polyRAD tutorial

Lindsay V. Clark
University of Illinois, Urbana-Champaign

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1 Introduction

polyRAD is an R package that assists with genotype calling from DNA sequence datasets such as genotyping-by-sequencing (GBS) or restriction site-associated DNA sequencing (RAD) in polyploids and diploids. Genotype likelihoods are estimated from allelic read depth, genotype prior probabilities are estimated from population parameters, and then genotype posterior probabilities are estimated from likelihoods and prior probabilities. Posterior probabilities can be used directly in downstream analysis, converted to weighted mean genotypes for analyses of additive genetic effects, or used for export of the most probable genotypes for analyses that require discrete genotypic data.

Analyses in polyRAD center around objects of an S3 class called "RADdata". A single "RADdata" object contains the entire dataset of read depth and locus information, as well as parameters that are estimated during the course of analysis.

2 Estimating genotype probabilities in a mapping population

In this example, we'll import some data from an F1 mapping population of *Miscanthus sinensis* that were output by the UNEAK pipeline. These data are from a study by Liu *et al.* (2015; doi:10.1111/gcbb.12275; data available at `http://hdl.handle.net/2142/79522`), and can be found in the "doc" folder of the polyRAD installation. *Miscanthus* is an ancient tetraploid that has undergone diploidization. Given the ability of the UNEAK pipeline to filter paralogs, we expect most loci to behave in a diploid fashion, but some may behave in an allotetraploid fashion.

We'll start by loading polyRAD and importing the data into a "RADdata" object. The `possiblePloidies` argument indicates the expected inheritance modes: diploid (2) and allotetraploid (2 2).

```
> library(polyRAD)
> mydata <- readHMC("HapMapClareMap1subset.hmc.txt",
+                   possiblePloidies = list(2, c(2, 2)))
> mydata
```

```
## RADdata object ##
299 taxa and 200 loci
2711740 total reads
Assumed sample cross-contamination rate of 0.001

Possible ploidies:
Autodiploid (2)
Allotetraploid (2 2)
```

We can view the imported taxa names (subsetted here for space).

```
> GetTaxa(mydata)[c(1:10,293:299)]
```

```
 [1] "IGR-2011-001"    "Kaskade-Justin"  "Map1-001"        "Map1-002"
 [5] "Map1-003"        "Map1-005"        "Map1-008"        "Map1-011"
 [9] "Map1-016"        "Map1-018"        "Map1-488"        "Map1-489"
[13] "Map1-490"        "Map1-491"        "Zebrinus-Justin" "p196-150A-c"
[17] "p877-348-b"
```

All names starting with "Map" are progeny. "Kaskade-Justin" and "Zebrinus-Justin" are the parents. "IGR-2011-001", "p196-150A-c", and "p877-348-b" aren't part of the population, but were doubled haploid lines that were used to screen for paralogous markers. We can tell polyRAD which taxa are the parents; since this is an F1 population it doesn't matter which is "donor" and which is "recurrent".

```
> mydata <- SetDonorParent(mydata, "Kaskade-Justin")
> mydata <- SetRecurrentParent(mydata, "Zebrinus-Justin")
```

Now we can run the pipeline. We'll specify that we don't want to include the parents or doubled haploid lines in the estimation of allele frequencies or evaluation of genotype freqnecies (`excludeTaxa` argument). The `allowedDeviation` argument indicates how different the apparent allele frequency (based on read depth ratios) can be from an expected allele frequency (determined based on ploidy and mapping population type) and still be classified as that allele frequency. The default settings assume an F1 population, but the population type can be adjusted using the `n.gen.backcrossing`, `n.gen.intermating`, and `n.gen.selfing` arguments.

```
> mydata <- PipelineMapping2Parents(mydata,
+                                   freqExcludeTaxa = c("Kaskade-Justin",
+                                                       "Zebrinus-Justin",
+                                                      "IGR-2011-001",
+                                                      "p196-150A-c",
+                                                      "p877-348-b"),
+                                   freqAllowedDeviation = 0.06)
```

