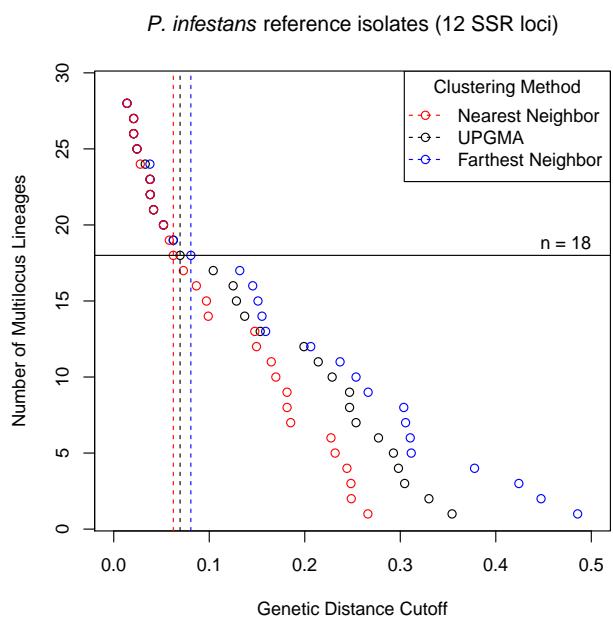
## DISCUSSION

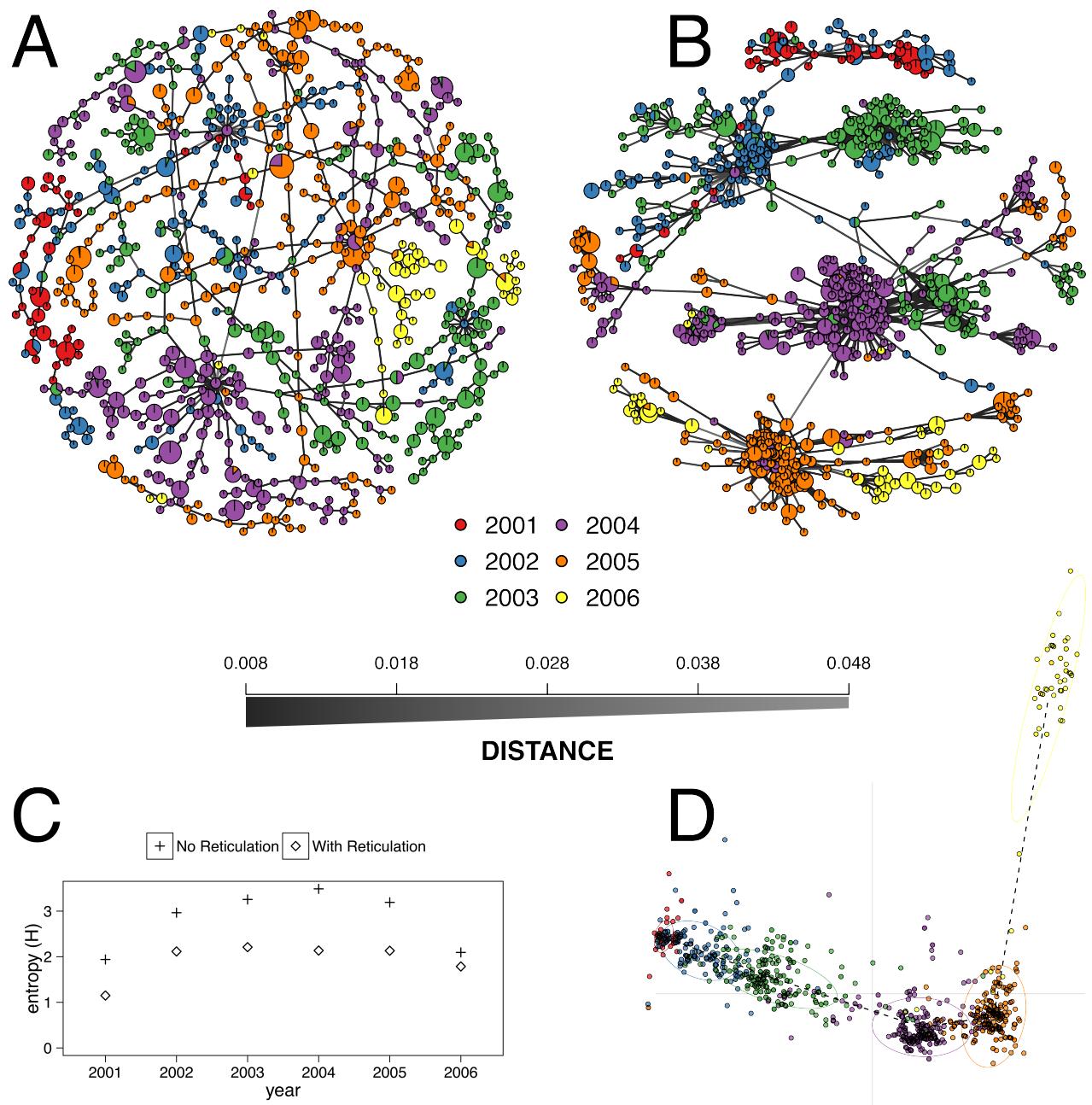
228 We have presented here new model-free tools for the analysis of clonal populations with emphasis on  
229 genomic-scale data. Especially important is `mlg.filter`, which

