

polysat version 1.2 Tutorial Manual

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1 Introduction

The R package `polysat` provides useful tools for working with microsatellite data of any ploidy level, including populations of mixed ploidy. It can convert genotype data between different formats, including Applied Biosystems GeneMapper®, binary presence/absence data, ATetra, Tetra/Tetrasat, GenoDive, SPAGeDi, Structure, and POPDIST. It can also calculate pairwise genetic distances between samples, assist the user in estimating ploidy based on allele number, and estimate allele frequencies and F_{ST} . Due to the versatility of the R programming environment and the simplicity of how genotypes are stored by `polysat`, the user may find many ways to interface other R functions with this package, such as Principal Coordinate Analysis or AMOVA.

This manual is written to be accessible to beginning users of R. If you are a complete novice to R, it is recommended that you read through *An Introduction to R* (<http://cran.r-project.org/manuals.html>) before reading this manual or at least have both open at the same time. If you have the console open while reading the manual you can also look at the help files for base R functions (for example by typing `?save` or `?%in%`) and also get more detailed information on `polysat` functions (e.g. `?read.GeneMapper`).

The examples will be easiest to understand if you follow along with them and think about the purpose of each line of code. A file called “`polysattutorial.R`” in the “`doc`” subdirectory of the package installation can be opened with a text editor and contains all of the R input found in this manual.

2 Obtaining and installing `polysat`

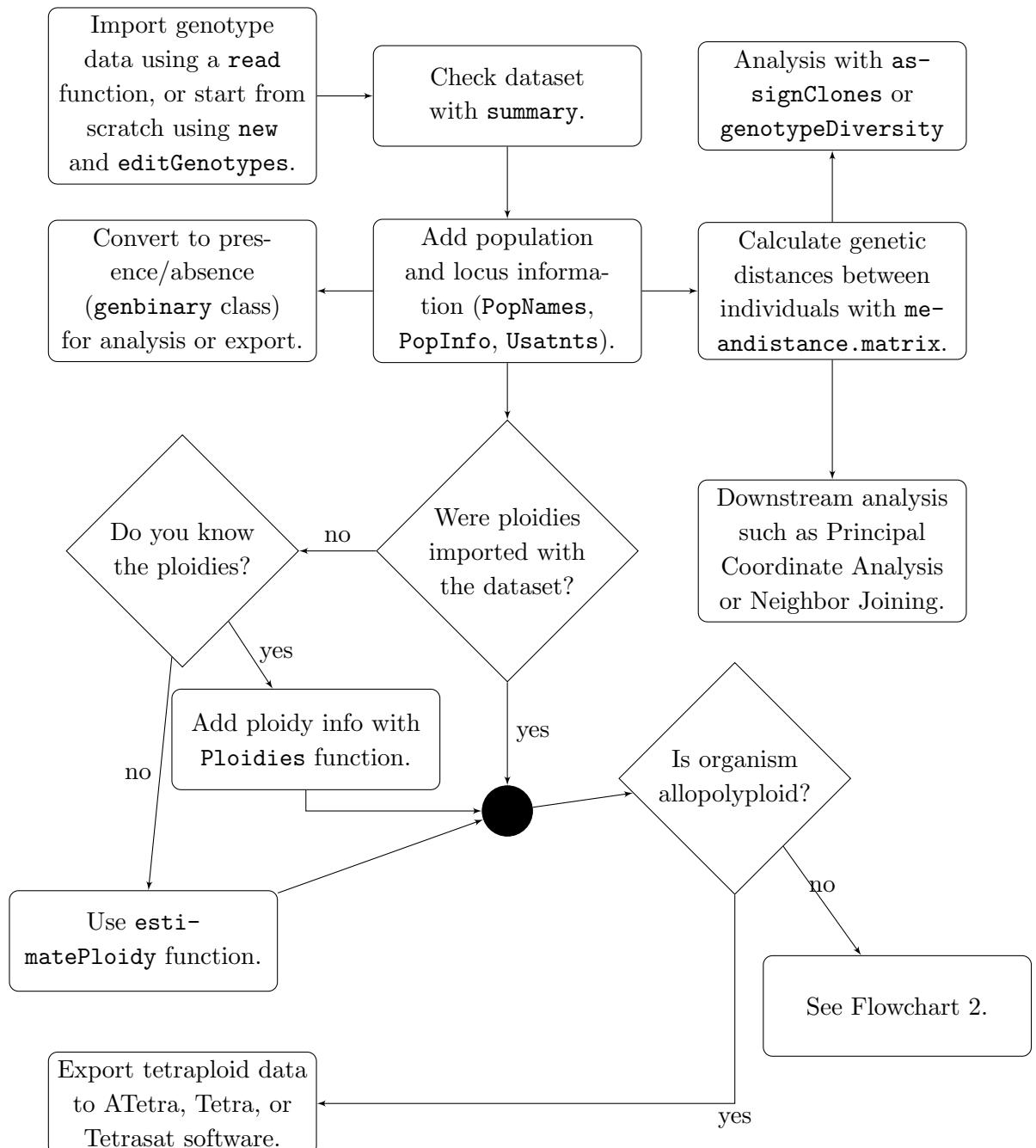
The R console and base system can be obtained at <http://www.r-project.org/>. Once installed, `polysat` can be installed and loaded by typing the following commands into the R console:

```
> install.packages("combinat")
> install.packages("polysat")
> library("polysat")
```

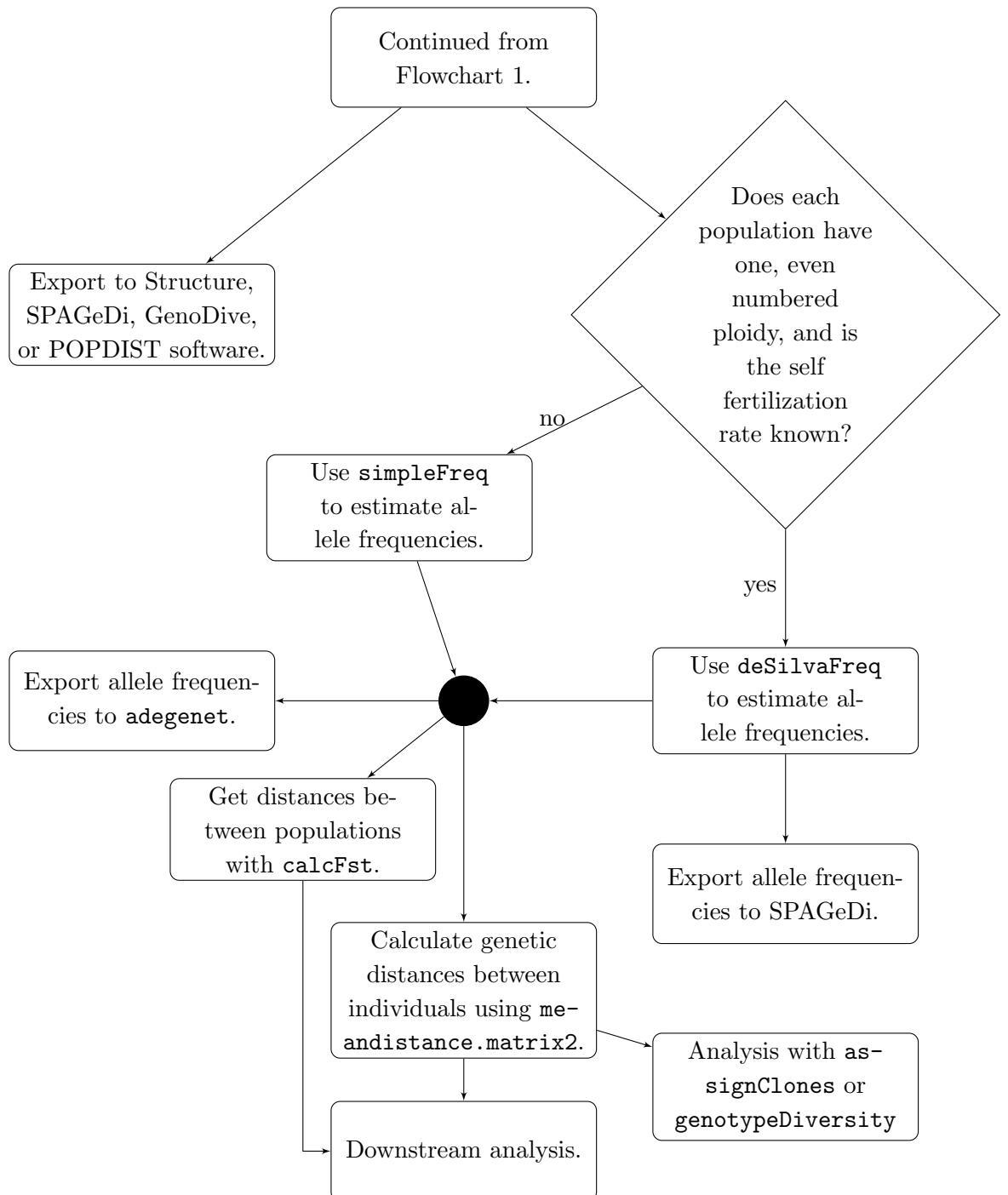
If you quit and restart R, you will not have to re-install the package but you might need to load it again (using the `library` function as shown above).

3 Workflow overview

The flowcharts on the next two pages give an overview of the steps required for the most common analyses performed in `polysat`. The first steps will always be importing or inputting the genotype data and making sure that the dataset contains information about populations and microsatellite repeat lengths. Different analysis and export functions are then available depending on whether the ploidy is known, whether the organism is autopolyploid or allopolyploid, and whether the selfing rate is known.



Flowchart 1. Functions for allopolyplloid or autopolyplloid data.



Flowchart 2. Functions for autopolypliod data only.

