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Statistical Genetics: Analyses with R

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Preface

This book is supposed to give an introduction to different methods used in Statistical Genetics and how to run them in the R universe. Statistical Genetics benefits a lot from the increase in computing power and data storage possibilities, because it allows us to run more complex models and include vastly more information into our analyses.

Why read this book

This book will follow a hands-on approach, including code chunks and exercises for each chapter. This makes it useful for using as a compendium to Statistical Genetics courses for students and for everybody looking to get into Statistical Genetics analyses in R.

Structure of the book

Chapter 1 introduces Statistical Genetics.

Acknowledgments

A lot of people helped me when I was writing the book.

Shirin Glander

About the Author

I am a biologist turned bioinformatician turned data scientist from Münster in Germany. I've earned my PhD from the University of Münster, where I worked on the link between flowering time and immune defense in plants using quantitative genetics and RNA-sequencing. My overarching interest has been evolutionary biology and genetics. Following my PhD, I continued as a Postdoc for two years; during this time, I was working as a bioinformatician for Next-Generation-Sequencing analysis on questions relating to immunology - specifically inflammation, immune tolerance and autoinflammatory diseases. Since July of 2017 I have switched to industry, where I work as a data scientist for codecentric AG.

I'm a big fan of R and I write a blog[1] where I explore different data sets and techniques in R. I also teach ballroom and Latin dance courses.

You can find me and my package for gene expression analysis (exprAnalysis) on Github[2].

[1] www.shirin-glander.de
[2] https://github.com/ShirinG

The Basics of R

> "R is an integrated suite of software facilities for data manipulation, calculation and graphical display." An Introduction to R - Notes on R: A Programming Environment for Data Analysis and Graphics version 3.4.0 (2017-04-21) by W. N. Venables, D. M. Smith and the R Core Team[3]

R has originally been developed for statistical analysis and is a powerful toolbox for a wide range of tasks pertaining to data handling, statistical modeling and visualisation. It consists of a core distribution that contains basic functions. You can use additional packages for specific analyses.

This books assumes a basic knowledge about getting data into R, installing and loading packages, calling functions, etc. It is beyond the scope of this book to provide an in depth introduction to R. Luckily, there are a large number of free online resources available, like

- An Introduction to R - Notes on R: A Programming Environment for Data Analysis and Graphics version 3.4.0 (2017-04-21) by W. N. Venables, D. M. Smith and the R Core Team[4]
- Data Camp[5]
- Penn State Statistics resources[6]
- 'Introducin R' by Germán Rodríguez from Princeton University[7]
- etc.

[3] https://cran.r-project.org/doc/manuals/r-release/R-intro.pdf
[4] https://cran.r-project.org/doc/manuals/r-release/R-intro.pdf
[5] https://www.datacamp.com/courses/free-introduction-to-r
[6] https://onlinecourses.science.psu.edu/statprogram/r
[7] http://data.princeton.edu/R/introducingR.pdf

Software information and conventions

Package names are written in bold text (e.g., **rmarkdown**), and inline code and filenames are formatted in a typewriter font (e.g., `plot()`). Function names are followed by parentheses (e.g., `bookdown::render_book()`).

Some packages are available via CRAN[8], while others are hosted at Bioconductor[9]. I will provide package installation instructions at the beginning of each section, indicating where each package can be found. I will also be using the `library()` function, rather than `require()` for loading packages to make sure that we will get an error message in case packages have not been installed correctly.

The example workflows included are meant to illustrate the theoretical concepts and get you started on your own analysis. They are minimal examples of the necessary steps but are not meant to substitute the package manuals. When you want to apply the workflows to your own data, I highly recommend going back to the package documentation to find out about additional functions and using the `help()` function to explore parameter options. I will be using the same naming and code schemes as in the package manuals to make finding the relevant parts easy.

I used the **knitr** package and the **bookdown** package to compile this book. My R session information is shown below:

```
sessionInfo()
```

```
## R version 3.4.1 (2017-06-30)
## Platform: x86_64-apple-darwin15.6.0 (64-bit)
## Running under: macOS Sierra 10.12.6
##
## Matrix products: default
## BLAS: /Library/Frameworks/R.framework/Versions/3.4/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/3.4/Resources/lib/libRlapack.dylib
##
```

[8] https://cran.r-project.org/
[9] https://www.bioconductor.org/

```
## locale:
## [1] de_DE.UTF-8/de_DE.UTF-8/de_DE.UTF-8/C/de_DE.UTF-8/de_DE.UTF-8
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## loaded via a namespace (and not attached):
##  [1] compiler_3.4.1  backports_1.1.0 bookdown_0.5    magrittr_1.5
##  [5] rprojroot_1.2   tools_3.4.1     htmltools_0.3.6 rstudioapi_0.7
##  [9] yaml_2.1.14     Rcpp_0.12.12    stringi_1.1.5   rmarkdown_1.6
## [13] knitr_1.17      stringr_1.2.0   digest_0.6.12   evaluate_0.10.1
```

1

Introduction to Statistical Genetics

Statistical genetics describes the scientific field that uses statistics to gain insight from genetic data. Genetic data can help understand the basis and regulation of traits and diseases in humans, animals, plants and simpler organisms.

Statistical genetics makes use of large-scale datasets, e.g. from Genome-Wide Association Studies (GWAS) or from Next-Generation Sequencing approaches.

- 3rd generation of genetic markers (SNPs)

DNA sequence of numerous organisms, gene-statement information (transcriptional profiling), and an increasing amount of knowledge about gene function. Statisticians play an important role in the analysis and the design of genetic studies. Statistical genetics overlaps with fields such as biomathematics, bioinformatics, biology, epidemiology, genetics, etc. People in the department have worked and are working on methods in linkage analysis, allelic association tests, gene statement array data analysis, sequence analysis, comparative genomics, phylogenetic tree reconstruction, etc.

Statistical inference of genetic regulation needs sufficiently large study populations, especially if we analyse unrelated individuals. Family cohort analyses can be done on much smaller sample sizes, because we can largely assume Mendelian inheritance.

A solid understanding of inheritance mechanisms in populations is paramount to understanding statistical genetics. Therefore, this book will start with evolutionary and population genetics, the subfield that studies

evolutionary processes like speciation and adaptation or population-level phenomena like migration, drift, etc.

Closely related to population genetics is quantitative genetics. Here, the focus lies on analysing quantitative traits and their underlying genetic regulation.

> Genetic epidemiology is the study of the role of genetic factors in determining health and disease in families and in populations, and the interplay of such genetic factors with environmental factors. Genetic epidemiology seeks to derive a statistical and quantitative analysis of how genetics work in large groups.[1]

> Statistical genetics is concerned with the analysis of genetic data. Due to rapid progress in laboratory techniques, there is an ever-increasing slew of new data: the 3rd generation of genetic markers (SNPs), the DNA sequence of numerous organisms, gene-statement information (transcriptional profiling), and an increasing amount of knowledge about gene function. Statisticians play an important role in the analysis and the design of genetic studies. Statistical genetics overlaps with fields such as biomathematics, bioinformatics, biology, epidemiology, genetics, etc. People in the department have worked and are working on methods in linkage analysis, allelic association tests, gene statement array data analysis, sequence analysis, comparative genomics, phylogenetic tree reconstruction, etc.

> Statistical geneticists at HSPH develop statistical methods for understanding the genetic basis of human diseases and traits. These methods involve large-scale data sets from candidate-gene, genome-wide and resequencing studies, using both unrelated and related individuals. HSPH statistical geneticists collaborate with other investigators at HSPH and around the world on studies of cancer, heart disease, diabetes, respiratory disease, psychiatric disease, and health-related behaviors (e.g. smoking, diet). They

have close ties to the Program in Quantitative Genomics and Computational Biology and Bioinformatics group at HSPH. Training encompasses basic statistics; Mendelian and population genetics; design and analysis of genetic association studies; gene expression and epigenetic markers; and gene-environment interaction.

The human genome project is changing the practice of medicine and public health, and genetics is playing an ever more central role in all the biomedical sciences. This expanding role includes the mapping and sequencing of the human genome and the genomes of other organisms, the identification of all human genes and the cataloging of all common human genetic variants, the development of methods to assay the expression of many genes simultaneously, the investigation of the molecular evolution of human genes, and the translation of the resulting knowledge to address questions of human health and disease. These developments present remarkable opportunities for the prevention and cure of human disease, but require investigators working at the interface between human genetics and the mathematical sciences

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https://cran.r-project.org/web/views/Genetics.html https://www.mcgill.ca/statisticalgenetics/software

2

Evolutionary and Population Genetics

Evolutionary and population genetics study processes like adapation and speciation events from a genetic perspective. This means that they are interested in how genes and alleles are transmitted between parents and offspring (or in some cases even horizontally), how they change and adapt over time , how dominance, epistasis and epigenetics influence phenotypes and how this relate to population structure.

The basic concept of evolutionary biology is that in order for selection to lead to change in populations, there needs to be sufficient genetic and phenotypic variation in that population to select from. From a genetics perspective, this means that selection will favor certain alleles among the pool of genes in the population. Favorable alleles will therefore increase in frequency within the population over time, while disadvantageous alleles will eventually disappear. Depending on how stable the environment is, this change in allele frequencies can be subject to severe fluctuation.