We can examine the allele frequencies. Allele frequencies that fall outside of the expected ranges will be recorded as `NA`.

```
> table(mydata$alleleFreq)

0.125  0.25 0.375   0.5 0.625  0.75 0.875 
   11    89    47    94    47    89    11 
```

Genotype likelihood is also stored in the object for each possible genotype at each locus, taxon, and ploidy. This is the probability of seeing the observed distribution of reads.

```
> mydata$alleleDepth[3,1:10]

TP29_0 TP29_1 TP37_0 TP37_1 TP42_0 TP42_1 TP52_0 TP52_1 TP58_0 TP58_1 
     8      0      4      6      0     16      6      5     20      0 
```

```
> mydata$genotypeLikelihood[[1]][,3,1:10]

        TP29_0       TP29_1       TP37_0       TP37_1       TP42_0       TP42_1
0 2.328306e-26 9.970039e-01 6.614687e-11 5.121828e-20 9.900467e-01 1.529327e-55
1 3.914069e-03 3.914069e-03 2.048729e-01 2.048729e-01 1.519787e-05 1.519787e-05
2 9.970039e-01 2.328306e-26 5.121828e-20 6.614687e-11 1.529327e-55 9.900467e-01
        TP52_0       TP52_1       TP58_0       TP58_1
0 1.126520e-19 1.091423e-13 3.171212e-63 9.950119e-01
1 2.254729e-01 2.254729e-01 9.632565e-07 9.632565e-07
2 1.091423e-13 1.126520e-19 9.950119e-01 3.171212e-63
```

```
> mydata$genotypeLikelihood[[2]][,3,1:10]

        TP29_0       TP29_1       TP37_0       TP37_1       TP42_0       TP42_1
0 2.328306e-26 9.970039e-01 6.614687e-11 5.121828e-20 9.900467e-01 1.529327e-55
1 1.544286e-05 9.997951e-02 1.465818e-01 1.622200e-02 9.942715e-03 2.347003e-10
2 3.914069e-03 3.914069e-03 2.048729e-01 2.048729e-01 1.519787e-05 1.519787e-05
3 9.997951e-02 1.544286e-05 1.622200e-02 1.465818e-01 2.347003e-10 9.942715e-03
4 9.970039e-01 2.328306e-26 5.121828e-20 6.614687e-11 1.529327e-55 9.900467e-01
        TP52_0       TP52_1       TP58_0       TP58_1
0 1.126520e-19 1.091423e-13 3.171212e-63 9.950119e-01
1 2.676630e-02 8.078123e-02 9.465741e-13 3.171212e-03
2 2.254729e-01 2.254729e-01 9.632565e-07 9.632565e-07
3 8.078123e-02 2.676630e-02 3.171212e-03 9.465741e-13
4 1.091423e-13 1.126520e-19 9.950119e-01 3.171212e-63
```

Above, for one individal (Map1-001), we see its read depth at the first ten alleles (first five loci), followed by the genotype likelihoods under diploid and tetraploid models. For example, at locus TP29 we see that homozygosity for allele 0 is the most likely, although heterozygosity is not impossible, and homozygosity for allele 1 could happen in the very, very unlikely event that all eight of the reads are due to contamination. At TP37, heterozygosity is by far the most likely state, and if there is tetraploidy there are probably one or two copies of allele 0 rather than three copies.