4 Getting Started: A Tutorial

4.1 Creating a dataset

As with any genetic software, the first thing you want to do is import your data. For this tutorial, go into the “doc” directory of the polysat package installation, and find a file called “GeneMapperExample.txt”. Open this file in a text editor and inspect its contents. This file contains simulated genotypes of 300 diploid and tetraploid individuals at three loci. Move this text file into the R working directory. The working directory can be changed with the `setwd` function, or identified with the `getwd` function:

```
> getwd()  
[1] "c:/Users/lvclark/Rpackages/polysat/inst/doc"
```

Then read the file using the `read.GeneMapper` function, and assign the dataset a name of your choice (`simgen` in this example) by typing:

```
> simgen <- read.GeneMapper("GeneMapperExample.txt")
```

The dataset now exists as an object in R. The following commands display, respectively, some basic information about the dataset, the sample and locus names, a subset of the genotypes, and a list of which genotypes are missing.

```
> summary(simgen)  
  
Dataset with allele copy number ambiguity.  
Insert dataset description here.  
Number of missing genotypes: 5  
300 samples, 3 loci.  
1 populations.  
Ploidies: NA  
Length(s) of microsatellite repeats: NA
```

```
> Samples(simgen)
```

[1]	"A1"	"A2"	"A3"	"A4"	"A5"	"A6"	"A7"
[8]	"A8"	"A9"	"A10"	"A11"	"A12"	"A13"	"A14"
[15]	"A15"	"A16"	"A17"	"A18"	"A19"	"A20"	"A21"
[22]	"A22"	"A23"	"A24"	"A25"	"A26"	"A27"	"A28"
[29]	"A29"	"A30"	"A31"	"A32"	"A33"	"A34"	"A35"
[36]	"A36"	"A37"	"A38"	"A39"	"A40"	"A41"	"A42"
[43]	"A43"	"A44"	"A45"	"A46"	"A47"	"A48"	"A49"
[50]	"A50"	"A51"	"A52"	"A53"	"A54"	"A55"	"A56"
[57]	"A57"	"A58"	"A59"	"A60"	"A61"	"A62"	"A63"
[64]	"A64"	"A65"	"A66"	"A67"	"A68"	"A69"	"A70"
[71]	"A71"	"A72"	"A73"	"A74"	"A75"	"A76"	"A77"
[78]	"A78"	"A79"	"A80"	"A81"	"A82"	"A83"	"A84"
[85]	"A85"	"A86"	"A87"	"A88"	"A89"	"A90"	"A91"
[92]	"A92"	"A93"	"A94"	"A95"	"A96"	"A97"	"A98"
[99]	"A99"	"A100"	"B1"	"B2"	"B3"	"B4"	"B5"
[106]	"B6"	"B7"	"B8"	"B9"	"B10"	"B11"	"B12"
[113]	"B13"	"B14"	"B15"	"B16"	"B17"	"B18"	"B19"
[120]	"B20"	"B21"	"B22"	"B23"	"B24"	"B25"	"B26"
[127]	"B27"	"B28"	"B29"	"B30"	"B31"	"B32"	"B33"
[134]	"B34"	"B35"	"B36"	"B37"	"B38"	"B39"	"B40"
[141]	"B41"	"B42"	"B43"	"B44"	"B45"	"B46"	"B47"
[148]	"B48"	"B49"	"B50"	"B51"	"B52"	"B53"	"B54"
[155]	"B55"	"B56"	"B57"	"B58"	"B59"	"B60"	"B61"
[162]	"B62"	"B63"	"B64"	"B65"	"B66"	"B67"	"B68"
[169]	"B69"	"B70"	"B71"	"B72"	"B73"	"B74"	"B75"
[176]	"B76"	"B77"	"B78"	"B79"	"B80"	"B81"	"B82"
[183]	"B83"	"B84"	"B85"	"B86"	"B87"	"B88"	"B89"
[190]	"B90"	"B91"	"B92"	"B93"	"B94"	"B95"	"B96"
[197]	"B97"	"B98"	"B99"	"B100"	"C1"	"C2"	"C3"
[204]	"C4"	"C5"	"C6"	"C7"	"C8"	"C9"	"C10"
[211]	"C11"	"C12"	"C13"	"C14"	"C15"	"C16"	"C17"
[218]	"C18"	"C19"	"C20"	"C21"	"C22"	"C23"	"C24"
[225]	"C25"	"C26"	"C27"	"C28"	"C29"	"C30"	"C31"
[232]	"C32"	"C33"	"C34"	"C35"	"C36"	"C37"	"C38"
[239]	"C39"	"C40"	"C41"	"C42"	"C43"	"C44"	"C45"
[246]	"C46"	"C47"	"C48"	"C49"	"C50"	"C51"	"C52"

```

[253] "C53"  "C54"  "C55"  "C56"  "C57"  "C58"  "C59"
[260] "C60"  "C61"  "C62"  "C63"  "C64"  "C65"  "C66"
[267] "C67"  "C68"  "C69"  "C70"  "C71"  "C72"  "C73"
[274] "C74"  "C75"  "C76"  "C77"  "C78"  "C79"  "C80"
[281] "C81"  "C82"  "C83"  "C84"  "C85"  "C86"  "C87"
[288] "C88"  "C89"  "C90"  "C91"  "C92"  "C93"  "C94"
[295] "C95"  "C96"  "C97"  "C98"  "C99"  "C100"

> Loci(simgen)
[1] "loc1" "loc2" "loc3"

> viewGenotypes(simgen, samples = paste("A", 1:20,
+      sep = ""), loci = "loc1")

  Sample     Locus     Alleles
  A1      loc1      110      112      106
  A2      loc1      114      106      118      110
  A3      loc1      114      102      100      106
  A4      loc1      110      102      106      100
  A5      loc1      106      112
  A6      loc1      100      110      106
  A7      loc1      112      108
  A8      loc1      102      106
  A9      loc1      112
  A10     loc1      102      106      110      112
  A11     loc1      114      100      112
  A12     loc1      106      118
  A13     loc1      110      112
  A14     loc1      100      112      106
  A15     loc1      100      112      114
  A16     loc1      112      102      100
  A17     loc1      102      106
  A18     loc1      102      106
  A19     loc1      114      102      110      118
  A20     loc1      106      100      108

> find.missing.gen(simgen)

```

	Locus	Sample
1	loc1	B54
2	loc1	B80
3	loc2	B48
4	loc3	A42
5	loc3	C22

Additional information that isn't in "GeneMapperExample.txt" can be added directly to the dataset in R. The commands below add a description to the dataset, name three populations and assign 100 individuals to each, and indicate the length of the microsatellite repeats.

```
> Description(simgen) <- "Dataset for the tutorial"
> PopNames(simgen) <- c("PopA", "PopB", "PopC")
> PopInfo(simgen) <- rep(1:3, each = 100)
> Usatnts(simgen) <- c(2, 3, 2)
```

If you need help understanding what the `PopInfo` assignment means, type the following commands (results are hidden here for the sake of space):

```
> rep(1:3, each = 100)
> PopInfo(simgen)
```

Samples can now be retrieved by population. (Results hidden as above.)

```
> Samples(simgen, populations = "PopA")
```

The `Usatnts` assignment function above, indicates that loc1 and loc3 have dinucleotide repeats, while loc2 has trinucleotide repeats. The alleles are recorded here in terms of fragment length in nucleotides. If the alleles were instead recorded in terms of repeat number, the `Usatnts` values should be 1. These repeat lengths can be examined by typing:

```
> Usatnts(simgen)
```

loc1	loc2	loc3
2	3	2

To edit genotypes after importing the data:

Edit the alleles, then close the data editor window.

```
> simgen <- editGenotypes(simgen, maxalleles = 4)
```

Edit the alleles, then close the data editor window.

You can also add ploidy information to the dataset. The `estimatePloidy` function allows you to add or edit the ploidy information, using a table that shows you the mean and maximum number of alleles per sample. The samples in this dataset should be diploid or tetraploid, although many of them may have fewer alleles. Therefore, in the data editor that is generated by the command below, you should change `new.ploidy` values to 2 if the sample has a maximum of one allele per locus, and to 4 if a sample has a maximum of three alleles per locus. See `?Ploidies` or page 22 for a different way to edit ploidy values if they are already known.

```
> simgen <- estimatePloidy(simgen)
```

Edit the `new.ploidy` values, then close the data editor window.

Edit the `new.ploidy` values, then close the data editor window.

Take another look at the summary now that you have added this extra data.

```
> summary(simgen)
```

Dataset with allele copy number ambiguity.

Dataset for the tutorial

Number of missing genotypes: 5

300 samples, 3 loci.

3 populations.

Ploidies: 4 2

Length(s) of microsatellite repeats: 2 3

Now that you have your dataset completed, it is not a bad idea to save a copy of it. It will be automatically saved in your R workspace for use in subsequent R sessions. However, the `save` function creates a separate file containing a copy of the dataset (or any other R object), which can be useful as a backup against accidental changes or a copy to open on another computer. The file containing the dataset can be opened again at a later date using the `load` function.

```
> save(simgen, file = "simgen.RData")
```