Additional aspects like migration, mutation, recombination, inbreeding, drift, etc. make modeling of evolutionary and population genetic processes non-trivial.

https://en.wikipedia.org/wiki/Population_genetics

- Mendelian and population genetics;

This primer provides a concise introduction to conducting applied analyses of population genetic data in R, with a special emphasis on non-model populations including clonal or partially clonal organisms. It is not meant to be a textbook on population genetics. Quite to the contrary, we refer the reader to several textbooks on population genetics (Templeton, 2006; Hartl & Clark, 2007; Nielsen & Slatkin, 2013). Likewise, this book will not replace books on theory and statistics of population genetics (Weir, 1996). The reader is thus expected to have a basic understanding of population genetic theory and ap-

plications. Finally, this primer is focused on traditional population genetics based on allele frequencies, rather than more sophisticated coalescent approaches (Hein, Schierup & Wiuf, 2004; Wakeley, 2009; Nielsen & Slatkin, 2013), although some of the material covered here will apply. In a nutshell, this primer provides a valuable resource for tackling the nitty-gritty analysis of populations that do not necessarily conform to textbook genetics and might or might not be in Hardy-Weinberg equilibrium and panmixia.

This primer is geared towards biologists interested in analyzing their populations. This primer does not require extensive knowledge of programming in R, but the user is expected to install R and all packages required for this primer.

Why use R? Until recently, one of the more tedious aspects of conducting a population genetic analysis was the need for repeated reformatting data to conduct different, complimentary analyses in different programs. Often, these programs only ran on one platform. Now, R provides a toolbox with its packages that allows analysis of most data conveniently without tedious reformatting on all major computing platforms including Microsoft Windows, Linux, and Apple's OS X. R is an open source statistical programming and graphing language that includes tools for statistical, population genetic, genomic, phylogenetic, and comparative genomic analyses. This primer is for those of us that want to conduct applied analyses of populations and make full use of the palette of tools available in R including for example the R markdown code for this primer.

Note that the R user community is very active and that both R and its packages are regularly updated, critically modified, and noted as deprecated (no longer updated) as appropriate.

Any R user needs to make sure all components are up-to-date and that versions are compatible. Population genetics Traditional population genetics is based on analysis of observed allele frequencies compared to frequencies expected, assuming a population genetic model. For example, under a Wright-Fisher model you might expect to see populations of diploid individuals that reproduce sexually, with non-overlapping generations. This model ignores effects such as mutation, recombination, selection or changes in population size or structure. More complex models can incorporate different aspects of

effects observed in real populations. However, most of these models assume that populations reproduce sexually.

Here, we briefly review some terminology but assume that the reader has a basic understanding of theory and applications of population genetics.

A locus is a position in the genome where we can observe one or several alleles in different individuals. Loci used in population genetics are assumed to be selectively neutral and can be an anonymous or non-coding region such as a microsatellite locus (SSR), a single nucleotide polymorphism (SNP) or the presence/absence of a band on a gel. A genotype is the combination of alleles carried by a given individual at a particular set of loci. Individuals carrying the same set of alleles are considered to have the same multilocus genotype MLGMLG.

Basic metrics of interest in populations are polymorphisms, allele frequencies and genotype frequencies. Polymorphism can be estimated in several ways, such as the total number of loci observed that have more than one allele. The frequency of an allele is calculated as the number of allele copies observed in a population divided by the total number of individuals, times the ploidy (NN in haploids or 2N2N in diploids) in the population. For diploids we would observe frequency fAfA and fafa for alleles AA and aa, respectively:

fA=NA2NfA=NA2N and fa=Na2Nfa=Na2N

where NANA are the numbers of allele AA segregating in the population genotyped.

By definition, at a biallelic locus frequencies sum to 1:

fA+fa=1fA+fa=1.

Genotype frequencies are the relative frequencies of each MLGMLG observed in a population. Thus, for diploids at a biallelic locus we can observe three genotypes: AAAA, AaAa, and aaaa and their respective frequencies:

fAA=NAA2NfAA=NAA2N and fAa=NAa2NfAa=NAa2N and faa=Naa2Nfaa=Naa2N

These frequencies again add up to 1. In diploid organisms we can also calculate the frequency of homozygotes (AAAA or aaaa) or heterozygotes (AaAa) as the proportion of individuals falling into each class. The proportion of

individuals that are heterozygous is given by fAafAa while the homozygous proportion is given by 1–fAa=fAA+faa1–fAa=fAA+faa.

An important aspect of population structure is the heterozygosity observed within subdivided populations HSHS and across total populations HTHT. If we assume presence of two populations in Hardy-Weinberg equilibrium then frequencies of Allele AA in populations 1 and 2 pooled over both populations would be:

fA=2N1fA1+2N2fA22N1+2N2fA=2N1fA1+2N2fA22N1+2N2

where each allele frequency is multiplied by population size N1N1 and N2N2 and divided by total number of alleles 2N1+2N22N1+2N2. Remember, that in a diploid population there are 2 alleles per locus per individual and hence every frequency is multiplied by 2.

Genetic differentiation of two populations (e.g. subdivision of populations) occurs if we observe differences in polymorphism, allele frequencies, genotype frequencies, and heterozygosity. Wright's FstFst is a commonly used measure of differences in heterozygosity observed in the overall population relative to the subdivided populations and is defined as the differences between HTHT and HSHS relative to HTHT:

FST=HT–HSHTFST=HT–HSHT

If allele frequencies are identical in both subpopulations then HT=HSHT=HS. If, on the other hand, allele frequencies vary between subdivided populations, then HT>HSHT>HS and populations are considered to be genetically differentiated.

Besides calculation of FstFst other approaches can be used to assess population structure including clustering. These approaches will be explored in subsequent chapters.

The special case of clonal organisms Plants and microorganisms such as fungi, oomycetes, and bacteria often reproduce clonally or follow a mixed reproductive system, including various degrees of clonality and sexuality (Halkett, Simon & Balloux, 2005). Traditional population genetic theory does not apply and these populations violate basic assumptions in the corresponding analyses. Thus, a different set of tools are required for analyses of clonal populations (Anderson & Kohn, 1995; Milgroom, 1996; Balloux,

Lehmann & Meeûs, 2003; Halkett et al., 2005; De Meeûs, Lehmann & Balloux, 2006; Grünwald & Goss, 2011). Typically these focus on metrics that are model free and do not assume random mating or random sampling of alleles. Model free metrics suitable for analyzing these populations include measures of genotypic diversity or evenness, as well as cluster analysis based on genetic distance. Additional useful measures include indices of linkage disequiblibrium among markers that are used to infer if populations are reproducing sexually or clonally. We will explore these analyses bit by bit throughout this primer. The poppr R package was specifically developed to facilitate analyses of clonal populations (Kamvar, Tabima & Grünwald, 2014; Kamvar, Brooks & Grünwald, 2015).

Marker systems, ploidy and other kinds of data Population genetic data come in many shapes and forms and a good analysis needs to be tailored to the marker system, ploidy used and corresponding genetic models assumed (Grünwald & Goss, 2011). Organisms can be haploid, diploid, with or without known phase, or polyploid. In the case of diploid species, marker systems can be dominant (AFLP, RFLP or RAPD data) or co- dominant (SSR, SNPs, allozymes) (Grünwald et al., 2003). A locus can have two alleles (0/1 for AFLP; C/T for SNPs) or many alleles per locus (A/B/C…N for SSRs or allozymes). In each case the data has to be coded and analyzed differently. Mutation rates can also differ for different markers systems (SSR >> mitochondrial SNPs > nuclear SNPs) (Grünwald & Goss, 2011). All these aspects of a particular data set need to be considered in the data analyses.

Applications of population genetic analyses Population genetic analyses are tremendously valuable for answering questions ranging from applied to basic evolutionary questions (Grünwald & Goss, 2011). For example, a typical concern when finding a new, invasive organism is whether it is introduced to the area or emerged from a resident population. Other questions of interest might include:

Where is the center of origin? Does this organism reproduce sexually? Has the population gone through a genetic bottleneck? Are populations structured by region, geography, micro-environment? What are source and sink populations and what are the rates of migration? This primer will provide some of the basic tools needed to answer many of these questions for populations that are clonal or partially clonal. Note, however, that more powerful,

complementary coalescent methods will not be covered here. We hope you enjoy following along.

References Anderson JB., Kohn LM. 1995. Clonality in soilborne, plant-pathogenic fungi. Annual Review of Phytopathology 33:369–391. Available at: http://www.annualreviews.org/doi/abs/10.1146/annurev.py.33.090195.002101

Balloux F., Lehmann L., Meeûs T de. 2003. The population genetics of clonal and partially clonal diploids. Genetics 164:1635–1644. Available at: http://www.genetics.org/content/164/4/1635.full

De Meeûs T., Lehmann L., Balloux F. 2006. Molecular epidemiology of clonal diploids: A quick overview and a short dIY (do it yourself) notice. Infection, Genetics and Evolution 6:163–170. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16290062

Grünwald NJ., Goodwin SB., Milgroom MG., Fry WE. 2003. Analysis of genotypic diversity data for populations of microorganisms. Phytopathology 93:738–746. Available at: http://apsjournals.apsnet.org/doi/abs/10.1094/PHYTO.2003.93.6.738

Grünwald NJ., Goss EM. 2011. Evolution and population genetics of exotic and re-emerging pathogens: Novel tools and approaches. Annual Review of Phytopathology 49:249–267. Available at: http://www.annualreviews.org/doi/abs/10.1146/annurev-phyto-072910-095246?journalCode=phyto

Halkett F., Simon J-C., Balloux F. 2005. Tackling the population genetics of clonal and partially clonal organisms. Trends in Ecology & Evolution 20:194–201. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16701368

Hartl D., Clark A. 2007. Principles of population genetics. Sinauer Associates, Incorporated. Available at: http://books.google.com/books?id=SB1vQgAACAAJ

Hein J., Schierup M., Wiuf C. 2004. Gene genealogies, variation and evolution: A primer in coalescent theory. Oxford University Press, USA. Available at: http://books.google.com/books?id=QBC/_SFOamksC

Kamvar ZN., Brooks JC., Grünwald NJ. 2015. Novel R tools for analysis of genome-wide population genetic data with emphasis on clonality. Name:

Frontiers in Genetics 6:208. Available at: http://dx.doi.org/10.3389/fgene.2015.00208