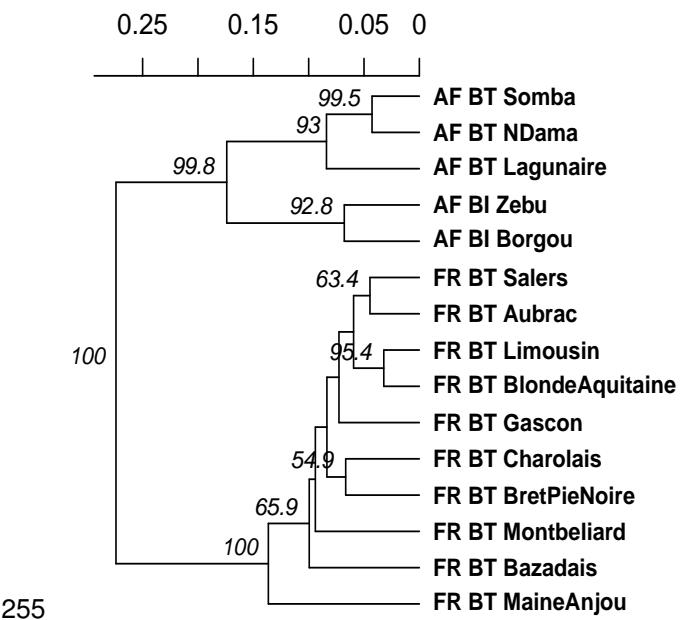
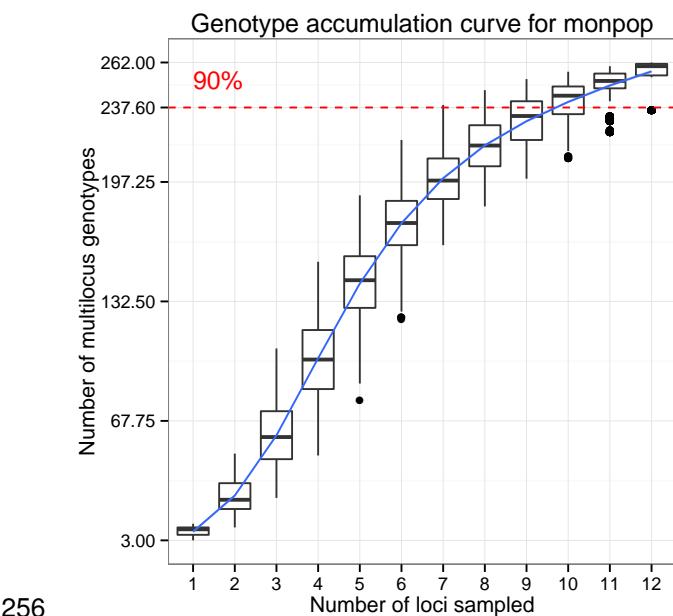
230 Creating structures like minimum spanning networks and dendrograms allow researchers to distill the  
231 most important information from large distance matrices, revealing patterns that could support hypotheses  
232 of differentiation or the lack thereof. Bifurcating dendrograms are most familiar to biologists as the  
233 interpretation of them is straightforward and bootstrap confidence values can easily be obtained due to the  
234 basic structure of the tree. Minimum spanning networks allow for a different view into populations, where  
235 samples themselves can be treated as internal nodes connecting other samples, which could effectively  
236 describe populations sampled through time. The drawback to these is that there is no clear method for a  
237 bootstrap procedure to obtain confidence intervals.