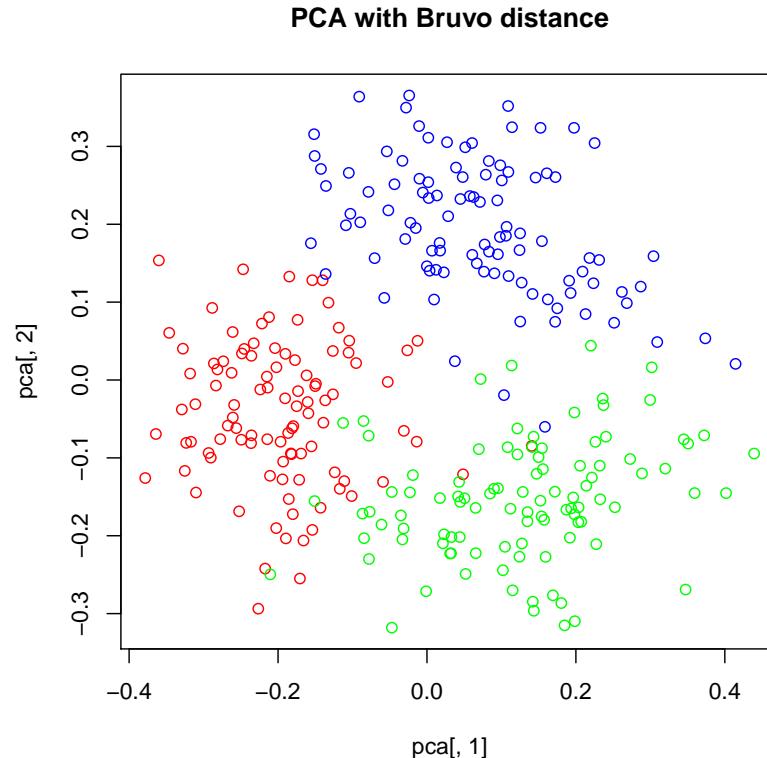
4.2 Data analysis and export

4.2.1 Genetic distances between individuals

The code below calculates a pairwise distance matrix between all samples (using the default distance measure `Bruvo.distance`), performs Principal Coordinate Analysis (PCA) on the matrix, and plots the first two principal coordinates, with each population represented by a different color.

```
> testmat <- meandistance.matrix(simgen)

> pca <- cmdscale(testmat)
> plot(pca[, 1], pca[, 2], col = rep(c("red", "green",
+ "blue"), each = 100), main = "PCA with Bruvo distance")
```



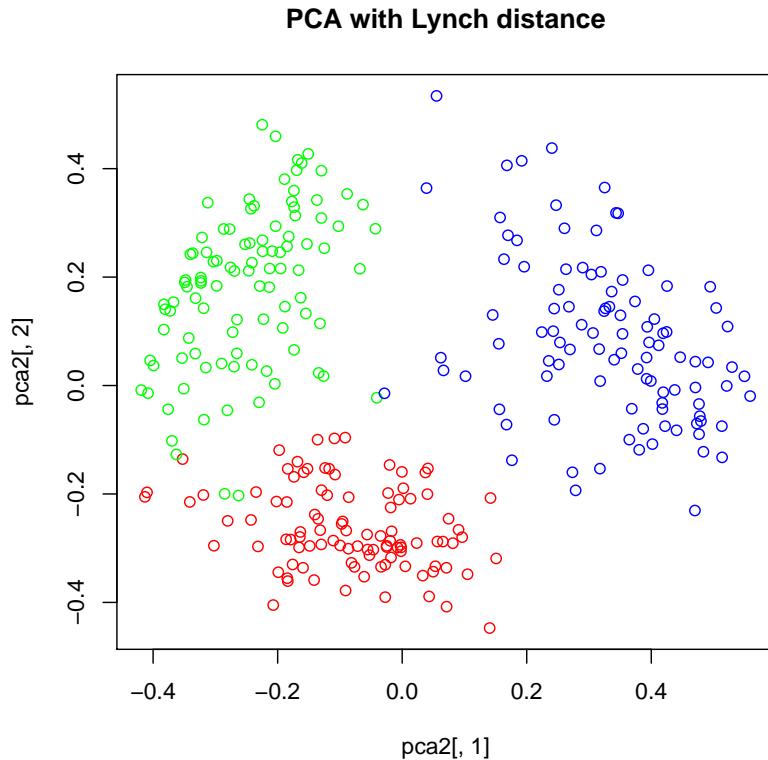
To conduct a PCA using the `Lynch.distance` measure, type:

```
> testmat2 <- meandistance.matrix(simgen, distmetric=Lynch.distance)
```

```

> pca2 <- cmdscale(testmat2)
> plot(pca2[, 1], pca2[, 2], col = rep(c("red",
+ "green", "blue"), each = 100), main = "PCA with Lynch distance")

```



`Bruvo.distance` takes mutation into account, while `Lynch.distance` does not. (See `?Bruvo.distance`, `?Lynch.distance`, and section 6.3.) Since mutation was not part of the simulation that generated this dataset, the latter measure works better here for distinguishing populations.

If your data are autopolyploid, also see `?meandistance.matrix2` for another method of calculating inter-individual distances.

4.2.2 Working with subsets of the data

It is likely that you will want to perform some analyses on just a subset of your data. There are several ways to accomplish this in `polysat`. The `deleteSamples` and `deleteLoci` functions are designed to be fairly intuitive.

```

> simgen2 <- deleteSamples(simgen, c("B59", "C30"))
> simgen2 <- deleteLoci(simgen2, "loc2")
> summary(simgen2)

```

Dataset with allele copy number ambiguity.

Dataset for the tutorial

Number of missing genotypes: 4

298 samples, 2 loci.

3 populations.

Ploidies: 4 2

Length(s) of microsatellite repeats: 2

There are also a couple methods that involve using vectors of samples and loci that you *do* want to use. Let's make a vector of samples in populations A and B that are tetraploid, and then exclude a few samples that we don't want to analyze.

```

> samToUse <- Samples(simgen2, populations = c("PopA",
+      "PopB"), ploidies = 4)
> exclude <- c("A50", "A78", "B25", "B60", "B81")
> samToUse <- samToUse[!samToUse %in% exclude]
> samToUse

[1] "A1"   "A2"   "A3"   "A4"   "A6"   "A10"  "A11"
[8] "A14"  "A15"  "A16"  "A19"  "A20"  "A24"  "A26"
[15] "A28"  "A29"  "A33"  "A34"  "A36"  "A37"  "A38"
[22] "A39"  "A41"  "A42"  "A43"  "A46"  "A48"  "A49"
[29] "A51"  "A57"  "A60"  "A61"  "A62"  "A63"  "A64"
[36] "A66"  "A68"  "A69"  "A70"  "A76"  "A79"  "A81"
[43] "A82"  "A83"  "A85"  "A86"  "A89"  "A90"  "A92"
[50] "A94"  "A97"  "A98"  "A99"  "B2"   "B3"   "B5"
[57] "B6"   "B10"  "B11"  "B12"  "B18"  "B19"  "B21"
[64] "B22"  "B23"  "B24"  "B26"  "B28"  "B29"  "B31"
[71] "B33"  "B37"  "B38"  "B40"  "B42"  "B43"  "B44"
[78] "B45"  "B46"  "B47"  "B48"  "B51"  "B53"  "B55"
[85] "B56"  "B63"  "B66"  "B67"  "B69"  "B70"  "B71"
[92] "B75"  "B76"  "B78"  "B79"  "B83"  "B87"  "B88"
[99] "B90"  "B91"  "B92"  "B95"  "B100"

```

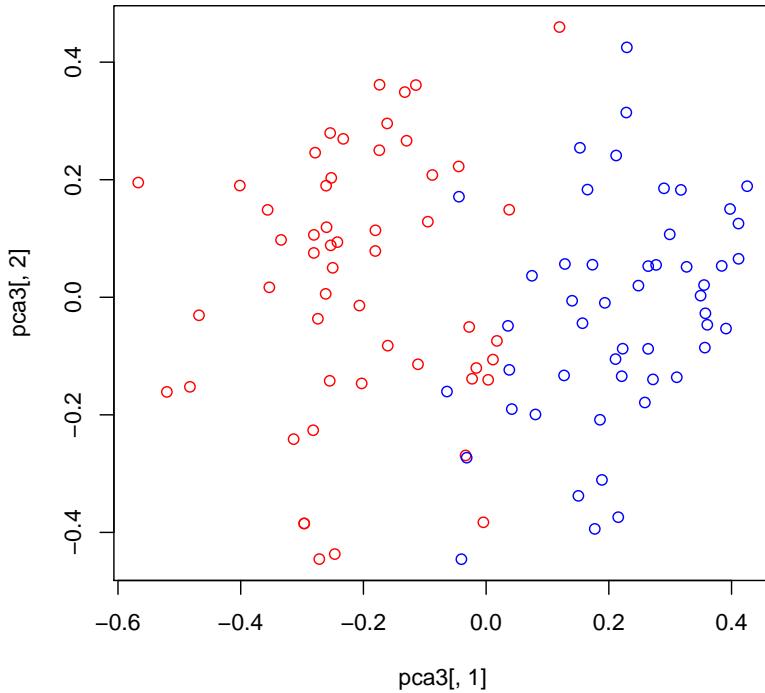
You can subscript the dataset with square brackets, like you can with many other R objects. Note, however, that in this case you can't use square brackets to replace a subset of the dataset, just to access a subset of the dataset. A vector of samples should be placed first in the brackets, followed by a vector of loci.

```
> summary(simgen2[samToUse, "loc1"])

Dataset with allele copy number ambiguity.
Dataset for the tutorial
Number of missing genotypes: 0
103 samples, 1 loci.
2 populations.
Ploidies: 4
Length(s) of microsatellite repeats: 2
```

The analysis and data export functions all have optional `samples` and `loci` arguments where vectors of sample and locus names can indicate that only a subset of the data should be used.

```
> testmat3 <- meandistance.matrix(simgen2, samples = samToUse,
+      distmetric = Lynch.distance, progress = FALSE)
> pca3 <- cmdscale(testmat3)
> plot(pca3[, 1], pca3[, 2], col = c("red", "blue")) [PopInfo(simgen2)[samToUse]]
```



(If you are confused about how I got the color vector, I would encourage dissecting it: See what `PopInfo(simgen2)` gives you, what `PopInfo(simgen2)[samToUse]` gives you, and lastly what the result of `c("red", "blue") [PopInfo(simgen2)[samToUse]]` is.)