Kamvar ZN., Tabima JF., Grünwald NJ. 2014. PopprPoppr: An R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2:e281. Available at: http://dx.doi.org/10.7717/peerj.281

Milgroom MG. 1996. Recombination and the multilocus structure of fungal populations. Annual Review of Phytopathology 34:457–477. Available at: http://www.annualreviews.org/doi/abs/10.1146/annurev.phyto.34.1.457

Nielsen R., Slatkin M. 2013. An introduction to population genetics: Theory and applications. Sinauer Associates, Incorporated. Available at: http://books.google.com/books?id=Iy08kgEACAAJ

Templeton A. 2006. Population genetics and microevolutionary theory. Wiley. Available at: http://books.google.com/books?id=tJVreQjInt0C

Wakeley J. 2009. Coalescent theory: An introduction. Roberts & Company Publishers. Available at: http://books.google.com/books?id=x30RAgAACAAJ

Weir B. 1996. Genetic data analysis 2. Sinauer Associates. Available at: http://books.google.com/books?id=e9QPAQAAMAAJ

```
pkg = c("poppr", "treemap", "magrittr")
sapply(pkg, function(x) {if (system.file(package = x) == '') install.packages(x)})
```

```
## $poppr
## NULL
##
## $treemap
## NULL
##
## $magrittr
## NULL
```

You can then load the package:

```
library(poppr)
```

This section will briefly go over the basics of data import into poppr. For this section, we will focus on the GenAlEx format. Other formats are supported and details are given in the R help page for the adegenet function

import2genind. We will show examples of haploid, diploid, and polyploid data sets and show you how you can format your data if it's grouped into multiple stratifications.

To import GenAlEx formatted data into poppr, you should use the function read.genalex. Below is an example using monpop.csv.

```
monpop <- read.genalex("datasets/monpop.csv")
monpop
```

```
##
## This is a genclone object
## -------------------------
## Genotype information:
##
##    264 original multilocus genotypes
##    694 haploid individuals
##     13 codominant loci
##
## Population information:
##
##      1 stratum - Pop
##     12 populations defined -
## 26_09_BB, 26_09_FR, 26_10_BB, ..., 7_09_FR, 79_10_BB, 79_10_FR
```

Other data formats Given dependence of poppr on adegenet, users can import data from the following formats

FSTAT (file.dat) GENETIX (file.gtx) GENEPOP (file.gen) STRUCTURE (file.str) The adegenet function import2genind will import all of these formats. If you have sequence data, you can use the read.FASTA function from the ape package. If your data is in any other format, type help("df2genind") for guidance.

GenAlEx data format GenAlEx is a very popular add-on for Microsoft Excel. It is relatively easy to use because of its familiar, menu-driven interface. It also gives the user the option to include information on population groupings, regional groupings, and xy coordinates. The flexibility of this format made it a clear choice for import into poppr.

The data format is standard in that individuals are defined in the rows and

loci are defined in the columns. The first two rows are reserved for metadata and the first two columns are reserved for the individual names and population names, respectively. The examples we will be using include haploid, diploid and polyploid data.

Basic Format Below is what the monpop (haploid) data looks like. Highlighted in red is how missing data should be coded for SSR markers. Highlighted in blue are the parts of the metadata rows used by poppr. These three numbers represent:

The columns of the metadata beyond those three rows define the number of individuals contained within each population. Since this data is redundant with the second column, it is not necessary. Notice, also, that the second column, reserved for the population assignments, has a pattern of underscores in the populations. This will be important at the end of this section. Below is a modified version of the input format that should make it easier to format.

Highlighted in blue is the cell that defines the number of columns highlighted in red. If we set this number to 1, then we do not have to enter in any information in those columns. Try it for yourself.

Diploids Diploid data is only different in the fact that you will have two alleles per locus. This is coded such that each allele is in a separate column. Below is an example of the nancycats data set (from the adegenet package), exported like above. Highlighted in blue and red are the first and second loci, respectively.

Polyploids GenAlEx does not handle polyploids, but since poppr can do it, we have set up a scheme to allow import of polyploids via this format. The limitation is that all of your loci have to have the same observed ploidy. Below is the example of Phytophthora infestans in the data set Pinf where some genotypes had observed tetraploid loci (Goss et al., 2014).

Highlighted in blue is the first locus and highlighted in red are two samples at that locus, an observed diploid and observed triploid. Note the extra zeroes needed to make the genotype tetraploid.

Population strata A hierarchical sampling approach is necessary to infer structure of populations in space or time. Poppr facilitates definition of stratified data by concatenating the different stratifications into a single

column by a common separator ("_" by default). Here's an example of the three stratifications of the monpop data set introduced above:

```
splitStrata(monpop) <- ~Tree/Year/Symptom
monpop # After (Three distinct levels)
```

```
##
## This is a genclone object
## -------------------------
## Genotype information:
##
##    264 original multilocus genotypes
##    694 haploid individuals
##     13 codominant loci
##
## Population information:
##
##      3 strata - Tree, Year, Symptom
##     12 populations defined -
## 26_09_BB, 26_09_FR, 26_10_BB, ..., 7_09_FR, 79_10_BB, 79_10_FR
```

References Everhart S., Scherm H. 2015. Fine-scale genetic structure of Monilinia fructicola during brown rot epidemics within individual peach tree canopies. Phytopathology 105:542–549. Available at: https://doi.org/10.1094/PHYTO-03-14-0088-R

Goss EM., Tabima JF., Cooke DEL., Restrepo S., Fry WE., Forbes GA., Fieland VJ., Cardenas M., Grünwald NJ. 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. Available at: http://www.pnas.org/content/early/2014/05/29/1401884111.abstract

Populations are best sampled hierarchically on a range of scales from subpopulations (e.g. fields, valleys, ranges) to regions (e.g. valleys, states, countries or continents) or across time (years or decades). This approach is useful because population structure and evolutionary processes may not be discernible a priori. Most of the times we do not know if population are differentiated spatially or temporally. Thus, a combination of targeted local sampling with sampling over larger spatial or temporal scales is necessary

to detect population structure over different scales, without using intense sampling throughout the entire range.

The methods implemented in poppr allow specification of which strata you want to analyze. This is a rapid way of working with subsets of your data without having to perform any data manipulation or changing the input file. In this tutorial, we will show you how to define the hierarchical structure of your data and how to specify specific levels that you might want to analyze.

Data used in this example For this example, we will use the monpop data set (Everhart & Scherm, 2015). This microsatellite data consists of 13 loci for 694 individuals of the haploid fungal pathogen Monilinia fructicola that infects peach flowers and fruits in commercial orchards. The monpop population came from four trees within a single orchard (trees 26, 45, and 7). Each tree was sampled in 2009, 2010, and/or 2011. Additionally, each sample was noted as to whether it came from a blossom or a fruit. This example data set is included with the poppr package.

Working with stratified data The steps for working with stratified data include:

Import data set with samples labeled according to strata Define the stratifications for the data Setting the stratification(s) that you want to have analyzed Importing data labeled according to a stratum The easiest way to work with stratified data is to label each sample using an underscore "_" to separate each level. This was already done for the monpop data, where each sample was coded hierarchically by tree, year, and symptom in the following format: "Tree_Year_Symptom".

Let's load the hierarchically labeled example data:

Genotype information shows us that the data contains 264 multilocus genotypes among 694 haploid individuals with 13 loci. Population information has two items, the stratifications and the populations defined. You can think of stratifications as the index names for each of the hierarchical levels within your data (so for our data it should be Tree, Year, and Symptom). By default, however, no stratifications are defined and so this is "Pop", which is the entire dataset of 694 individuals. Because we labeled each sample according to stratification, populations defined shows us our data has 12 groups defined:

7_09_BB, 26_09_BB, 26_09_FR, 7_09_FR, 26_10_BB, 45_10_BB, 79_10_BB, 79_10_FR, 26_10_FR, 45_10_FR, 26_11_BB, and 26_11_FR.

Assigning stratifications We imported the data that has three stratifications "Tree_Year_Symptom". In order to analyze our data according to any combination of those three stratifications, we need to tell poppr that the 12 groups should be split by tree, year, and/or symptom. Thus, the first step is to split our data according to strata so that we can access each of the three hierarchical levels in the data. The splitStrata command is used to index the three stratifications:

After splitting the data populations are specified by stratification: "Tree Year Symptom".

We can look at how the stratifications are distributed by using a treemap plot. This is a plot that allows us to visualize hierarchical stratifications. The function we'll use is called treemap() from the treemap package.

The treemap() function needs only a data frame containing the strata and their respective counts. This can easily be done with the dplyr package where we will:

Group our strata by Tree, Year, and Symptom Summarize the data by counting how many times we see each specific combination

```
library(dplyr)
```

```
##
## Attaching package: 'dplyr'

## The following objects are masked from 'package:stats':
##
##     filter, lag

## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
```

```
monstrata <- strata(monpop) %>%
  group_by(Tree, Year, Symptom) %>%
  summarize(Count = n())
```

```
monstrata
```

```
## # A tibble: 12 x 4
## # Groups:   Tree, Year [?]
##      Tree   Year Symptom Count
##    <fctr> <fctr>  <fctr> <int>
##  1      7      9      BB    23
##  2      7      9      FR    73
##  3     26      9      BB    41
##  4     26      9      FR   132
##  5     26     10      BB     5
##  6     26     10      FR    85
##  7     26     11      BB    30
##  8     26     11      FR    97
##  9     45     10      BB    13
## 10     45     10      FR   130
## 11     79     10      BB     1
## 12     79     10      FR    64
```

Now we can use the treemap() function to plot the data. Note that it has a lot of arguments to allow you to properly manipulate the graphic, but we will only use the very necessary components to visualize the distribution of the strata:

dtf - The data frame containing the stratifications and counts index - The variables used for nesting (in order) vSize - The variable to use to compute the size of the blocks All the other arguments we are using here give various aesthetics. If you want to know more about how they work, you can peruse the manual for the treemap function by typing help("treemap", "treemap").