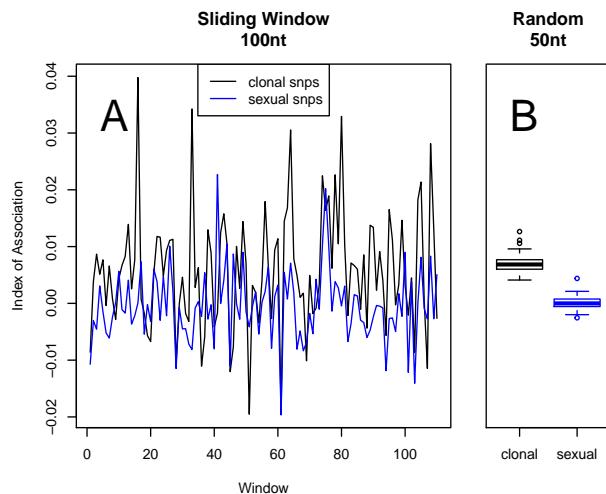
238 Reticulate minimum spanning networks are very important for clonal organisms where a minimum  
239 spanning tree would become a chain, implying that the clones were derived in a progressive and linear  
240 fashion. This presents but one potential scenario for clonal organisms, but does not account for any other  
241 biologically relevant process. Reticulations in the minimum spanning networks allow for a representation  
242 of uncertainty that goes along with clonal organisms. The current implementation in *poppr* has been  
243 successfully used in analyses such as reconstruction of the *P. ramorum* epidemic in Curry County, OR  
244 (Kamvar et al., 2014a, 2015). Reticulated networks also allow for the application of graph community  
245 detection algorithms such as the infoMAP algorithm (Rosvall and Bergstrom, 2008). As shown in the *P.*  
246 *ramorum* and H3N2 data, while it is possible to utilize these graph walking algorithms on non-reticulate  
247 minimum spanning trees, the results derived from these are limited to explain populations derived from  
248 serial cloning events.

- 249
- bootstrapping methods encourage future developers to write distance implementations in common  
250 format
  - moving towards open source, modular tools is the direction that population genetics and plant  
251 pathology needs to go.
- 252

**FIGURES AND TABLES****FIGURE 1**

**FIGURE 2**

**FIGURE 3****FIGURE 4**

**FIGURE 5**

257

**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	.	.	.	1	3	.	.	.	.	.	.	.	.	.	.	.	.	.
US-8	.	.	1	1	.	2	.	.	.	.	.	.	.	.	.	.	.	.

**FIGURE AND TABLE LEGENDS****FIGURE 1**

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

**FIGURE 2**

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

**FIGURE 3**

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

**FIGURE 4**

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

**TABLE 1**

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

**REFERENCES**

- 282 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.
- 284 Anderson, J. B., and Kohn, L. M. (1995). Clonality in soilborne, plant-pathogenic fungi. *Annual review of phytopathology* 33, 369–391.
- 286 Arnaud-Hanod, S., Duarte, C. M., Alberto, F., and Serro, E. A. (2007). Standardizing methods to address clonality in population studies. *Molecular Ecology* 16, 5115–5139.
- 288 Brown, A., Feldman, M., and Nevo, E. (1980). MULTILOCUS sTRUCTURE oF nATURAL pOPULATIONS oF *Hordeum spontaneum*. *Genetics* 96, 523–536. Available at: <http://www.genetics.org/content/96/2/523.abstract>.
- 291 Bruno, R., Michiels, N. K., D'Souza, T. G., and Schulenburg, H. (2004). A simple method for the calculation of microsatellite genotype distances irrespective of ploidy level. *Molecular Ecology* 13, 2101–2106.
- 294 Csardi, G., and Nepusz, T. (2006). The igraph software package for complex network research. *InterJournal Complex Systems*, 1695. Available at: <http://igraph.org>.
- 296 Dagum, L., and Menon, R. (1998). OpenMP: An industry standard API for shared-memory programming. *Computational Science & Engineering, IEEE* 5, 46–55.