The prior genotype probabilities (expected genotype distributions) are also stored in the object for each possible ploidy. These distributions are estimated based on the most likely parent genotypes. Low confidence parent genotypes can be ignored by increasing the `minLikelihoodRatio` argument to `PipelineMapping2Parents`.

```
> mydata$priorProb[[1]][,1:10]

  TP29_0 TP29_1 TP37_0 TP37_1 TP42_0 TP42_1 TP52_0 TP52_1 TP58_0 TP58_1
0   0.25   0.25    0.0    0.5    0.0    0.5    0.5    0.0      0      0
1   0.50   0.50    0.5    0.5    0.5    0.5    0.5    0.5      1      1
2   0.25   0.25    0.5    0.0    0.5    0.0    0.0    0.5      0      0
```

```
> mydata$priorProb[[2]][,1:10]

  TP29_0 TP29_1 TP37_0 TP37_1 TP42_0 TP42_1 TP52_0 TP52_1 TP58_0 TP58_1
0      0      0    0.0    0.0      0      0    0.5    0.0      0      0
1      0      0    0.0    0.5      0      1    0.5    0.0      0      0
2      1      1    0.5    0.5      0      0    0.0    0.0      1      1
3      0      0    0.5    0.0      1      0    0.0    0.5      0      0
4      0      0    0.0    0.0      0      0    0.0    0.5      0      0
```

Here we see some pretty big differences under the diploid and allotetrapliod models. For example, if TP29 is behaving in a diploid fashion we expect F2-like segregation since both parents were heterozygous. However, if TP29 is behaving in an allotetraploid fashion, we expect that both parents are homozygous at differing paralogous loci, resulting in no segregation.

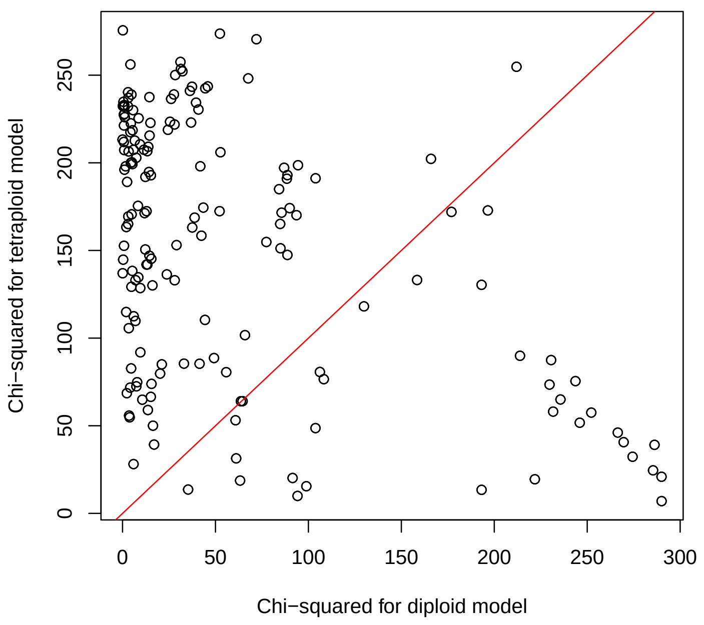
Now we want to determine which ploidy is the best fit for each locus. This is done by comparing genotype prior probabilities to genotype likelihoods and estimating a χ^2 statistic. Lower values indicate a better fit.

```
> mydata$ploidyChiSq[,1:10]

        TP29_0    TP29_1     TP37_0     TP37_1    TP42_0    TP42_1   TP52_0
[1,]  12.93418  12.93418   8.498903   8.498903  37.38438  37.38438 15.20986
[2,] 172.40358 172.40358 134.680604 134.680604 243.42034 243.42034 66.47934
       TP52_1    TP58_0    TP58_1
[1,] 15.20986  77.36922  77.36922
[2,] 66.47934 154.83699 154.83699
```

We can make a plot to get an overall sense of how well the markers fit the diploid versus tetraploid model.

```
> plot(mydata$ploidyChiSq[1,], mydata$ploidyChiSq[2,],
+      xlab = "Chi-squared for diploid model",
+      ylab = "Chi-squared for tetraploid model")
> abline(a = 0, b = 1, col = "red")
```



Alleles above the red line fit the diploid model better, and alleles below the red line fit the tetraploid model better.