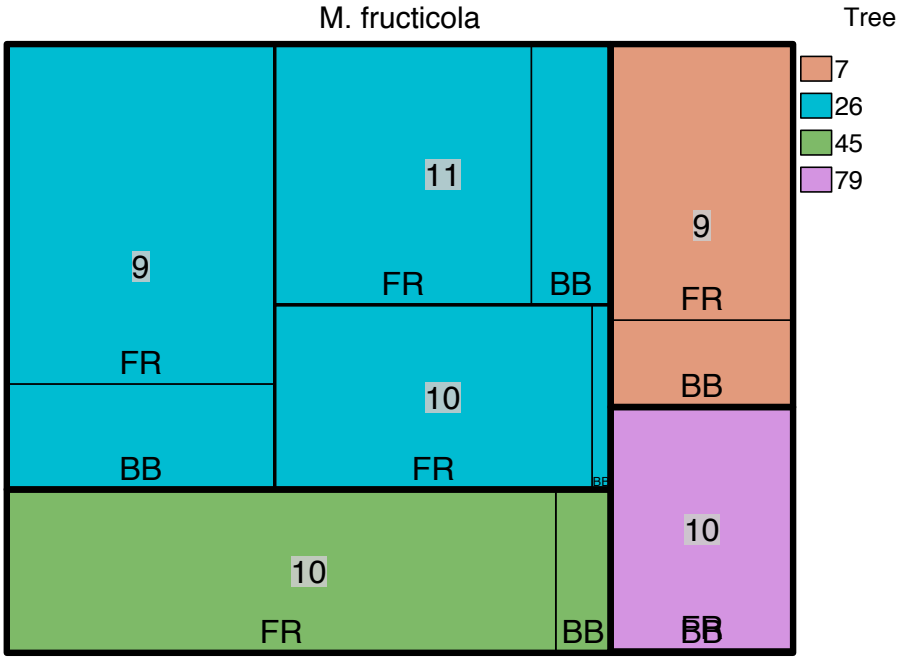
```
library(treemap)
nameStrata(monpop) # The order of our variables
```

```
## [1] "Tree"    "Year"    "Symptom"
```

```
monstrata$Count    # The variable used for the block size
```

```
##  [1]  23  73  41 132   5  85  30  97  13 130   1  64
```

```
# Adjusting the aesthetics for the labels
label_position <- list(c("center", "top"), c("center", "center"), c("center", "bottom"))
label_size     <- c(Tree = 0, Year = 15, Symptom = 15)

# Plotting, First three arguments are necessary.
treemap(dtf = monstrata, index = nameStrata(monpop), vSize = "Count",
        type = "categorical", vColor = "Tree", title = "M. fructicola",
        align.labels = label_position, fontsize.labels = label_size)
```




```
itreemap(dtf = monstrata, index = nameStrata(monpop), vSize = "Count",
        type = "categorical", vColor = "Tree")
```

Next, we analyze the data according to Tree and Year:

```
setPop(monpop) <- ~Tree/Year
monpop
```

```
##
## This is a genclone object
## -------------------------
## Genotype information:
```

```
##
##    264 original multilocus genotypes
##    694 haploid individuals
##     13 codominant loci
##
## Population information:
##
##      3 strata - Tree, Year, Symptom
##      6 populations defined - 7_9, 26_9, 26_10, 45_10, 79_10, 26_11
```

To analyze the data according to Symptom:

```
setPop(monpop) <- ~Symptom
monpop
```

```
##
## This is a genclone object
## -------------------------
## Genotype information:
##
##    264 original multilocus genotypes
##    694 haploid individuals
##     13 codominant loci
##
## Population information:
##
##      3 strata - Tree, Year, Symptom
##      2 populations defined - BB, FR
```

Order of the levels that you define is important, so if we wanted to define the symptoms according to tree, we would use the following:

```
setPop(monpop) <- ~Symptom/Tree
monpop
```

```
##
## This is a genclone object
## -------------------------
## Genotype information:
##
```

```
##    264 original multilocus genotypes
##    694 haploid individuals
##     13 codominant loci
##
## Population information:
##
##      3 strata - Tree, Year, Symptom
##      8 populations defined - BB_7, BB_26, FR_26, ..., BB_79, FR_79, FR_45
```

Now that we have laid out the basics of manipulating data by strata, we will now apply strata for clone correction.

Clone correction When dealing with clonal populations, analyses are typically conducted with and without clone correction. Clone correction is a method of censoring a data set such that only one individual per MLG is represented per population (Milgroom, 1996; Grünwald et al., 2003; Grünwald & Hoheisel, 2006). This technique is commonly used with the index of association and genotypic diversity measures since clone corrected populations approximate behavior of sexual populations. Since we want to only observe unique genotypes per population, clone correction requires specification of the stratifications at which clones should be censored. This section will show how to clone correct at a specific stratification and also compare the results with uncorrected data.

Question: Will allelic diversity increase or decrease with clone-censored data? Using monpop as an example, if we wanted to know the diversity of alleles within each tree per year, how should we go about correcting for the clones? We use the function clonecorrect specifying the "Tree/Year" strata:

```
mcc_TY <- clonecorrect(monpop, strata = ~Tree/Year, keep = 1:2)
mcc_TY
```

```
##
## This is a genclone object
## -------------------------
## Genotype information:
##
##    264 original multilocus genotypes
##    278 haploid individuals
##     13 codominant loci
```

```
##
## Population information:
##
##      3 strata - Tree, Year, Symptom
##      6 populations defined - 7_9, 26_9, 26_10, 45_10, 79_10, 26_11
```

Notice that the number of samples reduced from 694 to 278, but is still more than the number of MLGs. This indicates that there are duplicated genotypes that cross trees and years, but that's okay because of our definition of a clone-corrected data set as having one representative genotype per population. Before we continue, we should set the original data to the same strata:

```
setPop(monpop) <- ~Tree/Year
```

Now we can compare the diversity of alleles at each locus using Simpson's index (1–D1–D) as implemented in the function locus_table (Detailed in our chapter on locus based statistics). We will do this in three steps:

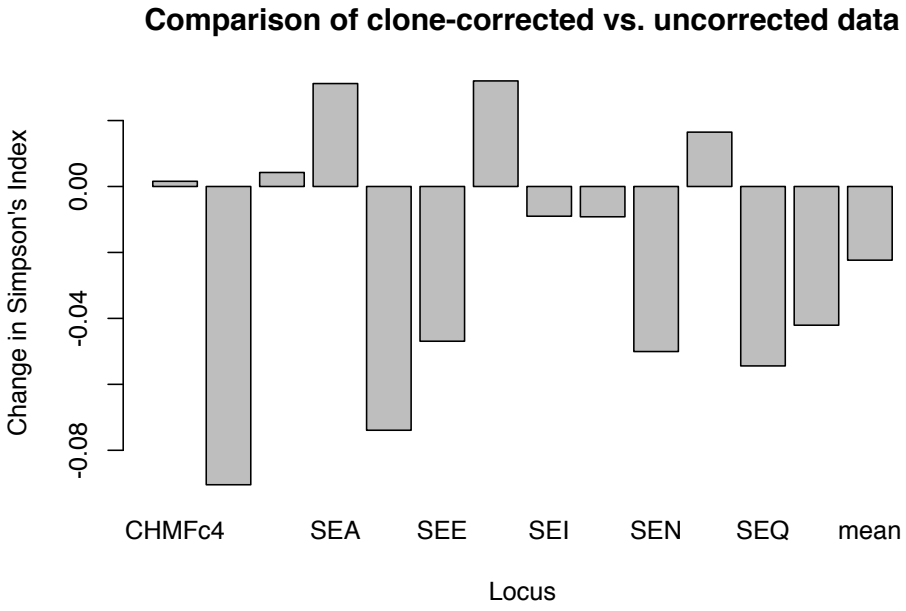
Calculate diversity of the clone corrected data Calculate diversity of the uncorrected data Take the difference of step 1 from step 2.

```
cc <- locus_table(mcc_TY, info = FALSE)
mp <- locus_table(monpop, info = FALSE)
mp - cc
```

```
##          summary
## locus     allele      1-D     Hexp Evenness
##   CHMFc4       .  0.00158  0.00031  0.00160
##   CHMFc5       . -0.09045 -0.09114 -0.04291
##   CHMFc12      .  0.00425  0.00312  0.03873
##   SEA          .  0.03122  0.02983  0.08509
##   SED          . -0.07392 -0.07564 -0.07563
##   SEE          . -0.04693 -0.04770 -0.02642
##   SEG          .  0.03202  0.03071  0.04818
##   SEI          . -0.00902 -0.01053  0.03060
##   SEL          . -0.00919 -0.01071 -0.05397
##   SEN          . -0.05006 -0.05176 -0.07643
##   SEP          .  0.01648  0.01511  0.04087
##   SEQ          . -0.05442 -0.05620 -0.08707
##   SER          . -0.04207 -0.04374 -0.02363
```

```
##   mean         . -0.02235 -0.02372 -0.01085
```

```
locus_diff <- mp - cc

# Note that I need to select the column containing Simpson's Index. That's
# labeled as "1-D".
barplot(locus_diff[, "1-D"], ylab = "Change in Simpson's Index", xlab = "Locus",
        main = "Comparison of clone-corrected vs. uncorrected data")
```



We can see quite a difference in some loci after clone correcting based on tree in the overall data set showing that, while some loci show a decrease, many loci show an increase in allelic diversity after clone-correction.

Advanced R: writing your own functions for analysis by stratum Of course, we still want to analyze each tree/year combination separately. Instead of typing those commands above for each combination, we can write a function to do it for us.