4.2.3 Population statistics

Allele frequencies are estimated in the example below. The example then uses these allele frequencies to calculate pairwise Wright's F_{ST} [14] values, first using all loci and then just two of the loci. See Section 6.4.1 for important information about allele frequency estimation.

```
> simfreq <- deSilvaFreq(simgen, self = 0.1, initNull = 0.01,
+                               samples = Samples(simgen, ploidies = 4))

Starting loc1
Starting loc1 PopA
```

```

64 repetitions for loc1 PopA
Starting loc1 PopB
106 repetitions for loc1 PopB
Starting loc1 PopC
84 repetitions for loc1 PopC
Starting loc2
Starting loc2 PopA
54 repetitions for loc2 PopA
Starting loc2 PopB
94 repetitions for loc2 PopB
Starting loc2 PopC
89 repetitions for loc2 PopC
Starting loc3
Starting loc3 PopA
104 repetitions for loc3 PopA
Starting loc3 PopB
117 repetitions for loc3 PopB
Starting loc3 PopC
105 repetitions for loc3 PopC

```

> *simfreq*

Genomes	loc1.100	loc1.102	loc1.104	loc1.106	
PopA	212	0.1202992	0.12041013	0.00000000	0.2196366
PopB	208	0.00000000	0.16964161	0.09127732	0.0666518
PopC	180	0.1546742	0.01733696	0.24074235	0.00000000
	loc1.108	loc1.110	loc1.112	loc1.114	loc1.116
PopA	0.03591695	0.14287772	0.1542292	0.1251016	0.00000000
PopB	0.00000000	0.12865007	0.00000000	0.1251792	0.09286717
PopC	0.10203928	0.03436444	0.1477607	0.0749076	0.18553453
	loc1.118	loc1.null	loc2.143	loc2.146	loc2.149
PopA	0.07118362	0.01034496	0.00000000	0.16292064	0.00000000
PopB	0.30132333	0.02440948	0.39112389	0.05846641	0.1964645
PopC	0.02862591	0.01401403	0.09199651	0.12284567	0.1100339
	loc2.152	loc2.155	loc2.158	loc2.161	loc2.164
PopA	0.01937013	0.2277736	0.2318032	0.2269041	0.1208905
PopB	0.00000000	0.00000000	0.1737714	0.1586404	0.00000000

```

PopC 0.30329792 0.1475359 0.0000000 0.0000000 0.2080345
      loc2.null  loc3.210  loc3.212  loc3.214  loc3.216
PopA 0.01033780 0.08777834 0.0000000 0.1171561 0.07825934
PopB 0.02153341 0.0000000 0.15664870 0.0000000 0.0000000
PopC 0.01625563 0.21567201 0.06139389 0.0000000 0.13814503
      loc3.218  loc3.220  loc3.222  loc3.224  loc3.226
PopA 0.27813128 0.0000000 0.15201002 0.0000000 0.0000000
PopB 0.37855398 0.0000000 0.15477761 0.15861852 0.0000000
PopC 0.09445973 0.1538148 0.06183346 0.08256635 0.1684937
      loc3.228  loc3.230  loc3.null
PopA 0.05675610 0.20737987 0.02252894
PopB 0.02972989 0.08606954 0.03560175
PopC 0.00000000 0.00000000 0.02362112

> simFst <- calcFst(simfreq)
> simFst

          PopA      PopB      PopC
PopA 0.00000000 0.05068795 0.05453103
PopB 0.05068795 0.00000000 0.07098261
PopC 0.05453103 0.07098261 0.00000000

> simFst12 <- calcFst(simfreq, loci = c("loc1",
+      "loc2"))
> simFst12

          PopA      PopB      PopC
PopA 0.00000000 0.06004514 0.05597902
PopB 0.06004514 0.00000000 0.07356898
PopC 0.05597902 0.07356898 0.00000000

```

4.2.4 Genotype data export

Lastly, you may want to export your data for use in another program. Below is a simple example of data export for the software Structure. Additional export functions are described in sections 6.2 and 7.1. More details on the options for all of these functions are found in their respective help files.

In this example, both diploid and tetraploid samples are included in the file. The `ploidy` argument indicates how many lines per individual the file should have.

```
> write.Structure(simgen, ploidy = 4, file = "simgenStruct.txt")
```

5 How data are stored in polysat

In the tutorial above, you learned some ways of creating, viewing, and editing a dataset in polysat. This section goes into more details of the underlying data structure in polysat. This is particularly useful to understand if you want to extend the functionality of the package, but it may clear up some confusion for basic polysat users as well.

polysat uses the S4 class system in R. “Class” and “object” are two computer science terms that are introduced in Section 3 of *An Introduction to R*. Whenever you create a vector, data frame, matrix, list, etc. you are creating an object, and the class of the object defines which of these the object is. Furthermore, a class has certain “methods” defined for it so that the user can interact with the object in pre-specified ways. For example, if you use `mean` on a matrix, you will get the mean of all elements of the matrix, while if you use `mean` on a data frame, you will get the mean of each column; `mean` is a generic function with different methods for these two classes. S4 classes in R have “slots”, where each slot can hold an object of a certain class. Methods define how the user can access, replace, and manipulate the data in these slots.

5.1 The “genambig” class

The object that you created with the `read.GeneMapper` function in the tutorial is of the class “`genambig`”. This class has the slots `Description` (a character string or character vector describing the dataset), `Genotypes` (a two-dimensional list of vectors, where each vector contains all unique alleles for a particular sample at a particular locus), `Missing` (the symbol for a missing genotype), `Usatnts` (a vector containing the repeat length of each locus, or 1 if alleles for that locus are already in terms of repeat number rather than nucleotides), `Ploidies` (a vector containing the ploidy of each sample, or `NA` if unknown), `PopNames` (the name of each population), and `PopInfo`

(the population identity of each sample, using integers that correspond to the position of the population name in `PopNames`). You'll notice that there aren't slots to hold sample or locus names, which are stored as the `names` and `dimnames` of the objects in the other slots.

```
> showClass("genambig")

Class "genambig" [package "polysat"]

Slots:

Name:   Genotypes Description      Missing      Usatnts
Class:   array     character      ANY        integer

Name:   Ploidies   PopInfo   PopNames
Class: integer     integer   character

Extends: "gendata"
```

To create a "genambig" object from scratch without using one of the data import functions, first create two character vectors to contain sample and locus names, respectively. These vectors are then used as arguments to the `new` function.

```
> mysamples <- c("indA", "indB", "indC", "indD",
+      "indE", "indF")
> myloci <- c("loc1", "loc2", "loc3")
> mydataset <- new("genambig", samples = mysamples,
+      loci = myloci)
```

An object has now been created with all of the appropriate slots named according to sample and locus names.

```
> mydataset

An object of class "genambig"
Slot "Genotypes":
  loc1 loc2 loc3
```

```

indA -9    -9    -9
indB -9    -9    -9
indC -9    -9    -9
indD -9    -9    -9
indE -9    -9    -9
indF -9    -9    -9

Slot "Description":
[1] "Insert dataset description here."

Slot "Missing":
[1] -9

Slot "Usatnts":
loc1 loc2 loc3
NA   NA   NA

Slot "Ploidies":
indA indB indC indD indE indF
NA   NA   NA   NA   NA   NA

Slot "PopInfo":
indA indB indC indD indE indF
NA   NA   NA   NA   NA   NA

Slot "PopNames":
character(0)

```

In the tutorial you used some of the accessor and replacement functions for the "genambig" class. You can see a full list of them by typing:

```
> ?Samples
```

(Present and Absent are just for the "genbinary" class. More on that later.) Let's use some of these functions to fill in and examine the dataset.

```
> Loci(mydataset)
```

```

[1] "loc1" "loc2" "loc3"

> Loci(mydataset) <- c("L1", "L2", "L3")
> Loci(mydataset)

[1] "L1" "L2" "L3"

> Samples(mydataset)

[1] "indA" "indB" "indC" "indD" "indE" "indF"

> Samples(mydataset)[3] <- "indC1"
> Samples(mydataset)

[1] "indA" "indB" "indC1" "indD" "indE" "indF"

> PopNames(mydataset) <- c("Yosemite", "Sequoia")
> PopInfo(mydataset) <- c(1, 1, 1, 2, 2, 2)
> PopInfo(mydataset)

  indA  indB  indC1  indD  indE  indF
  1      1      1      2      2      2

> PopNum(mydataset, "Yosemite")

[1] 1

> PopNum(mydataset, "Sequoia") <- 3
> PopNames(mydataset)

[1] "Yosemite" NA          "Sequoia"

> PopInfo(mydataset)

  indA  indB  indC1  indD  indE  indF
  1      1      1      3      3      3

> Ploidies(mydataset) <- c(4, 4, 4, 4, 4, 6)
> Ploidies(mydataset)

```

```

indA  indB  indC1  indD  indE  indF
 4      4      4      4      4      6

> Ploidies(mydataset) ["indC1"] <- 6
> Ploidies(mydataset)

indA  indB  indC1  indD  indE  indF
 4      4      6      4      4      6

> Usatnts(mydataset) <- c(2, 2, 2)
> Usatnts(mydataset)

L1 L2 L3
 2  2  2

> Description(mydataset) <- "Tutorial, part 2."
> Description(mydataset)

[1] "Tutorial, part 2."

> Genotypes(mydataset, loci = "L1") <- list(c(122,
+      124, 128), c(124, 126), c(120, 126, 128, 130),
+      c(122, 124, 130), c(128, 130, 132), c(126,
+      130))
> Genotype(mydataset, "indB", "L3") <- c(150, 154,
+      160)
> Genotypes(mydataset)

          L1          L2 L3
indA  Numeric,3 -9 -9
indB  Numeric,2 -9 Numeric,3
indC1 Numeric,4 -9 -9
indD  Numeric,3 -9 -9
indE  Numeric,3 -9 -9
indF  Numeric,2 -9 -9

> Genotype(mydataset, "indD", "L1")

```

```
[1] 122 124 130
> Missing(mydataset)
[1] -9
> Missing(mydataset) <- -1
> Genotypes(mydataset)

      L1      L2 L3
indA Numeric,3 -1 -1
indB Numeric,2 -1 Numeric,3
indC1 Numeric,4 -1 -1
indD Numeric,3 -1 -1
indE Numeric,3 -1 -1
indF Numeric,2 -1 -1
```

If you know a little bit more about S4 classes, you know that you can access the slots directly using the `@` symbol, for example:

```
> mydataset@Genotypes

      L1      L2 L3
indA Numeric,3 -1 -1
indB Numeric,2 -1 Numeric,3
indC1 Numeric,4 -1 -1
indD Numeric,3 -1 -1
indE Numeric,3 -1 -1
indF Numeric,2 -1 -1

> mydataset@Genotypes[["indB", "L1"]]

[1] 124 126
```

However, I STRONGLY recommend against accessing the slots in this way in order to replace (edit) the data. The replacement functions are designed to prevent multiple types of errors that could happen if the user edited the slots directly.