Now we'll examine the posterior genotype probabilities. These are still estimated separately for each ploidy.

```
> mydata$posteriorProb[[1]][,3,1:10]

        TP29_0       TP29_1       TP37_0       TP37_1       TP42_0       TP42_1
0 2.317110e-26 9.922095e-01 0.000000e+00 2.500003e-19 0.000000e+00 1.006277e-50
1 7.790494e-03 7.790494e-03 1.000000e+00 1.000000e+00 1.000000e+00 1.000000e+00
2 9.922095e-01 2.317110e-26 2.500003e-19 0.000000e+00 1.006277e-50 0.000000e+00
        TP52_0       TP52_1 TP58_0 TP58_1
0 4.996257e-19 0.000000e+00      0      0
1 1.000000e+00 1.000000e+00      1      1
2 0.000000e+00 4.996257e-19      0      0

> mydata$posteriorProb[[2]][,3,1:10]

  TP29_0 TP29_1     TP37_0     TP37_1 TP42_0 TP42_1       TP52_0       TP52_1
0      0      0 0.00000000 0.00000000      0      0 4.208727e-18 0.000000e+00
1      0      0 0.00000000 0.07337121      0      1 1.000000e+00 0.000000e+00
2      1      1 0.92662879 0.92662879      0      0 0.000000e+00 0.000000e+00
3      0      0 0.07337121 0.00000000      1      0 0.000000e+00 1.000000e+00
4      0      0 0.00000000 0.00000000      0      0 0.000000e+00 4.208727e-18
```

```
  TP58_0 TP58_1
0      0      0
1      0      0
2      1      1
3      0      0
4      0      0
```

We can export the results for use in downstream analysis. The function below weights possible ploidies for each allele based on the results in `mydata$ploidyChiSq`, and for each taxon outputs a continuous, numerical genotype that is the mean of all possible genotypes weighted by genotype posterior probabilities. By default, one allele per locus is discarded in order to avoid mathematical singularities in downstream analysis. The continuous genotypes also range from zero to one by default, which can be changed with the `minval` and `maxval` arguments.

```
> mywm <- GetWeightedMeanGenotypes(mydata)
> mywm[3:6, 1:10]
```

```
              TP29_1    TP37_1     TP42_1    TP52_1 TP58_1 TP86_1   TP110_1
Map1-001 0.03851695 0.4989112 0.46671674 0.5465480    0.5    0.5 0.5888637
Map1-002 0.03489354 0.0148696 0.03328326 0.5465480    0.5    0.5 0.3695186
Map1-003 0.03489444 0.2574198 0.25000000 0.9991572    0.5    0.5 0.3947991
Map1-005 0.96510646 0.4979527 0.46671674 0.9998517    0.5    0.5 0.4022034
         TP111_1   TP116_1   TP154_1
Map1-001      NA 0.4927330 0.5000000
Map1-002      NA 0.4882984 0.9958151
Map1-003      NA 0.0427502 0.9879935
Map1-005      NA 0.0126574 0.9958151
```

Note that the parent weighted mean genotypes are not necessarily correct, since they were estimated using the genotype priors for the progeny. We can view parent genotypes with `GetLikelyGen`, which was used internally by `AddGenotypePriorProb_Mapping2Parents` to estimate the parent genotypes.

```
> kaskadeGen <- GetLikelyGen(mydata, "Kaskade-Justin")
> zebrinusGen <- GetLikelyGen(mydata, "Zebrinus-Justin")
> kaskadeGen[,1:10]
```

```
  TP29_0 TP29_1 TP37_0 TP37_1 TP42_0 TP42_1 TP52_0 TP52_1 TP58_0 TP58_1
2      1      1      2      0      2      0      0      2      2      0
4      2      2      4      0      4      0      0      4      4      0
```

```
> zebrinusGen[,1:10]
```

```
  TP29_0 TP29_1 TP37_0 TP37_1 TP42_0 TP42_1 TP52_0 TP52_1 TP58_0 TP58_1
2      1      1      1      1      1      1      1      1      0      2
4      2      2      1      3      2      2      1      3      0      4
```