A function can be thought of as a set of instructions that tells the computer what to do. You have been using functions before such as poppr(). A function is written like this:

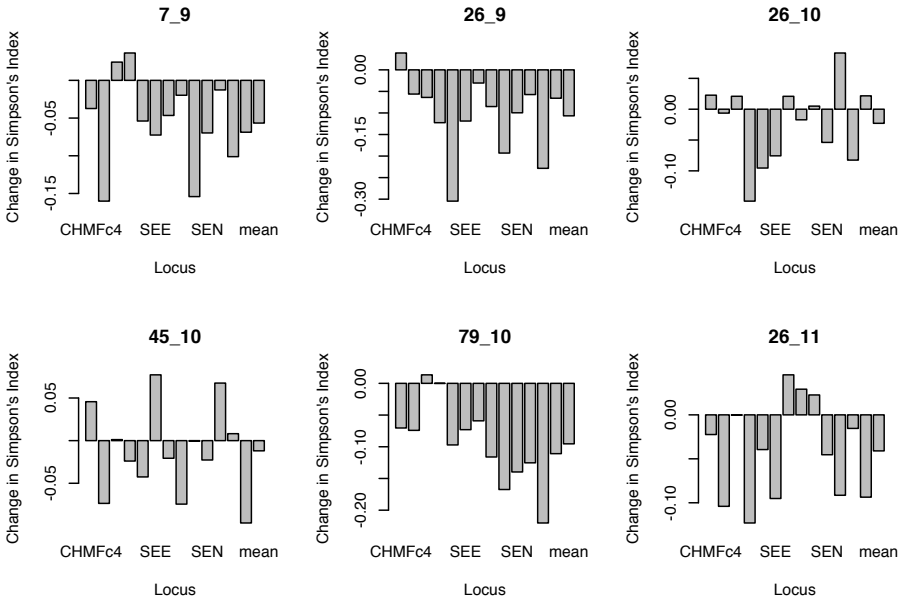
Writing a function for comparing clone corrected and uncorrected data

We will simply take the steps from above and turn them into a function to calculate the difference in Simpson's diversity for a given population. In order to do that, we will need to compute three steps:

the name of the population the clone-corrected data set the uncorrected data set From there, we can construct the function.

```
plot_simp_diff <- function(pop_name, clone_corrected, un_corrected){

  # Step 1: calculate diversity for clone-corrected data
  cc <- locus_table(clone_corrected, pop = pop_name, info = FALSE)

  # Step 2: calculate diversity for uncorrected data
  uc <- locus_table(un_corrected, pop = pop_name, info = FALSE)

  # Step 3: Take the difference
  res <- uc - cc

  # Step 4: Plot Simpson's index.
  barplot(res[, "1-D"], main = pop_name, ylab = "Change in Simpson's Index", xlab = "Locus")
}
```

```
par(mfrow = c(2, 3)) # Set up the graphics to have two rows and three columns

for (i in popNames(monpop)){
  plot_simp_diff(i, mcc_TY, monpop)
}
```




```
par(mfrow = c(1, 1)) # Next we reset the graphics to have one row and one column
```

These barplots show the difference in Simpson's index of original minus clone corrected data for each population per locus. We can see that allelic diversity generally is lower in the total data set (containing some repeated MLGs) relative to clone corrected data.

Conclusions This was a brief introduction to the easiest way to create stratifications and apply them in poppr to more rapidly analyze your data. By indexing the stratifications of your data, you can set the stratification(s) you want to have analyzed in a single command. This approach avoids having to create new sub-sets of the data for each analysis and simultaneously reduces the chance of error when manipulating data sets by hand.

References Everhart S., Scherm H. 2015. Fine-scale genetic structure of Monilinia fructicola during brown rot epidemics within individual peach tree canopies. Phytopathology 105:542–549. Available at: https://doi.org/10.1094/PHYTO-03-14-0088-R

Grünwald NJ., Goodwin SB., Milgroom MG., Fry WE. 2003. Analysis of genotypic diversity data for populations of microorganisms. Phytopathology 93:738–746. Available at: http://apsjournals.apsnet.org/doi/abs/10.1094/PHYTO.2003.93.6.738

Grünwald NJ., Hoheisel G-A. 2006. Hierarchical analysis of diversity, selfing, and genetic differentiation in populations of the oomycete Aphanomyces euteiches. Phytopathology 96:1134–1141. Available at: http://apsjournals.apsnet.org/doi/abs/10.1094/PHYTO-96-1134

Milgroom MG. 1996. Recombination and the multilocus structure of fungal populations. Annual Review of Phytopathology 34:457–477. Available at: http://www.annualreviews.org/doi/abs/10.1146/annurev.phyto.34.1.457

Nielsen R., Slatkin M. 2013. An introduction to population genetics: Theory and applications. Sinauer Associates, Incorporated. Available at: http://books.google.com/books?id=Iy08kgEACAAJ

Locus stats, heterozygosity, HWE ZN Kamvar, SE Everhart, and NJ Grünwald A rigorous population genetic analysis looks closely at the data to assess quality and identify outliers or problems in the data such as erroneous allele calls. This chapter focuses on analysis on a per-locus level. While there are statistics that analyze populations across loci, it is important to analyze each locus independently to make sure that one locus is not introducing bias or spurious errors into the analysis.

Note: Many of these statistics are specific to co-dominant data. Locus summary statistics A quick way to assess quality of the data is to determine the number, diversity, expected heterozygosity, and evenness of the alleles at each locus. As an example, we will use data for the fungal-like protist Phytophthora infestans from (Goss et al., 2014). First, we'll use the function locus_table to get all of the statistics mentioned above. For documentation on this function type ?locus_table. Here is a first look at each locus:

```
library(magrittr)  # We will also use magrittr for part of this chapter
data(Pinf)         # P. infestans data set from Mexico and South America
locus_table(Pinf)
```

```
##
## allele = Number of observed alleles
## 1-D = Simpson index
## Hexp = Nei's 1978 gene diversity
## ----------------------------------------

##       summary
## locus  allele    1-D   Hexp Evenness
```

```
##   Pi02 10.000  0.633  0.637    0.663
##   D13  25.000  0.884  0.889    0.587
##   Pi33  2.000  0.012  0.012    0.322
##   Pi04  4.000  0.578  0.582    0.785
##   Pi4B  7.000  0.669  0.672    0.707
##   Pi16  6.000  0.403  0.406    0.507
##   G11  21.000  0.839  0.844    0.544
##   Pi56  3.000  0.361  0.363    0.707
##   Pi63  3.000  0.413  0.415    0.641
##   Pi70  3.000  0.279  0.281    0.580
##   Pi89 11.000  0.615  0.619    0.578
##   mean  8.636  0.517  0.520    0.602
```

We can see here that we have a widely variable number of alleles per locus and that we actually have a single locus that only has two alleles, Pi33. This locus also has low diversity, low expected heterozygosity and is very uneven in allele distribution. This is a sign that this might be a phylogenetically uninformative locus, where we have two alleles and one is occurring at a minor frequency. We will explore analysis with and without this locus. Let's first see if both of these alleles exist in both populations of this data set.

```
locus_table(Pinf, pop = "North America")
```

```
##
## allele = Number of observed alleles
## 1-D = Simpson index
## Hexp = Nei's 1978 gene diversity
## ----------------------------------------

##       summary
## locus  allele    1-D   Hexp Evenness
##   Pi02  9.000  0.690  0.697    0.653
##   D13  21.000  0.895  0.906    0.684
##   Pi33  2.000  0.021  0.021    0.353
##   Pi04  4.000  0.545  0.551    0.764
##   Pi4B  5.000  0.596  0.603    0.736
##   Pi16  6.000  0.425  0.430    0.498
##   G11  15.000  0.824  0.833    0.625
##   Pi56  3.000  0.335  0.338    0.647
```

```
##   Pi63  3.000  0.310  0.313    0.568
##   Pi70  2.000  0.203  0.205    0.595
##   Pi89 11.000  0.627  0.634    0.549
##   mean  7.364  0.497  0.503    0.607
```

```
locus_table(Pinf, pop = "South America")
```

```
##
## allele = Number of observed alleles
## 1-D = Simpson index
## Hexp = Nei's 1978 gene diversity
## ----------------------------------------
```

```
##       summary
## locus  allele   1-D  Hexp Evenness
##   Pi02   5.00  0.54  0.54     0.83
##   D13   13.00  0.83  0.84     0.67
##   Pi33   1.00     .     .
##   Pi04   4.00  0.61  0.62     0.81
##   Pi4B   7.00  0.70  0.71     0.78
##   Pi16   3.00  0.35  0.36     0.69
##   G11   14.00  0.80  0.81     0.63
##   Pi56   2.00  0.39  0.39     0.81
##   Pi63   3.00  0.50  0.51     0.73
##   Pi70   3.00  0.37  0.37     0.62
##   Pi89   2.00  0.48  0.49     0.97
##   mean   5.18  0.51  0.51     0.75
```

Phylogenetically uninformative loci We can see that the South American populations is fixed for one allele, thus it would not be a bad idea to remove that locus from downstream analyses. We can do this using the function informloci. This will remove loci that contain less than a given percentage of divergent individuals (the default is 2/N2/N, where NN equals the number of individuals in the data set).

```
nLoc(Pinf)  # Let's look at our data set, note how many loci we have.
```

```
## [1] 11
```

```
iPinf <- informloci(Pinf)
```

```
## cutoff value: 2.32558139534884 % ( 2 samples ).
```

```
## MAF         : 0.01
```

```
##
##  Found 1 uninformative locus
##  ============================
##  1 locus found with a cutoff of 2 samples :
##  Pi33
##  0 loci found with MAF < 0.01
```

```
nLoc(iPinf) # Note that we have 1 less locus
```

```
## [1] 10
```

```
poppr(Pinf)
```

```
##             Pop  N MLG eMLG    SE    H    G lambda   E.5  Hexp    Ia
## 1 South America 38  29 29.0 0.000 3.27 23.3  0.957 0.883 0.513 2.873
## 2 North America 48  43 34.5 0.989 3.69 34.9  0.971 0.871 0.503 0.223
## 3         Total 86  72 34.6 1.529 4.19 57.8  0.983 0.875 0.520 0.652
##    rbarD File
## 1 0.3446 Pinf
## 2 0.0240 Pinf
## 3 0.0717 Pinf
```

```
poppr(iPinf)
```