In section 4.1 you were introduced to the `find.missing.gen` function. There is a related function called `isMissing` that may be more useful from a programming standpoint.

```

> isMissing(mydataset, "indA", "L2")
[1] TRUE

> isMissing(mydataset, "indA", "L1")
[1] FALSE

> isMissing(mydataset)

      L1     L2     L3
indA  FALSE  TRUE  TRUE
indB  FALSE  TRUE FALSE
indC1 FALSE  TRUE  TRUE
indD  FALSE  TRUE  TRUE
indE  FALSE  TRUE  TRUE
indF  FALSE  TRUE  TRUE

```

To add more samples or loci to your dataset, you can create a second "genambig" object and then use the `merge` function to join them.

```

> moredata <- new("genambig", samples = c("indG",
+      "indH"), loci = Loci(mydataset))
> Usatnts(moredata) <- Usatnts(mydataset)
> Description(moredata) <- Description(mydataset)
> PopNames(moredata) <- "Kings Canyon"
> PopInfo(moredata) <- c(1, 1)
> Ploidies(moredata) <- c(4, 4)
> Missing(moredata) <- Missing(mydataset)
> Genotypes(moredata, loci = "L1") <- list(c(126,
+      130, 136, 138), c(124, 126, 128))
> mydataset2 <- merge(mydataset, moredata)
> mydataset2

```

An object of class "genambig"

Slot "Genotypes":

	L1	L2	L3
indA	Numeric,3	-1	-1

```

indB  Numeric,2 -1 Numeric,3
indC1 Numeric,4 -1 -1
indD  Numeric,3 -1 -1
indE  Numeric,3 -1 -1
indF  Numeric,2 -1 -1
indG  Numeric,4 -1 -1
indH  Numeric,3 -1 -1

Slot "Description":
[1] "Tutorial, part 2."

Slot "Missing":
[1] -1

Slot "Usatnts":
L1 L2 L3
2 2 2

Slot "Ploidies":
indA  indB indC1  indD  indE  indF  indG  indH
4      4      6      4      4      6      4      4

Slot "PopInfo":
indA  indB indC1  indD  indE  indF  indG  indH
1      1      1      3      3      3      4      4

Slot "PopNames":
[1] "Yosemite"      NA                  "Sequoia"
[4] "Kings Canyon"

```

5.2 The “gendata” and “genbinary” classes

The “genambig” class is actually a subclass of another class called “gendata”. The `Description`, `PopInfo`, `PopNames`, `Ploidies`, `Missing`, and `Usatnts` slots, and their access and replacement methods, are all defined for “gendata”, and are inherited by “genambig”. The “genambig” class adds the `Genotypes` slot and the methods for interacting with it.

A second subclass of "gendata" is "genbinary". This class also has a **Genotypes** slot, but formatted as a matrix indicating the presence and absence of alleles. (See `?genbinary-class` for more details.) It also adds a slot called **Present** and one called **Absent** to indicate the symbols used to represent the presence or absence of the alleles, the same way the **Missing** slot holds the symbol used to indicate missing data. Like "genambig", "genbinary" inherits all of the slots from "gendata", as well as the methods for accessing them.

The code below creates a "genbinary" object using a conversion function, then demonstrates how the genotypes are stored differently and how the functions from "gendata" remain the same.

```
> simgenB <- genambig.to.genbinary(simgen)
> Genotypes(simgenB, samples = paste("A", 1:20,
+     sep = ""), loci = "loc1")

  loc1.100 loc1.102 loc1.104 loc1.106 loc1.108 loc1.110
A1      0      0      0      1      0      1
A2      0      0      0      1      0      1
A3      1      1      0      1      0      0
A4      1      1      0      1      0      1
A5      0      0      0      1      0      0
A6      1      0      0      1      0      1
A7      0      0      0      0      1      0
A8      0      1      0      1      0      0
A9      0      0      0      0      0      0
A10     0      1      0      1      0      1
A11     1      0      0      0      0      0
A12     0      0      0      1      0      0
A13     0      0      0      0      0      1
A14     1      0      0      1      0      0
A15     1      0      0      0      0      0
A16     1      1      0      0      0      0
A17     0      1      0      1      0      0
A18     0      1      0      1      0      0
A19     0      1      0      0      0      1
A20     1      0      0      1      1      0
```

	loc1.112	loc1.114	loc1.116	loc1.118
A1	1	0	0	0
A2	0	1	0	1
A3	0	1	0	0
A4	0	0	0	0
A5	1	0	0	0
A6	0	0	0	0
A7	1	0	0	0
A8	0	0	0	0
A9	1	0	0	0
A10	1	0	0	0
A11	1	1	0	0
A12	0	0	0	1
A13	1	0	0	0
A14	1	0	0	0
A15	1	1	0	0
A16	1	0	0	0
A17	0	0	0	0
A18	0	0	0	0
A19	0	1	0	1
A20	0	0	0	0

> *PopInfo(simgenB)* [Samples(*simgenB*, ploidies = 2)]

A5	A7	A8	A9	A12	A13	A17	A18	A21	A22	A23	A25
1	1	1	1	1	1	1	1	1	1	1	1
A27	A30	A31	A32	A35	A40	A44	A45	A47	A50	A52	A53
1	1	1	1	1	1	1	1	1	1	1	1
A54	A55	A56	A58	A59	A65	A67	A71	A72	A73	A74	A75
1	1	1	1	1	1	1	1	1	1	1	1
A77	A78	A80	A84	A87	A88	A91	A93	A95	A96	A100	B1
1	1	1	1	1	1	1	1	1	1	1	2
B4	B7	B8	B9	B13	B14	B15	B16	B17	B20	B25	B27
2	2	2	2	2	2	2	2	2	2	2	2
B30	B32	B34	B35	B36	B39	B41	B49	B50	B52	B54	B57
2	2	2	2	2	2	2	2	2	2	2	2
B58	B59	B61	B62	B64	B65	B68	B72	B73	B74	B77	B80

2	2	2	2	2	2	2	2	2	2	2	2	2	2
B82	B84	B85	B86	B89	B93	B94	B96	B97	B98	B99	C1		
2	2	2	2	2	2	2	2	2	2	2	2	2	3
C3	C4	C6	C7	C8	C10	C11	C14	C16	C17	C20	C21		
3	3	3	3	3	3	3	3	3	3	3	3	3	3
C23	C25	C27	C28	C31	C32	C36	C37	C38	C39	C40	C44		
3	3	3	3	3	3	3	3	3	3	3	3	3	3
C46	C47	C48	C50	C56	C57	C59	C61	C64	C67	C68	C71		
3	3	3	3	3	3	3	3	3	3	3	3	3	3
C74	C75	C76	C77	C79	C80	C82	C83	C84	C85	C86	C87		
3	3	3	3	3	3	3	3	3	3	3	3	3	3
C90	C92	C93	C95	C96	C98								
3	3	3	3	3	3								

The "genbinary" class exists to facilitate the import and export of genotype data formatted in a binary presence/absence format, for example:

```
> write.table(Genotypes(simgenB), file = "simBinaryData.txt")
```

The "genbinary" class is also used by polysat to make some of the allele frequency calculations easier. `simpleFreq` internally converts a "genambig" object to a "genbinary" object in order to tally allele counts in populations.

The class system in polysat is set up so that anyone can extend it to better suit their needs. There seem to be as many ways of formatting genotype data as there are population genetic software, and so a new subclass of "gendata" could be created with genotypes formatted in a different way. A user could also create a subclass of "genambig", for example to hold GPS or phenotypic data in addition to the data already stored in a "genambig" object. (See `?setClass`, `?setMethod`, and [2].)

6 Functions for autopolyploid data

In order to properly utilize polysat (and other software for polyploid data) it is important to understand the inheritance mode in your system. In an autopolyploid, all homologous chromosomes are equally capable of pairing with each other at meiosis, and thus at a given microsatellite locus, gametes can receive any combination of alleles from the parent. The same is not true

of allopolyploids. This affects the distribution of genotypes in the population, and as a result affects all aspects of population genetic analysis.

The functions described below are specifically for autopolyploid data. Their potential (or lack thereof) for use on allopolyploid data is described in the next section.

6.1 Data import

Four other population genetic programs that I am aware of can handle polyploid microsatellite data with allele copy number ambiguity under polysomic inheritance (autopolyploidy): Structure [5, 4, 15, 8], SPAGeDi [7], GenoDive [13] (<http://www.bentleydrummer.nl/software/software/GenoDive.html>), and POPDIST [6][18].

In the “doc” directory of the polysat installation there are files called “structureExample.txt”, “spagediExample.txt”, “genodiveExample.txt”, “POPDISTExample1.txt” and “POPDISTExample2.txt”. To import these into “genambig” objects, first copy them into your working directory, then perform the assignments:

```
> GDdata <- read.Genodive("genodiveExample.txt")
> Structdata <- read.Structure("structureExample.txt",
+      ploidy = 8)
> Spagdata <- read.SPAGeDi("spagediExample.txt")
> PDdata <- read.POPDIST(c("POPDISTExample1.txt",
+      "POPDISTExample2.txt"))
```

Use `summary`, `viewGenotypes`, and the accessor functions (section 5.1) to examine the contents of the three “genambig” objects that you have just created. All four of these functions take population information from the file and put it into the object. The Structure, SPAGeDi, and POPDIST files are coded in a way that indicates the ploidy of each individual, so this information is written to the “genambig” object as well.