To subset parent genotypes in order to match the progeny genotypes:

```
> kaskadeGenSubset <- sweep(kaskadeGen[,-OneAllelePerMarker(mydata)],
+                           1, c(2,4), "/")
> zebrinusGenSubset <- sweep(zebrinusGen[,-OneAllelePerMarker(mydata)],
+                           1, c(2,4), "/")
> kaskadeGenSubset[,1:10]
```

```
  TP29_1 TP37_1 TP42_1 TP52_1 TP58_1 TP86_1 TP110_1 TP111_1 TP116_1 TP154_1
2    0.5      0      0      1      0      1    0.50    0.50       0    0.50
4    0.5      0      0      1      0      1    0.25    0.75       0    0.75
```

```
> zebrinusGenSubset[,1:10]
```

```
  TP29_1 TP37_1 TP42_1 TP52_1 TP58_1 TP86_1 TP110_1 TP111_1 TP116_1 TP154_1
2    0.5   0.50    0.5   0.50      1      0    0.50     0.5    0.50     0.5
4    0.5   0.75    0.5   0.75      1      0    0.75      NA    0.75     0.5
```

3 Estimating genotype probabilities in a diversity panel

Pipelines in polyRAD for processing a diversity panel (i.e. a germplasm collection, a set of samples collected in the wild, or a panel for genome-wide association analysis or genomic prediction) use iterative algorithms. Essentially, allele frequencies are re-estimated with each iteration until convergence is reached.

Here we'll import a RAD-seq dataset from a large collection of wild and ornamental *Miscanthus* from Clark *et al.* (2014; doi:10.1093/aob/mcu084, data available at `http://hdl.handle.net/2142/49963`).

```
> mydata <- readHMC("HapMapMsiSubset1.hmc.txt",
+                   possiblePloidies = list(2, c(2,2)))
```

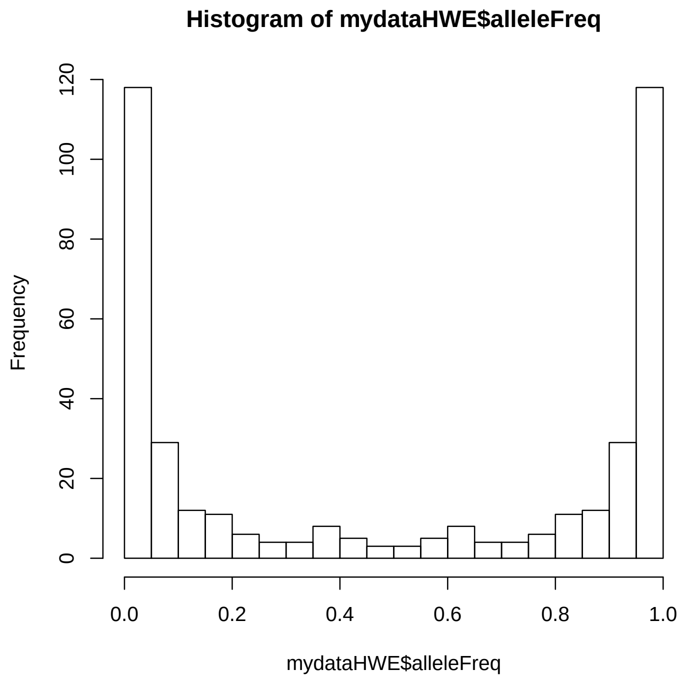
We can iteratively estimate genotype probabilities assuming Hardy-Weinberg equilibrium. The argument `tol` is set to a higher value than the default here in order to help the tutorial run more quickly.

```
> mydataHWE <- IterateHWE(mydata, tol = 1e-3)
```

```
Starting iteration 1
Mean difference in allele frequencies of 0.00883270513429876
Starting iteration 2
Mean difference in allele frequencies of 0.004211651772166
Starting iteration 3
Mean difference in allele frequencies of 0.00219754259949768
Starting iteration 4
Mean difference in allele frequencies of 0.00121707625086124
Starting iteration 5
Mean difference in allele frequencies of 0.000738011961142255
```

Let's take a look at allele frequencies:

```
> hist(mydataHWE$alleleFreq, breaks = 20)
```



There are bumps around 0.4 and 0.6, which may be artefactual due to paralogs in the dataset.