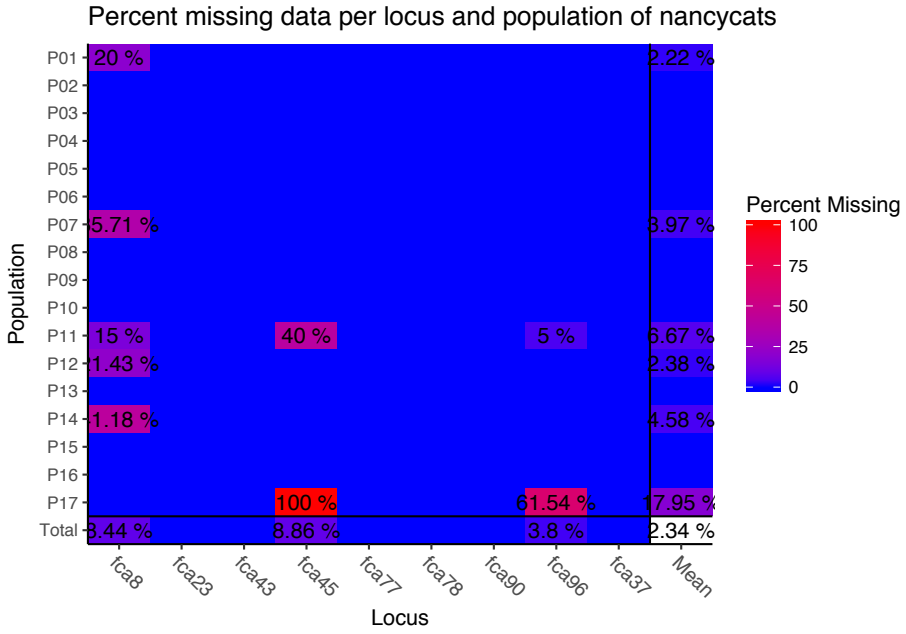
```
##             Pop  N MLG eMLG    SE    H    G lambda   E.5  Hexp    Ia
## 1 South America 38  29 29.0 0.000 3.27 23.3  0.957 0.883 0.565 2.873
## 2 North America 48  43 34.5 0.989 3.69 34.9  0.971 0.871 0.551 0.225
## 3         Total 86  72 34.6 1.529 4.19 57.8  0.983 0.875 0.571 0.655
##    rbarD  File
## 1 0.3446 iPinf
## 2 0.0255 iPinf
## 3 0.0750 iPinf
```

We can see that it increased ever so slightly for the “North America” and “To-

tal" populations, but not the "South America" population as expected given the fixed alleles at locus P33.

Missing data It is often important to asses the percentage of missing data. The poppr function info_table will help you visualize missing data so that you can assess how to treat these further using the function missingno. For this example, we will use the nancycats data set as it contains a wide variety of possibilities for missing data:

```
data(nancycats)
info_table(nancycats, plot = TRUE)
```




```
##           Locus
## Population  fca8 fca23 fca43 fca45 fca77 fca78 fca90 fca96 fca37  Mean
##       P01   0.200     .     .     .     .     .     .     .     . 0.022
##       P02       .     .     .     .     .     .     .     .     .     .
##       P03       .     .     .     .     .     .     .     .     .     .
##       P04       .     .     .     .     .     .     .     .     .     .
##       P05       .     .     .     .     .     .     .     .     .     .
##       P06       .     .     .     .     .     .     .     .     .     .
##       P07   0.357     .     .     .     .     .     .     .     . 0.040
##       P08       .     .     .     .     .     .     .     .     .     .
```

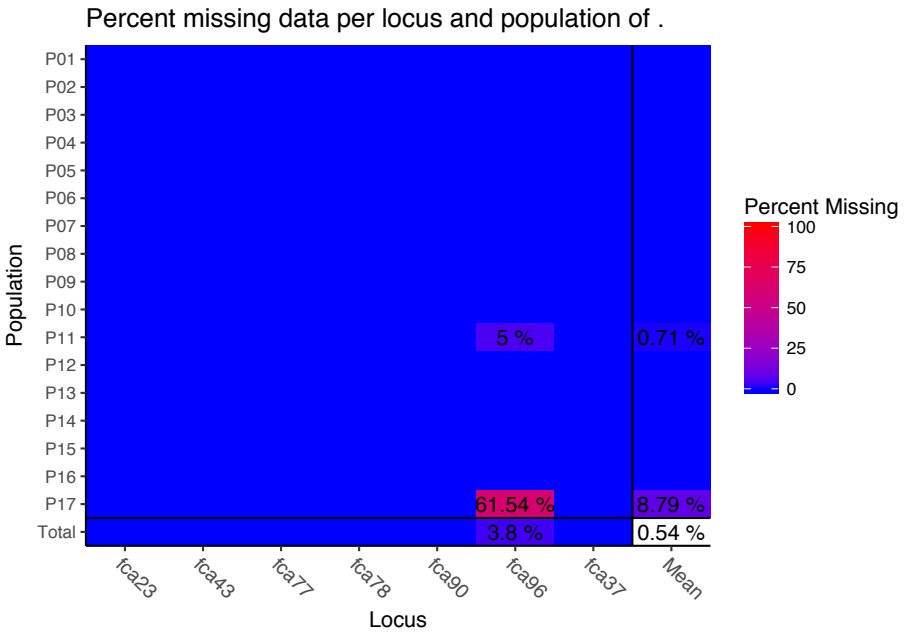
```
##      P09       .     .     .     .     .     .     .     .     .     .
##      P10       .     .     .     .     .     .     .     .     .     .
##      P11   0.150     .     . 0.400     .     .     . 0.050     . 0.067
##      P12   0.214     .     .     .     .     .     .     .     . 0.024
##      P13       .     .     .     .     .     .     .     .     .     .
##      P14   0.412     .     .     .     .     .     .     .     . 0.046
##      P15       .     .     .     .     .     .     .     .     .     .
##      P16       .     .     .     .     .     .     .     .     .     .
##      P17       .     .     . 1.000     .     .     . 0.615     . 0.179
##      Total 0.084     .     . 0.089     .     .     . 0.038     . 0.023
```

Here we see a few things. The data set has an average of 2.34% missing data overall. More alarming, perhaps is the fact that population 17 has not been genotyped at locus fca45 at all and that locus fca8 shows missing data across many populations. Many analyses in poppr can be performed with missing data in place as it will be either considered an extra allele in the case of MLG calculations or will be interpolated to not contribute to the distance measure used for the index of association. If you want to specifically treat missing data, you can use the function missingno to remove loci or individuals, or replace missing data with zeroes or the average values of the locus.

Removing loci and genotypes When removing loci or genotypes, you can specify a cutoff representing the percent missing to be removed. The default is 0.05 (5%).

```
nancycats %>% missingno("loci") %>% info_table(plot = TRUE, scale = FALSE)
```

```
##
## Found 617 missing values.
##
## 2 loci contained missing values greater than 5%
##
## Removing 2 loci: fca8, fca45
```




```
##           Locus
## Population fca23 fca43 fca77 fca78 fca90  fca96 fca37   Mean
##       P01      .     .     .     .     .      .     .      .
##       P02      .     .     .     .     .      .     .      .
##       P03      .     .     .     .     .      .     .      .
##       P04      .     .     .     .     .      .     .      .
##       P05      .     .     .     .     .      .     .      .
##       P06      .     .     .     .     .      .     .      .
##       P07      .     .     .     .     .      .     .      .
##       P08      .     .     .     .     .      .     .      .
##       P09      .     .     .     .     .      .     .      .
##       P10      .     .     .     .     .      .     .      .
##       P11      .     .     .     .     . 0.0500     . 0.0071
##       P12      .     .     .     .     .      .     .      .
##       P13      .     .     .     .     .      .     .      .
##       P14      .     .     .     .     .      .     .      .
##       P15      .     .     .     .     .      .     .      .
##       P16      .     .     .     .     .      .     .      .
##       P17      .     .     .     .     . 0.6154     . 0.0879
##       Total    .     .     .     .     . 0.0380     . 0.0054
```

2.1 Evolution of Genetic Systems

2.2 Phylogenetics

2.3 Game Theory

2.4 Genetic Algorithms

2.5 Evolutionary Algorithms

2.6 Hardy-Weinberg

2.7 Linkage Disequilibrium

3

Quantitative Genetics

- gene expression and epigenetic markers
- gene-environment interaction

http://nitro.biosci.arizona.edu/zbook/book.html

3.1 QTL analysis

Quantitative Trait Loci (QTL) are regions in the genome that are associated with variation in a quantitative trait. Quantitative traits are phentoypes that can be measured on a continuous scale, like height, weight, etc.

QTL analysis (or QTL mapping) is typically done on experimental populations to find genes which contribute to the heritability of traits. Phenotype and genetic marker data are collected from every individual in the population. The general concept of QTL mapping is that we can then calculate the correlation between genotypes and phenotypes at each marker position and test whether they show a statistically significant association.