- 298     299     Davey, J. W., and Blaxter, M. L. (2010). RADSeq: Next-generation population genetics. *Briefings in Functional Genomics* 9, 416–423. doi:10.1093/bfgp/elq031.
- 300     301     302     Davey, J. W., Hohenlohe, P. A., Etter, P. D., Boone, J. Q., Catchen, J. M., and Blaxter, M. L. (2011). Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nature Reviews Genetics* 12, 499–510.
- 303     304     Dobzhansky, T. (1973). Nothing in biology makes sense except in the light of evolution. *The American Biology Teacher* 75, 87–91.
- 305     306     307     Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., and Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (gBS) approach for high diversity species. *PloS one* 6, e19379.
- 308     309     Everhart, S., and Scherm, H. (2015). Fine-scale genetic structure of *Monilinia fructicola* during brown rot epidemics within individual peach tree canopies. *Phytopathology* 105, 542–549.
- 310     311     312     Excoffier, L., Smouse, P. E., and Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among dNA haplotypes: Application to human mitochondrial dNA restriction data. *Genetics* 131, 479–491.
- 313     314     315     Goss, E. M., Tabima, J. F., Cooke, D. E., Restrepo, S., Fry, W. E., Forbes, G. A., Fieland, V. J., Cardenas, M., and Grnwald, 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.
- 316     317     Grunwald, 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.
- 318     319     Grnwald, 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.
- 320     321     322     Grnwald, 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>.
- 323     324     Jombart, T. (2008). Adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* 24, 1403–1405. doi:10.1093/bioinformatics/btn129.
- 325     326     Jombart, T., and Ahmed, I. (2011). Adegenet 1.3-1: New tools for the analysis of genome-wide sNP data. *Bioinformatics* 27, 3070–3071.
- 327     328     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.
- 329     330     331     Kamvar, Z. N., Larsen, M. M., Kanaskie, A. M., Hansen, E. M., and Grnwald, 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.
- 332     333     334     Kamvar, Z. N., Larsen, M. M., Kanaskie, A., Hansen, E., and Grnwald, N. J. (2015). Spatial and temporal analysis of populations of the sudden oak death pathogen in oregon forests. *Phytopathology*, in press.
- 335     336     Kamvar, Z. N., Tabima, J. F., and Grnwald, N. J. (2014b). Poppr: An r package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* 2, e281.
- 337     338     Lalo, 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.
- 339     340     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.

- 341 Li, Y., Cooke, D. E., Jacobsen, E., and Lee, T. van der (2013). Efficient multiplex simple sequence repeat  
342 genotyping of the oomycete plant pathogen *phytophthora infestans*. *Journal of microbiological methods*  
343 92, 316–322.
- 344 Linde, C., Zhan, J., and McDonald, B. (2002). Population structure of mycosphaerella graminicola:  
345 From lesions to continents. *Phytopathology* 92, 946–955.
- 346 Luikart, G., England, P. R., Tallmon, D., Jordan, S., and Taberlet, P. (2003). The power and promise of  
347 population genomics: From genotyping to genome typing. *Nature Reviews Genetics* 4, 981–994.
- 348 Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer*  
349 research 27, 209–220.
- 350 Mastretta-Yanes, A., Arrigo, N., Alvarez, N., Jorgensen, T. H., Piero, D., and Emerson, B.  
351 (2015). Restriction site-associated dNA sequencing, genotyping error estimation and de novo assembly  
352 optimization for population genetic inference. *Molecular ecology resources* 15, 28–41.
- 353 McDonald, B. A., and Linde, C. (2002). The population genetics of plant pathogens and breeding  
354 strategies for durable resistance. *Euphytica* 124, 163–180. doi:10.1023/A:1015678432355.
- 355 Milgroom, M. G. (1996). Recombination and the multilocus structure of fungal populations. *Annual*  
356 *review of phytopathology* 34, 457–477.
- 357 Milgroom, M. G., Levin, S. A., and Fry, W. E. (1989). Population genetics theory and fungicide  
358 resistance. *Plant disease epidemiology* 2, 340–367.
- 359 Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National*  
360 *Academy of Sciences* 70, 3321–3323.
- 361 Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L.,  
362 Solymos, P., Stevens, M. H. H., and Wagner, H. (2015). *Vegan: Community ecology package*. Available  
363 at: <http://CRAN.R-project.org/package=vegan>.
- 364 Paradis, E. (2010). Pegas: an R package for population genetics with an integrated–modular approach.  
365 *Bioinformatics* 26, 419–420.
- 366 R Core Team (2015). *R: A language and environment for statistical computing*. Vienna, Austria: R  
367 Foundation for Statistical Computing Available at: <http://www.R-project.org/>.
- 368 Rosvall, M., and Bergstrom, C. T. (2008). Maps of random walks on complex networks reveal  
369 community structure. *Proceedings of the National Academy of Sciences* 105, 1118–1123.
- 370 Shannon, C. (2001). A mathematical theory of communication. *ACM SIGMOBILE Mobile Computing*  
371 *and Communications Review* 5, 3–55. Available at: <http://cm.bell-labs.com/cm/ms/what/shannonday/shannon1948.pdf>.
- 373 Smith, J. M., Smith, N. H., O'Rourke, M., and Spratt, B. G. (1993). How clonal are bacteria?  
374 *Proceedings of the National Academy of Sciences* 90, 4384–4388. doi:10.1073/pnas.90.10.4384.
- 375 Sokal, R. R. (1958). A statistical method for evaluating systematic relationships. *Univ Kans Sci Bull* 38,  
376 1409–1438.
- 377 Wickham, H., and Chang, W. (2015). *Devtools: Tools to make developing r packages easier*. Available  
378 at: <http://CRAN.R-project.org/package=devtools>.