The data import functions have some additional options for input and output, which are described in more detail in the help files. In particular, any extra columns can optionally be extracted from a Structure file, and the spatial coordinates can optionally be extracted from a SPAGeDi file.

```
> ?read.Structure
> ?read.SPAGeDi
```

polysat also supports two genotype formats that work for either autopolyploids or allopolyploids, but do not contain any population, ploidy, or other information: GeneMapper, and binary presence/absence. The tutorial in the beginning of this manual uses `read.GeneMapper` to import data. The “GeneMapperExample.txt” file contains the minimum amount of information needed in order to be read by the function. Full “Genotypes Table” files as exported from ABI GeneMapper® can also be read by `read.GeneMapper`, and further, the function can take a vector of file names rather than a single file name if the data are spread across multiple files. There are three additional GeneMapper example files in the “doc” directory, which can be read into a “genambig” object in this way:

```
> GMdata <- read.GeneMapper(c("GeneMapperCBA15.txt",
+ "GeneMapperCBA23.txt", "GeneMapperCBA28.txt"))
```

A binary presence/absence matrix can be read into R using the base function `read.table`. Arguments to this function give options about how the file is delimited and whether it has headers and/or row labels. The example file in the “doc” directory can be read in the following way:

```
> domdata <- read.table("dominantExample.txt", header = TRUE,
+ sep = "\t", row.names = 1)
```

Examine the data frame produced, and notice in particular that the column names are formatted as the locus and allele separated by a period. After this data frame is converted to a matrix, it can be used to create a “genbinary” object.

```
> domdata
```

	ABC1.123	ABC1.126	ABC1.129	ABC1.132	ABC1.135	ABC2.201
ind1	1	0	0	0	1	0
ind2	0	1	1	0	1	1
ind3	0	0	0	0	0	0
	ABC2.203	ABC2.205	ABC2.207	ABC2.209		
ind1	1	1	0	0		
ind2	1	1	1	0		
ind3	0	1	0	1		

```

> domdata <- as.matrix(domdata)
> PAdat <- new("genbinary", samples = c("ind1",
+     "ind2", "ind3"), loci = c("ABC1", "ABC2"))
> Genotypes(PAdat) <- domdata

```

A few functions in polysat will work directly on a "genbinary" object, but for most functions you will want to convert to a "genambig" object. Addition of population and other information can be done either before or after the conversion.

```

> PopInfo(PAdat) <- c(1, 1, 2)
> PAdat <- genbinary.to.genambig(PAdat)

```

6.2 Data export

Autopolyplloid data can also be exported in the same six formats that are available for import.

The `write.Structure` function requires that an overall ploidy for the file be specified, to indicate how many rows per individual to write. Individuals with higher ploidy than the overall ploidy will have alleles randomly removed, and individuals with lower ploidy will have the missing data symbol inserted in the extra rows. Additional arguments give the options to specify extra columns to include, to omit or include population information, and to specify the missing data symbol. The row of missing data symbols that is automatically written underneath marker names is the RECESSIVEALLELES row in Structure, indicating that allele copy number is ambiguous.

`write.Structure` was used in the tutorial in section 4.2.4, but below is another example with some of the options changed (see `?write.Structure` for more information). Here, `myexcol` is an array of data to be written into extra columns in the file.

```

> myexcol <- array(c(rep(0:1, each = 150), seq(0.1,
+     30, by = 0.1)), dim = c(300, 2), dimnames = list(Samples(simgen),
+     c("PopFlag", "Something")))
> myexcol[1:10, ]

```

	PopFlag	Something
A1	0	0.1

```

A2      0      0.2
A3      0      0.3
A4      0      0.4
A5      0      0.5
A6      0      0.6
A7      0      0.7
A8      0      0.8
A9      0      0.9
A10     0      1.0

```

```

> write.Structure(simgen, ploidy = 4, file = "simgenStruct2.txt",
+   writepopinfo = FALSE, extracols = myexcol,
+   missingout = -1)

```

The `write.Genodive` function is fairly straightforward, with the only option being whether to code alleles as two or three digits. All alleles are converted to repeat number, using the information contained in the `Usatnts` slot of the "genambig" object.

```
> write.Genodive(simgen, file = "simgenGD.txt")
```

`write.SPAGeDi` has options for the number of digits used to code alleles as well as the character (or lack thereof) used to separate alleles. Alleles are converted to repeat numbers as in `write.Genodive`. Additionally, a data frame of spatial coordinates can be supplied to the function to be written to the file. By default, the function will create two dummy columns for spatial coordinates, which the user can then fill in using a text editor or spreadsheet software. (See `?write.SPAGeDi`)

```
> write.SPAGeDi(simgen, file = "simgenSpag.txt")
```

If you are using SPAGeDi to calculate relationship and kinship coefficients, also see the function `write.freq.SPAGeDi` for exporting allele frequencies from polysat to SPAGeDi for use in these calculations.

The `write.POPDIST` function does not have any options for formatting. In the example below, the `samples` argument is used to ensure that each population has uniform ploidy, which is a requirement of the POPDIST software.

```
> write.POPDIST(simgen, samples = Samples(simgen,
+     ploidies = 4), file = "simgenPOPDIST.txt")
```

`write.GeneMapper` is very straightforward, without any special formatting options. This function was used to create the “GeneMapperExample.txt” file that is provided with the package. I do not know of any other software that will read the GeneMapper format, but it may be a convenient way for the user to store and edit genotypes.

```
> write.GeneMapper(simgen, file = "simgenGM.txt")
```

To export a table of genotypes in binary presence/absence format, first convert the “genambig” object to a “genbinary” object, then write the `Genotypes` slot to a text file, adjusting the options of `write.table` to suit your needs. (See `?write.table`.)

```
> simgenPA <- genambig.to.genbinary(simgen)
> write.table(Genotypes(simgenPA), file = "simgenPA.txt",
+     quote = FALSE, sep = ",")
```

6.3 Individual-level statistics

6.3.1 Estimating and exporting ploidies

The `estimatePloidy` function, which was demonstrated in section 4.1, is equally appropriate for autopolyploid and allopolyploid data. If you want to export the ploidy data, one method is the following:

```
> write.table(data.frame(Ploidies(simgen), row.names = Samples(simgen)),
+     file = "simgenPloidies.txt")
```

6.3.2 Inter-individual distances

A matrix of pairwise distances between individuals can be generated using the `meandistance.matrix` function, which was demonstrated in section 4.2.1. The most important argument is `distmetric`, or the distance measure that is used. The two options that are provided with `polysat` are `Bruvo.distance`, which takes mutational distance between alleles into account [1], and `Lynch.distance`, which is a simple band-sharing measure

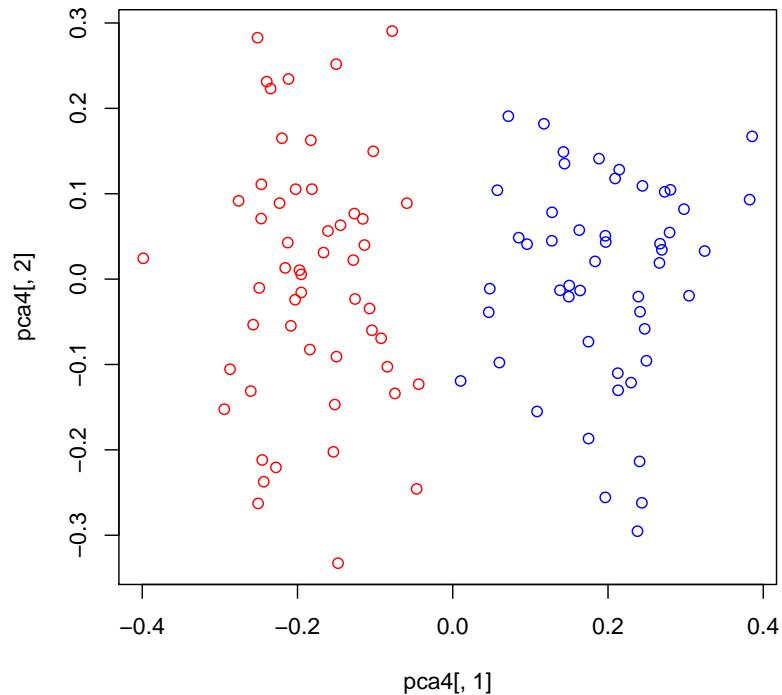
[11]. (The user can create functions to serve as additional distance measures, as long as the arguments are the same as those for `Bruvo.distance` and `Lynch.distance`.) The `progress` argument can be set to TRUE or FALSE to indicate whether the progress of the computation should be printed to the screen. The `all.distances` argument can also be set to TRUE or FALSE to indicate whether, in addition to the mean distance matrix, a three-dimensional array of distances by locus should be returned. There is also a `max1` argument to indicate the threshold for `Bruvo.distance` to skip calculations that are too computationally intensive (see `?Bruvo.distance`).