Let's consider the famous example of Doebley and Stec (1991), who assessed the variation of traits that discriminate commercial maize from its native relative teosinte. Teosinte is much smaller than maize as we know it today and one teosinte plant produces many ears, each of which has only two rows of seeds. But even though maize and teosinte look so completely different, they are still able to produce viable offspring together. Doebley and Stec (1991) utilized this and crossed the two plant species to produce an F1 generation, which were in turn self-pollinated. The resulting F2 population of maize-teosinte-hybrids showed a wide range of intermediate parental mor-

phologies. Each of the F2 offspring was then genotyped at 58 locations in the genome, so that the quantitative trait information on morphology could be correlated with the genetic map. This analysis revealed that most of the morphological variation between maize and teosinte were the result of changes in only a handful of genes, one of which is the *tb1* (*teosinte branched 1*) gene.

3.1.1 Recombinant Inbred Lines (RILs)

RILs are experimental sister populations that have been produced by a very specific back-crossing scheme. The process is similar to Doebley and Stec's crossing of maize and teosinte: two homozygous parents are crossed to produce an F1 generation. Following the laws of genetics, each offspring's genome consists of a random combination of parental alleles and crossover (or recombination) events. Depending on the design, F1 offspring are usually either selfed or mated with a sibling to introduce another level of genetic recombination. The final generation is then inbred for many generations to obtain a collection of homozygous sister lines, each with a unique mosaic genome of parental alleles (Pollard, 2012).

3.1.2 QTL analysis in R

3.1.2.1 The qtl package

The most established R package for QTL mapping is Karl Broman's **qtl** package[1] (Broman et al., 2017). It implements several techniques for finding QTLs, like Hidden Markov Models (HMM), interval mapping, Haley-Knott regression and multiple imputation. It is very well documented and comes with extensive example data and code.

Here, I will introduce you to a basic QTL mapping workflow using the examples given in the package documentation and refer you to more complex analysis options where applicable.

[1] http://www.rqtl.org/

3.1.2.1.1 *Installation and loading the package*

If this is the first time you are using the **qtl** package, you need to install it from CRAN. The following line of code checks whether you already have the package, and if not installs it.

```
pkg = "qtl"
if (system.file(package = pkg) == '') install.packages(pkg)
```

You can then load the package:

```
library(qtl)
```

3.1.2.1.2 *Loading the data*

I will be using the example data on murine hypertension that is provided in the package (Sugiyama et al., 2001). In this dataset, we find information on 250 male mice from a reciprocal backcross between two strains: 1) the salt-sensitive C57BL/6J strain or 2) the inbred normotensive A/J strain. These mice were then given 1% salt water over two weeks and their hypertension blood pressure levels were measured.

```
data(hyper)
```

It is - as always - a good idea to familiarize yourself with the data to identify potential problems or errors before spending hours or days on an analysis that leads nowhere. The `summary()` function shows you the main properties of the data, like number of individuals and phenotypes, genotype information and proportion of missing data.

```
summary(hyper)
```

```
##     Backcross
##
##     No. individuals:    250
##
##     No. phenotypes:     2
```

```
##     Percent phenotyped: 100 100
##
##     No. chromosomes:    20
##         Autosomes:      1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
##         X chr:          X
##
##     Total markers:      174
##     No. markers:        22 8 6 20 14 11 7 6 5 5 14 5 5 5 11 6 12 4 4 4
##     Percent genotyped:  47.7
##     Genotypes (%):
##           Autosomes:    BB:50.1  BA:49.9
##        X chromosome:    BY:53.0  AY:47.0
```

```
plot(hyper)
```

The `plot()` function produces plots that show missing genotypes, marker positions and the distribution of phenotypes or traits. Figure 3.1 will give you a first idea about the quality of your data.

The package manual[2] includes a description of various additional plotting functions, which I won't cover here.

3.1.2.1.3 Genetic map estimation

Before we proceed with the analysis, I typically recommend replacing the existing genetic map with an estimated one to reduce the potential errors. The genetic map represents all markers on a chromosome in a linear fashion. The 'est.map() function applies a Hidden Markov Model (Lander and Green, 1987) to estimate the map with an assumed genotyping error rate (*error.prob*).

Here, we can also specify the mapping function (*map.function*) that we want to use to convert genetic distance to recombination fraction. The distance between two markers is usually given as a unit of genetic linkage, called *centimorgan (cM)* One cM represents the distance with an average of 0.01 crossover events in one generation (i.e. 1% recombination). However, this representation of distance underestimates the actual recombination frac-

[2] http://www.rqtl.org/tutorials/rqtltour.pdf

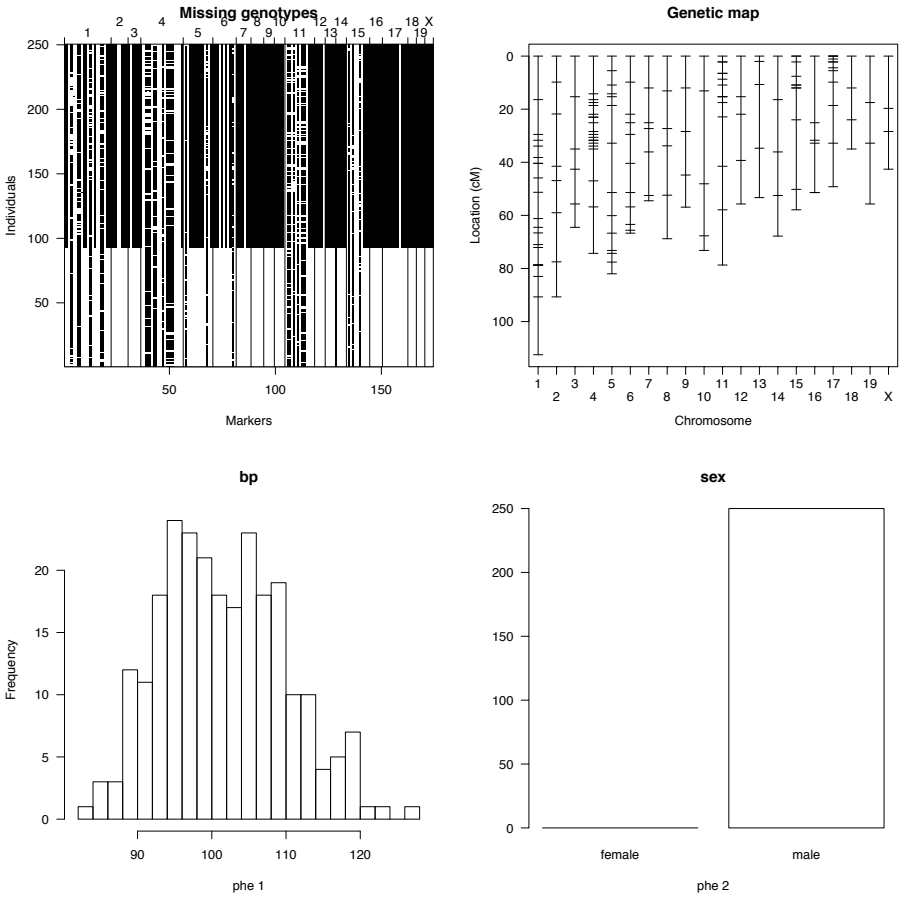


FIGURE 3.1

Plots providing an overview over missing genotypes, marker positions and the distribution of phenotypes or traits. Plots were generated with `qtl::plot()`. In the upper left plot markers are shown against individuals, with black areas showing missing information. As we can see here, there is quite a bit of missing data. The upper right plot shows the marker positions on the chromosomes. Here, we can easily see that chromosomes 1, 4, 11 and 17 have clusters of more densely positioned markers. The lower plots show phenotype information, either as a histogram for continuous values or as a bar chart for categorical factors.

tion, which is inherently not additive. With increasing distance the chance of double crossovers increases, so that they are in a way "invisible" to the traditional estimation of recombination distance.

Another reason why genetic maps based on recombination are biased is crossover interference, which described the phenomenon that a crossover event reduces the likelihood of another recombination event occur close by.

To correct for such biases, we can choose from the following mapping functions:

- **Haldane's** is the simplest mapping function and assumes a Poisson distribution for crossover events and does not consider interference.
- **Kosambi's** mapping function also considers interference and double crossovers but it can not calculate joint recombination probabilities for more than three loci.
- **Carter-Falconer's** mapping function can be extended to more complex interference rates.
- **Morgan's** mapping function assumes complete interference.

The two most widely used mapping functions are Haldane's (the default) and Kosambi's. For this example, using Haldane's should be sufficient. Because our example is a backcross, we can assume no interference, meaning that all crossovers are independent (Lynch and Walsh, 1998).

```
newmap <- est.map(hyper, error.prob = 0.0001,
                  map.function = "haldane")
hyper <- replace.map(hyper, newmap)
```

We can now estimate the recombination fractions between all pairs of markers. The `est.rf()` function also calculates the LOD scores. LOD stands for "likelihood of the odds" and is a measure of linkage. In QTL mapping we calculate LOD scores for the genetic markers and a threshold, above which we consider a QTL statistically significant in its association with the trait.

```
hyper <- est.rf(hyper)
```

The `calc.errorlod()` function calculates the genotyping errors according to Lincoln and Lander (1992). We can see which markers have an error LOD above a certain threshold (cutoff) with the `top.errorlod()` function (Table 3.1).

```
hyper <- calc.errorlod(hyper, error.prob = 0.0001)
te <- top.errorlod(hyper, cutoff = 3)
```

TABLE 3.1: Markers with an error LOD bigger than 3 under an error probability of 0.0001 indicate potential genotyping errors.

chr	id	marker	errorlod
4	102	D4Mit288	3.324582
4	107	D4Mit111	3.262205
4	216	D4Mit214	3.261092
11	57	D11Mit82	3.021105
11	118	D11Mit82	3.021105

These potentially erroneous markers can then be removed or set to NA.

```
hyper.clean <- hyper
for (i in 1:nrow(te)) {
  chr <- te$chr[i]
  id <- te$id[i]
  mar <- te$marker[i]
  hyper.clean$geno[[chr]]$data[hyper$pheno$id == id, mar] <- NA
}
```

3.1.2.1.4 *Finding QTLs*

Now, we can proceed with the central step: mapping the QTLs.