We can do a different genotype probability estimation that models population structure and variation in allele frequencies among populations. We don't need to specify populations, since principal components analysis is used to assess population structure assuming an isolation-by-distance model, with gradients of gene flow across many groups of individuals. This dataset includes a very broad sampling of *Miscanthus* across Asia, so it is very appropriate to model population structure in this case.

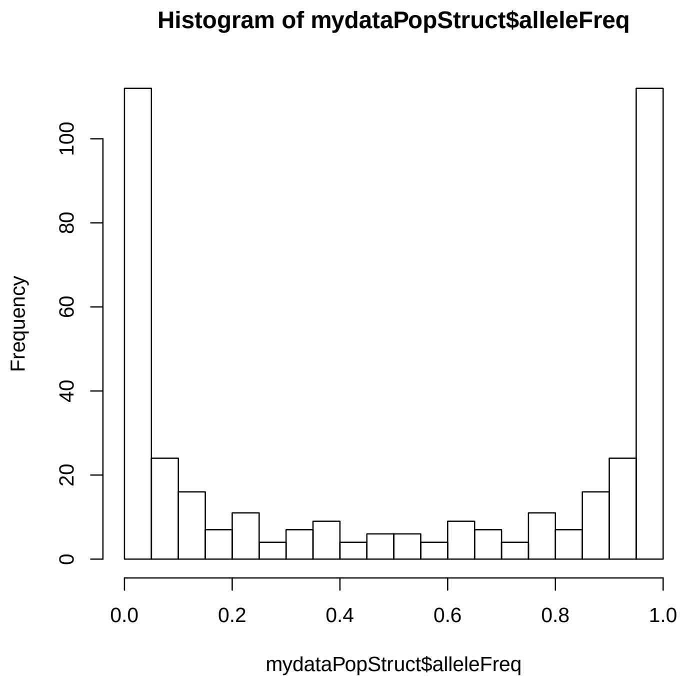
```
> mydataPopStruct <- IteratePopStruct(mydata)
```

```
Starting iteration 1
PCs used: 8
Mean difference in allele frequencies of 0.0161767929725412
Starting iteration 2
PCs used: 8
Mean difference in allele frequencies of 0.00692698899546912
Starting iteration 3
```

```
PCs used: 8
Mean difference in allele frequencies of 0.00414833854709348
Starting iteration 4
PCs used: 8
Mean difference in allele frequencies of 0.00288682724685397
Starting iteration 5
PCs used: 8
Mean difference in allele frequencies of 0.00217032671518564
Starting iteration 6
PCs used: 8
Mean difference in allele frequencies of 0.00172564305730193
Starting iteration 7
PCs used: 8
Mean difference in allele frequencies of 0.00139150240616677
Starting iteration 8
PCs used: 8
Mean difference in allele frequencies of 0.0011617686105707
Starting iteration 9
PCs used: 8
Mean difference in allele frequencies of 0.000993736467149977
```

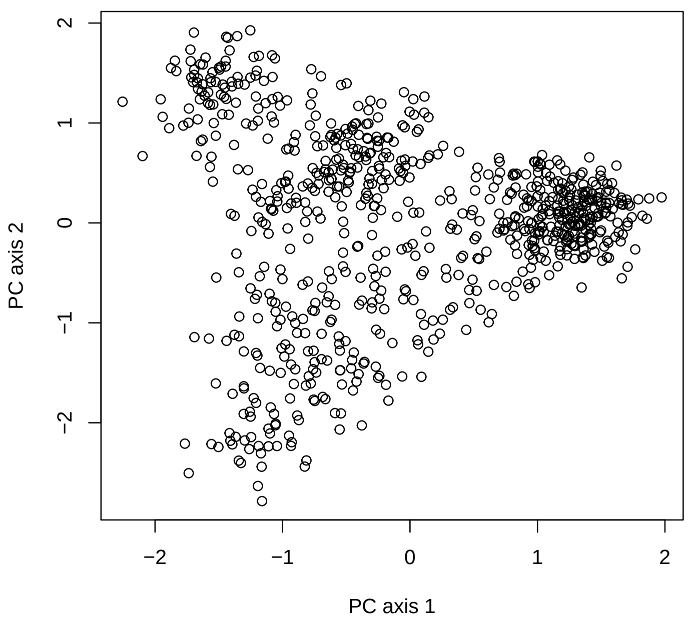
The allele frequency bumps have somewhat gone away:

```
> hist(mydataPopStruct$alleleFreq, breaks = 20)
```