A second means of calculating inter-individual distances is provided with polysat 1.2 and is called `meandistance.matrix2`. Whereas `meandistance.matrix` passes genotypes directly to `distmetric` with each allele present in only one copy, `meandistance.matrix2` uses ploidy, selfing rate, and allele frequencies to calculate the probabilities that a given ambiguous genotype represents any possible unambiguous genotype. Unambiguous genotypes are then passed to `distmetric`. The distance is a weighted average across all possible combinations of unambiguous genotypes. There is no advantage to using `Lynch.distance` with this function, but it may give improved results for `Bruvo.distance` or a user-defined distance measure.

```
> testmat4 <- meandistance.matrix2(simgen, samples=samToUse, freq=simfreq,
+                                         self=0.2)

> pca4 <- cmdscale(testmat4)
> plot(pca4[, 1], pca4[, 2], col = c("red", "blue") [PopInfo(simgen)[samToUse]], 
+       main = "Bruvo distance with meandistance.matrix2")
```

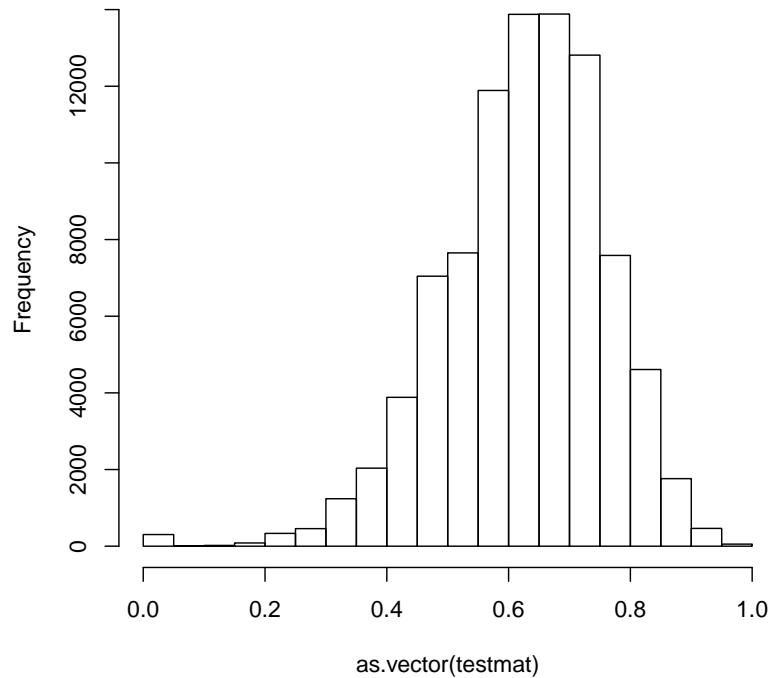
Bruvo distance with meandistance.matrix2



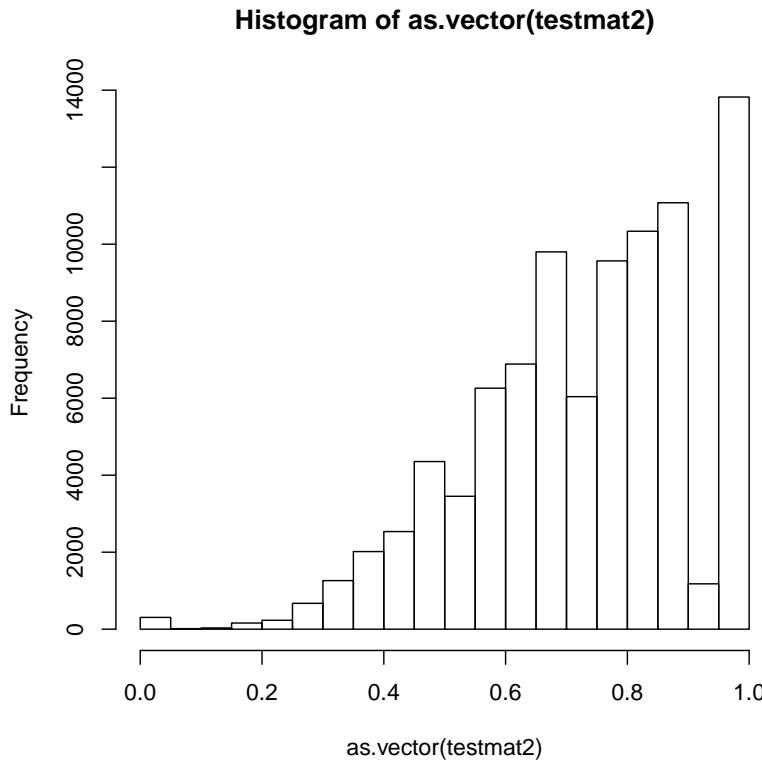
Besides the `cmdscale` function for performing Principal Coordinate Analysis on the resulting matrix, you may want to create a histogram to view the distribution of distances, or you may want to export the distance matrix for use in other software.

```
> hist(as.vector(testmat))
```

Histogram of as.vector(testmat)



> *hist(as.vector(testmat2))*



```
> write.table(testmat2, file = "simgenDistMat.txt")
```

`meandist.from.array` can take a three-dimensional array such as that produced when `all.distances=TRUE` and recalculate a mean distance matrix from it. This could be useful, for example, if you want to try omitting loci from your analysis. If `Bruvo.distance` skips some calculations because `max1` is exceeded, you may also want to estimate these distances and fill them into the array manually, then recalculate the mean distance matrix. See the help file for `meandist.from.array` for some additional functions that can help to locate missing values in the three-dimensional distance array.

The following example first creates a vector indicating the subset of samples to use, both to save on computation time for the example and because missing data can be a problem for Principal Coordinate Analysis if fewer than three loci are used. An array of distances is then calculated, followed by the mean distance matrix for each combination of two loci.

```

> subsamples <- Samples(simgen, populations = 1)
> subsamples <- subsamples[!isMissing(simgen, subsamples,
+     "loc1") & !isMissing(simgen, subsamples, "loc2") &
+     !isMissing(simgen, subsamples, "loc3")]
> Larray <- meandistance.matrix(simgen, samples = subsamples,
+     progress = FALSE, distmetric = Lynch.distance,
+     all.distances = TRUE)[[1]]
> mdist1.2 <- meandist.from.array(Larray, loci = c("loc1",
+     "loc2"))
> mdist2.3 <- meandist.from.array(Larray, loci = c("loc2",
+     "loc3"))
> mdist1.3 <- meandist.from.array(Larray, loci = c("loc1",
+     "loc3"))

```

As before, you can use `cmdscale` to perform Principal Coordinate Analysis and `plot` to visualize the results. Differences between plots reflect the effects of excluding loci.

6.3.3 Determining groups of asexually-related samples

Very similarly to the software GenoType [13], polysat can use a matrix of inter-individual distances to assign samples to groups of asexually-related individuals. This analysis can be performed on any matrix of distances calculated with `meandistance.matrix`, `meandistance.matrix2`, or a user-defined function that produces matrices in the same format. As in GenoType, a histogram such as those produced above may be useful for determining a distance threshold for distinguishing sexually- and asexually-related pairs of individuals. The data in `simgen` were simulated in a sexually-reproducing population, but let's pretend for the moment that there was some asexual reproduction, and we saw a bimodal distribution of distances with a cutoff of 0.2 between modes.

```

> clones <- assignClones(testmat, samples = paste("A",
+     1:100, sep = ""), threshold = 0.2)
> clones

```

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
1	2	3	4	5	6	7	8	9	10	11	12

A13	A14	A15	A16	A17	A18	A19	A20	A21	A22	A23	A24
13	14	15	16	17	18	19	20	21	22	23	24
A25	A26	A27	A28	A29	A30	A31	A32	A33	A34	A35	A36
25	26	27	28	15	29	30	31	32	33	34	35
A37	A38	A39	A40	A41	A42	A43	A44	A45	A46	A47	A48
36	37	38	39	40	37	36	7	21	41	42	43
A49	A50	A51	A52	A53	A54	A55	A56	A57	A58	A59	A60
44	45	46	47	48	49	27	50	14	51	17	28
A61	A62	A63	A64	A65	A66	A67	A68	A69	A70	A71	A72
52	53	36	54	55	56	31	57	58	59	60	22
A73	A74	A75	A76	A77	A78	A79	A80	A81	A82	A83	A84
61	22	62	63	64	65	66	67	68	69	3	55
A85	A86	A87	A88	A89	A90	A91	A92	A93	A94	A95	A96
70	36	71	72	35	24	73	26	74	75	76	77
A97	A98	A99	A100								
78	37	79	80								

Some of the individuals with similar genotypes have been assigned to the same clonal group.

Diversity statistics based on genotype frequencies are also available; see section 6.4.2.

6.4 Population statistics

6.4.1 Allele frequencies

There are two functions in polysat for estimating allele frequencies. If all of your individuals are the same, even-numbered ploidy and if you have a reasonable estimate of the selfing rate in your system, `deSilvaFreq` will give the most accurate estimate. For mixed ploidy systems, the `simpleFreq` function is available, but will be biased toward underestimating common allele frequencies and overestimating rare allele frequencies, which will cause an underestimation of F_{ST} . `deSilvaFreq` uses an iterative algorithm to estimate genotype frequencies based on allele frequencies and “allelic phenotype” frequencies, then recalculate allele frequencies from genotype frequencies [3]. `simpleFreq` simply assumes that in a partially heterozygous genotype, all alleles have an equal chance of being present in more than one copy.

Both allele frequency estimators take as the first argument a "genambig" or "genbinary" object, which must have the `PopInfo` and `Ploidies` slots filled in. The `self` argument for supplying the selfing rate is only applicable for `deSilvaFreq`. (See `?deSilvaFreq` for some other arguments that can be adjusted.) Both functions produce a data frame of allele frequencies, with populations in rows and alleles in columns. `deSilvaFreq` adds a null allele for each locus, while `simpleFreq` does not. In both cases the data frame will also have a column indicating the population size in number of genomes (*e.g.* four hexaploid individuals = 24 genomes).

The function `calcFst` takes the data frame produced by either allele frequency estimation, and produces a matrix containing pairwise F_{ST} values according to the original calculation by Wright [14]. Population sizes are weighted by number of genomes, rather than number of individuals.