Because the individuals in QTL studies are genotyped at specific marker locations throughout the genome, we inherently have to deal with the missing information about genotypes between markers. Hidden Markov Models (HMM) can help us overcome this problem by calculating genotype probabilities between markers based on the joint genotype distribution.

We first need to calculate these genotype probabilities using the `calc.genoprob()` function. We can define several parameters, like step size, the amount of error we want to allow for, the mapping function

and step width. Here, we want to calculate genotype probabilities for every cM (step = 1), with a fixed step width and an error probability of 0.0001. As above, we are again using Haldane's mapping function.

```
hyper.clean <- calc.genoprob(hyper.clean, step = 1,
                             error.prob = 0.0001,
                             map.function = "haldane",
                             stepwidth = "fixed")
```

The simplest QTL model, we can run is single-QTL marker regression or interval mapping using the `scanone()` function.

These simple methods can give a good estimation of QTLs but they can also introduce bias, especially with multiple QTL in close proximity. More advanced mapping approaches, like Composite Interval Mapping (CIM) are discussed later on.

The first parameter we want to specify is the phenotype(s) and model (e.g. parametric or non-parametric) for mapping. Here, we want to use the first phenotype, i.e. the first column in our phenotype matrix, which follows a normal distribution. We can see the phenotype matrix by calling:

```
hyper.clean$pheno
```

Then, we need to specify the mapping algorithm we want to use. We can choose from several options. Here, I will only present the practical implications for each method. For a full discussion of the mathematical principles, see Lynch and Walsh (1998).

- **Marker regression**: Marker regression is by far the simplest approach to QTL mapping. Here, we calculate the association between phenotype and genotype at each marker position independently.

Because it is so simple, marker regression is seldom recommended to use. With interval mapping, a phenotype ~ genotype association analysis is performed for each flanking marker pair independently. This improves the approximation and gives confidence regions around QTL.

- **EM (Expectation-Maximization) algorithm**: EM is usually applied to maximum likelihood (ML) analyses of mixed models. It is an iterative process of calculating conditional probabilities and updating the ML estimates. This process is repeated until the estimates converge (Lander

and Botstein, 1989). If we have a reasonably dense marker map, the EM algorithm will converge on the global maximum.

- **(Extended) Haley-Knott regression**: Haley-Knott regression uses a simpler model than the EM algorithm (Haley and Knott, 1992). The extended Haley-Knott regression also considers variance is therefore gives improved approximations. Haley-Knott regression can give good approximations of the likelihood profiles for ML interval mapping but with more complex cases, it can be heavily biased.
- **Multiple imputation**: This method uses multiple rounds of imputing the unknown genotypes between markers and combines them into a final imputation model (Sen and Churchill, 2001). This allows us to perform a simple analysis of variance at each position in the genome. Multiple imputation needs much more computational power than simpler methods and will usually not outperform them with single-QTL models (it is much more advantageous with more complex multi-QTL models, however).

Here, I will show QTL mapping examples for the EM algorithm and for multiple imputation:

```
# EM algorithm
out.em <- scanone(hyper.clean, pheno.col = 1, model = "normal",
                  method = "em")
```

The multiple imputation method requires the use of the `sim.geno()` function before we call the mapping function. It calculates the joint genotype distribution based on the available marker information and uses it to perform the imputation of missin genotypes. With the `n.draws` parameter, we define how many imputations will be run. The more imputations steps we run, the more precise the genotypes but with increasing cost of computational time and power.

```
hyper.clean <- sim.geno(hyper.clean, step = 1, n.draws = 100,
                        error.prob = 0.0001,
                        map.function = "haldane",
                        stepwidth = "fixed")

out.imp <- scanone(hyper.clean, pheno.col = 1, model = "normal",
                   method = "imp")
```

Now that we have a LOD score for each marker position, we want to know

which positions are significantly associated with the phenotype. To determine this, we will use the **scanone()** function again, but this time we want to calculate the genome-wide LOD score threshold with a permutation test. Above this threshold we can consider a QTL to be statistically significant. Here, I am using a similar call as before, but I am specifying that we want to use 1000 permutations.

```
operm.imp <- scanone(hyper.clean, pheno.col = 1, model = "normal",
                     method = "imp", n.perm = 1000)
```

The `summary()` function will tell us our genome-wide LOD score threshold for a given significance value (here 0.05).

```
lod <- summary(operm.imp, alpha = 0.05)
lod
```

```
## LOD thresholds (1000 permutations)
##     lod
## 5% 2.34
```

And now we can also refine our QTL results by including the significance threshold. This will give us the LOD score and estimated p-values for each marker above the threshold, around which we can now assume to have found a QTL for our trait of interest.

```
summary(out.imp, perms = operm.imp, alpha = 0.05, pvalues = TRUE)
```

```
##           chr   pos  lod  pval
## c1.loc97    1 100.3 3.69 0.004
## D4Mit164    4  41.6 8.08 0.000
## D15Mit152  15  37.3 2.34 0.050
```

Now that we have our QTL, we can plot them with the `plot()` function. Here, I am plotting the results from both, the EM algorithm and the multiple imputation method. As expected, they do not differ much. The horizontal dotted line shows the genome-wide LOD threshold and our two significant QTL pop up nicely on chromosomes 1 and 4.

```
plot(out.em, col = "blue")
plot(out.imp, col = "red", add = TRUE)
abline(h = lod[1], lty = 2)
```

```
legend("topright", c("EM algorithm","Mult. imputation"),
       col = c("blue", "red"), lty = 1, lwd = 3)
```

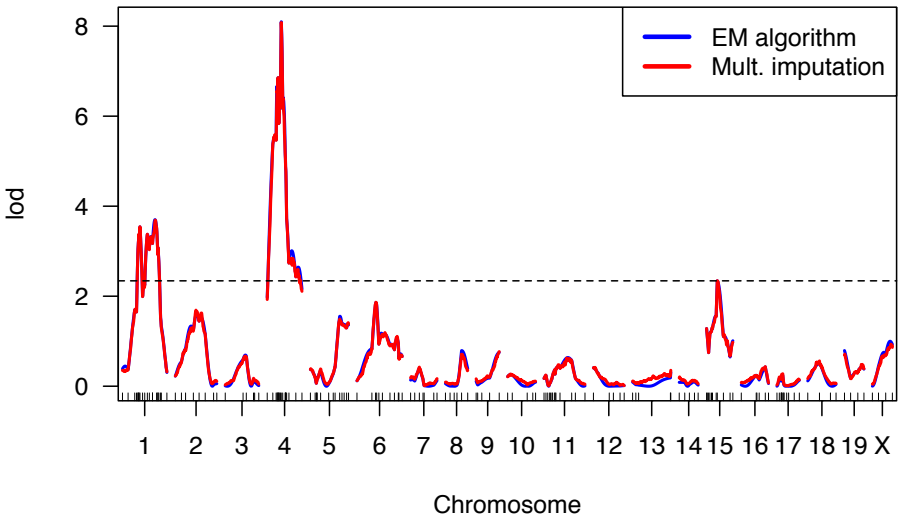


FIGURE 3.2
QTL plot showing LOD scores and QTL positions with EM algorithm and the multiple imputation. The dotted line indicates the genome-wide LOD score threshold for a $p < 0.05$. Every peak above this threshold is considered a QTL.

3.1.2.1.5 QTL interaction mapping

3.1.2.1.6 Covariates in QTL models

3.1.2.2 QTL Analysis using Bayesian Interval Mapping ("qtlbim" package)

3.2 Gene x Environment interactions

3.3 Variance and heritability

Exercises: Quantitative Genetics

4

Genetics of complex diseases

4.1 Genome Wide Association Studies (GWAS)

- design and analysis of genetic association studies

4.2 Pedigree analysis

5

Genetic Epidemiology

5.1 Infection models

A

More to Say

Yeah! I have finished my book, but I have more to say about some topics. Let me explain them in this appendix.

To know more about **bookdown**, see https://bookdown.org.

Bibliography

Broman, K. W., Wu, H., with ideas from Gary Churchill, Sen, S., contributions from Danny Arends, Corty, R., Flutre, T., Jansen, R., Prins, P., Ronnegard, L., Shah, R., Shannon, L., Tran, Q., Wolen, A., and Yandell, B. (2017). *qtl: Tools for Analyzing QTL Experiments*. R package version 1.41-6.

Doebley, J. and Stec, A. (1991). Genetic analysis of the morphological differences between maize and teosinte. *Genetics*, 129(1):285–295.

Haley, C. S. and Knott, S. A. (1992). A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity*, 69(4):315–324.

Lander, E. S. and Botstein, D. (1989). Mapping mendelian factors underlying quantitative traits using rflp linkage maps. *Genetics*, 121(1):185–199.

Lander, E. S. and Green, P. (1987). Construction of multilocus genetic linkage maps in humans. *Proceedings of the National Academy of Sciences*, 84(8):2363–2367.

Lincoln, S. E. and Lander, E. S. (1992). Systematic detection of errors in genetic linkage data. *Genomics*, 14(3):604 – 610.

Lynch, M. and Walsh, B. (1998). *Genetics and Analysis of Quantitative Traits*. Sinauer.

Pollard, D. A. (2012). *Design and Construction of Recombinant Inbred Lines*, pages 31–39. Humana Press, Totowa, NJ.

Sen, Ś. and Churchill, G. A. (2001). A statistical framework for quantitative trait mapping. *Genetics*, 159(1):371–387.

Sugiyama, F., Churchill, G. A., Higgins, D. C., Johns, C., Makaritsis, K. P., Gavras, H., and Paigen, B. (2001). Concordance of murine quantitative

trait loci for salt-induced hypertension with rat and human loci. *Genomics*, 71(1):70 – 77.

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