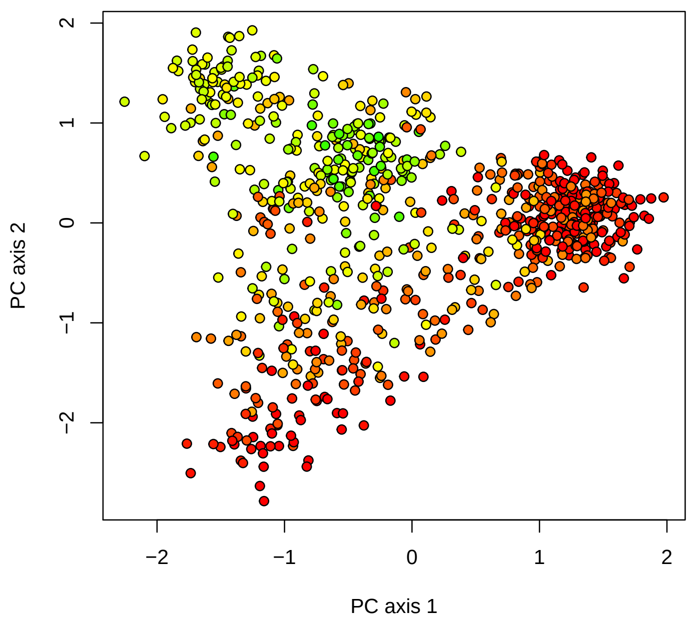
Here's some of the population structure that was used for modeling allele frequencies:

```
> plot(mydataPopStruct$PCA[,1], mydataPopStruct$PCA[,2],
+      xlab = "PC axis 1", ylab = "PC axis 2")
```



And here's an example of allele frequency varying across the environment. Allele frequencies were estimated for each taxon, and are stored in the `$alleleFreqByTaxa` slot.

```
> myallele <- 11
> freqcol <- rainbow(101)[round(mydataPopStruct$alleleFreqByTaxa[,myallele] * 100) + 1]
> plot(mydataPopStruct$PCA[,1], mydataPopStruct$PCA[,2],
+      xlab = "PC axis 1", ylab = "PC axis 2", pch = 21,
+      bg = freqcol)
```



As before, we can export the weighted mean genotypes for downstream analysis.

```
> wmgenoPopStruct <- GetWeightedMeanGenotypes(mydataPopStruct)
> wmgenoPopStruct[1:10,1:5]

                    TP105_1      TP111_1   TP167_1   TP210_1   TP234_1
2p-558-157-b      0.9999000 9.999989e-01 0.9999932 0.9660920 0.9980988
Akeno             0.9996791 9.999155e-01 0.9999998 0.1831563 0.9868369
CANE9233-US47-0011 0.9944469 2.419826e-01 0.9998818 0.9845796 0.9913554
DC-2010-001-A     0.9926395 7.846835e-01 1.0000000 0.5132512 0.9841174
DC-2010-001-E     0.9999000 9.994290e-01 0.9999856 0.9953432 0.9831247
GZ-2010-007       0.9999000 4.252850e-08 0.9998942 1.0000000 0.9910130
Gunma             0.9999000 9.999999e-01 0.9999916 0.2189149 0.9968834
IGR-2011-001      0.9999000 9.999000e-01 0.9999580 0.6244779 0.9992742
JM0001-001        0.9992306 9.652316e-01 1.0000000 0.4989096 0.9947427
JM0001-002        0.9998938 9.999000e-01 0.9999996 0.4979062 0.9999000
```