Continuing the example from section 4.2.3, and comparing the results of `deSilvaFreq` and `simpleFreq`:

```
> simFst
      PopA      PopB      PopC
PopA 0.00000000 0.05068795 0.05453103
PopB 0.05068795 0.00000000 0.07098261
PopC 0.05453103 0.07098261 0.00000000

> simfreqSimple <- simpleFreq(simgen, samples = Samples(simgen,
+   ploidies = 4))
> simFstSimple <- calcFst(simfreqSimple)
> simFstSimple
      PopA      PopB      PopC
PopA 0.00000000 0.04738346 5.088305e-02
PopB 0.04738346 0.00000000 6.492718e-02
PopC 0.05088305 0.06492718 -1.323838e-16
```

Average allele frequencies can also be used by SPAGeDi for the calculation of relationship and kinship coefficients. SPAGeDi v1.3 can estimate allele frequencies using the same method as `simpleFreq`. However, if your data are appropriate for allele frequency estimation using `deSilvaFreq`, exporting the estimated allele frequencies to SPAGeDi should improve the accuracy of the relationship and kinship calculations. The `write.freq.SPAGeDi` function creates a file of allele frequencies in the format that is read by SPAGeDi.

```
> write.freq.SPAGeDi(simfreq, usatnts = Usatnts(simgen),
+   file = "SPAGfreq.txt")
```

The R package `adegenet`[9] can perform a number of calculations from allele frequencies, including five inter-population distance measures as well as Correspondance Analysis. The allele frequency tables produced by `polysat` can be converted to a format that can be read by `adegenet`.

```
> gpsimfreq <- freq.to.genpop(simfreq)
```

The object `gpsimfreq` that you just created can now be passed to the function `genpop` as the `tab` argument. See `?freq.to.genpop` for example code.

6.4.2 Genotype frequencies

For asexual organisms, you many want to calculate statistics based on the frequencies of genotypes in your populations. Two popular statistics for this, the Shannon index [16] and Simpson index [17], are provided with `polysat`. The function `genotypeDiversity` calculates either of these statistics or any user-defined statistic that can be calculated from a vector of frequencies. `genotypeDiversity` uses the function `assignClones` internally, so the same `threshold` argument may be set to allow for mutation or scoring error, or to group individuals by a larger distance threshold. This function examines loci individually as well as the mean distance across all loci. Where ordinary allelic diversity statistics are not available due to allele copy number ambiguity, genotype diversity statistics for individual loci may be useful.

```
> testmat5 <- meandistance.matrix(simgen, all.distances=TRUE)

> simdiv <- genotypeDiversity(simgen, d = testmat5,
+   threshold = 0.2, index = Shannon)
> simdiv

      loc1      loc2      loc3  overall
PopA 3.304798 2.654090 3.379927 4.303583
PopB 3.269491 2.582981 2.913112 4.002176
PopC 3.373131 3.009567 3.415639 4.521993
```

```

> simdiv2 <- genotypeDiversity(simgen, d = testmat5,
+     threshold = 0.2, index = Simpson)
> simdiv2

      loc1      loc2      loc3 overall
PopA 0.04700000 0.09220000 0.04336292  0.0150
PopB 0.04685548 0.09805122 0.06640000  0.0298
PopC 0.04240000 0.06320000 0.04091419  0.0112

```

7 Functions for allopolyploid data

In order to properly analyze microsatellites as codominant markers in allopolyploids, knowledge is required about which alleles belong to which genome. In an autopolyploid, all alleles for a given marker will segregate according to Mendelian laws. In an allopolyploid, a microsatellite marker represents two or more loci that are behaving in a Mendelian fashion, but if treated as one locus will not appear to behave according to random segregation. For example, an autotetraploid with the genotype ABCD that self fertilizes can produce offspring with the genotype AABB. An allotetraploid with the same four alleles, but distributed as AB and CD across two genomes, cannot self to produce an AABB individual as both of these alleles come from one genome.

If you have knowledge from other analyses about which alleles belong to which genomes, when importing your data you can code each microsatellite marker as multiple loci. As long as each “locus” in the `"genambig"` object is behaving according to random segregation, the analysis and export functions for autopolyploid data described in the previous section are appropriate.

Otherwise, the following functionality is available for allopolyploids in polysat:

7.1 Data import and export

Data can be formatted for the software Tetrasat [12], Tetra [10], and ATetra [19] using polysat. These programs are able to resolve ambiguity about how alleles are distributed across the two diploid genomes in an allotetraploid. From there, the programs can calculate allele frequencies and other statistics. See the help files for `write.Tetrasat` and `write.ATetra`.

`read.Tetrasat` (which produces a format readable by both `Tetrasat` and `Tetra`) and `read.ATetra` both take, as their only argument, the file name to be read. To import data from the example files “`ATetraExample.txt`” and “`tetrasatExample.txt`”, use the commands:

```
> ATdata <- read.ATetra("ATetraExample.txt")
> Tetdata <- read.Tetrasat("tetrasatExample.txt")
```

The functions for writing these two file formats only require a “`genambig`” object and a file name. `Ploidies` and `PopInfo` are required in the object for both functions. `write.Tetrasat` additionally requires information in the `Usatnts` slot. Since `ATetra` does not allow missing data, any missing genotypes that are encountered by `write.ATetra` are written to the console.

```
> write.ATetra(simgen, samples = Samples(simgen,
+     ploidies = 4), file = "simgenAT.txt")
```

```
Missing data: B48 loc2
Missing data: A42 loc3
Missing data: C22 loc3
```

```
> write.Tetrasat(simgen, samples = Samples(simgen,
+     ploidies = 4), file = "simgenTet.txt")
```

Data for allopolyploids can also be imported and exported in GeneMapper and binary presence/absence formats, as described in the sections 6.1 and 6.2.

7.2 Individual-level and population statistics

The `Bruvo.distance` measure of inter-individual distances is best suited to autopolyploids but may work for allopolyploids under a special case. `Bruvo.distance` measures distances between all alleles at a locus for the two individuals being compared, under the premise that these alleles could be closely related to each other by mutation. If two alleles belong to two different allopolyploid genomes, it is not possible for them to be closely related to each other even if their sizes are similar, since they are derived from different ancestral species. In the case where no allele from one allopolyploid genome is within three or four mutation steps of any allele from

the other genome, it is possible for the value produced by `Bruvo.distance` to accurately reflect the genetic similarity of two allopolyploid individuals. Along the same logic, `Lynch.distance` will only be appropriate if the two homeologous genomes have no alleles in common at a given locus. If either of these distance measures are appropriate for your data, see the description of the `meandistance.matrix` function in sections 4.2.1 and 6.3.2. The `meandistance.matrix2` function is never appropriate under allopolyploid inheritance, since it assumes random segregation of alleles when calculating genotype probabilities.

Assuming a distance matrix can be calculated using `meandistance.matrix`, all downstream analyses (principal coordinate analysis, clone assignment, genotype diversity) are appropriate.

The `estimatePloidy` function works equally well on autopolyploids and allopolyploids.

Both `simpleFreq` and `deSilvaFreq` work under the assumption of polysomic inheritance and should therefore not be used on allopolyploid data.

8 Treating microsatellite alleles as dominant markers

Both autopolyploid and allopolyploid microsatellite data can be converted to “allelic phenotypes” based on the presence and absence of alleles. Although much information is lost using this method, it can enable the user to perform a wider range of analyses, such as parentage analysis or AMOVA.

The `Lynch.distance` measure, described earlier, essentially treats alleles in this way. Alleles are assumed to be present in only one copy, and two alleles from two individuals are either identical or not. However, alleles are still grouped by locus and distances are averaged across all loci.

The “`genbinary`” class stores data in a binary presence/absence format, the same way that dominant data is typically coded. (See earlier description of the `genambig.to.genbinary` function in section 6.2.) This is intended to facilitate further analysis in R or other software that takes such a format. By default, 1 indicates that an allele is present, 0 indicates that an allele is absent, and -9 indicates that the data point is missing. There are replacement functions to change these symbols, for example (continuing from section 5.2):

```
> Present(simgenB) <- "P"
```

```

> Absent(simgenB) <- 2
> Missing(simgenB) <- 0
> Genotypes(simgenB) [1:10, 1:6]

  loc1.100 loc1.102 loc1.104 loc1.106 loc1.108 loc1.110
A1  "2"      "2"      "2"      "P"      "2"      "P"
A2  "2"      "2"      "2"      "P"      "2"      "P"
A3  "P"      "P"      "2"      "P"      "2"      "2"
A4  "P"      "P"      "2"      "P"      "2"      "P"
A5  "2"      "2"      "2"      "P"      "2"      "2"
A6  "P"      "2"      "2"      "P"      "2"      "P"
A7  "2"      "2"      "2"      "2"      "P"      "2"
A8  "2"      "P"      "2"      "P"      "2"      "2"
A9  "2"      "2"      "2"      "2"      "2"      "2"
A10 "2"      "P"      "2"      "P"      "2"      "P"

```

If you want to further manipulate the format of the genotype matrix, you can assign it to a new object name and then make the desired edits.

```

> genmat <- Genotypes(simgenB)
> dimnames(genmat)[[2]] <- paste("M", 1:dim(genmat)[2],
+      sep = "")
> genmat[1:10, 1:10]

```

	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
A1	"2"	"2"	"2"	"P"	"2"	"P"	"P"	"2"	"2"	"2"
A2	"2"	"2"	"2"	"P"	"2"	"P"	"2"	"P"	"2"	"P"
A3	"P"	"P"	"2"	"P"	"2"	"2"	"2"	"P"	"2"	"2"
A4	"P"	"P"	"2"	"P"	"2"	"P"	"2"	"2"	"2"	"2"
A5	"2"	"2"	"2"	"P"	"2"	"2"	"P"	"2"	"2"	"2"
A6	"P"	"2"	"2"	"P"	"2"	"P"	"2"	"2"	"2"	"2"
A7	"2"	"2"	"2"	"2"	"P"	"2"	"P"	"2"	"2"	"2"
A8	"2"	"P"	"2"	"P"	"2"	"2"	"2"	"2"	"2"	"2"
A9	"2"	"2"	"2"	"2"	"2"	"2"	"P"	"2"	"2"	"2"
A10	"2"	"P"	"2"	"P"	"2"	"P"	"P"	"2"	"2"	"2"

As demonstrated previously, the `write.table` function can write the matrix to a text file for use in other software. The arguments for `write.table`

allow the user to control which character is used to delimit fields, whether row and column names should be written to the file, and whether quotation marks should be used for character strings.

9 How to cite polysat

Clark, LV and Jasieniuk, M. POLYSAT: an R package for polyploid microsatellite analysis. *Molecular Ecology Resources* 11(3):562–566.

Feel free to email me at lvclark@ucdavis.edu with any questions, comments, or bug reports!

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