polyRAD tutorial

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August 29, 2017

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 was 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 will estimate allele frequencies. In this case the allele frequency estimation is mostly so we can get a reasonable estimate of how often alleles will show up due to contamination. In a diversity panel, the allele frequencies can also be used for estimating genotype frequencies, but here we are going to estimate genotype frequencies based on the parent genotypes. Since tetraploidy is possible, we will allow allele frequencies to be 0.125, 0.25, 0.375, and so on (`expectedFreqs` argument). We'll specify that we don't want to include the parents or double haploid lines in the estimation (`excludeTaxa` argument). The `allowedDeviation` argument indicates how different the apparent allele frequency (based on read depth ratios) can be from an expected allele frequency and still be classified as that allele frequencies. Allele frequencies that fall outside of the expected ranges will be recorded as `NA`.

```
> mydata <- AddAlleleFreqMapping(mydata,
+                                expectedFreqs = seq(0, 1, length.out = 9),
+                                 allowedDeviation = 0.06,
+                                 excludeTaxa = c("Kaskade-Justin",
+                                                 "Zebrinus-Justin",
+                                                 "IGR-2011-001",
+                                                 "p196-150A-c",
+                                                 "p877-348-b"))
> table(mydata$alleleFreq)

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

Now we can estimate the likelihood of the data for each possible genotype at each locus, taxon, and ploidy. This is the probability of seeing the observed distribution of reads.

```
> mydata <- AddGenotypeLikelihood(mydata)
> 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 next step is to estimate the prior genotype probabilities. Based on the likelihoods calculated above, the most likely genotypes for the parents are assumed to be correct, as long as the ratio of the highest likelihood to the second highest likelihood is sufficiently high (`minLikelihoodRatio` argument). 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 <- AddGenotypePriorProb_Mapping2Parents(mydata)
> 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.

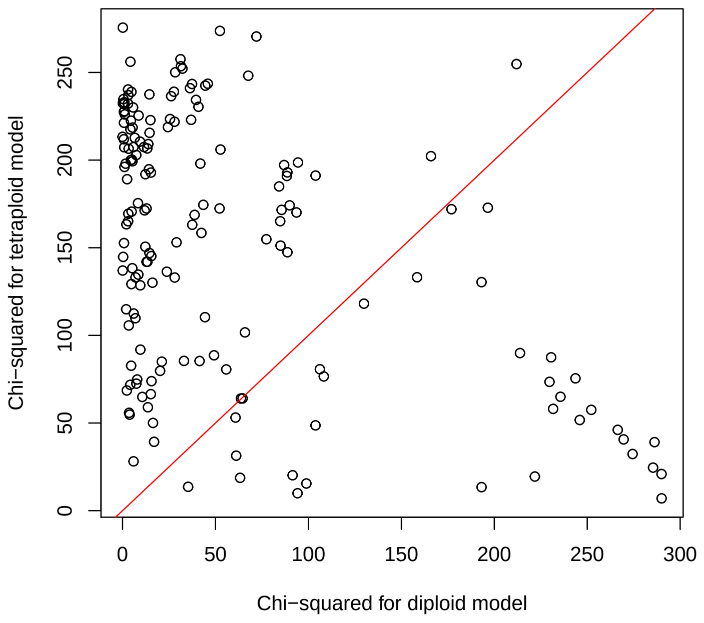
Now we want to determine which ploidy is the best fit for each locus. This is done by comparing genotype prior probabilities to genotype likelihoods and estimating a χ^2 statistic. Lower values indicate a better fit.

```
> mydata <- AddPloidyChiSq(mydata,
+                          excludeTaxa = c("Kaskade-Justin", "Zebrinus-Justin",
+                                          "IGR-2011-001", "p196-150A-c",
+                                          "p877-348-b"))
> 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 add the posterior genotype probabilities. These are still estimated separately for each ploidy.

```
> mydata <- AddGenotypePosteriorProb(mydata)
> 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
```

Now we can export the results for use in downstream analysis. The function below selects the best-fit ploidy 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
Map1-001 3.895247e-03 5.000000e-01 5.00000e-01 0.5000000    0.5    0.5
Map1-002 5.945789e-11 7.721368e-06 1.76762e-21 0.5000000    0.5    0.5
Map1-003 9.656690e-07 2.500000e-01 2.50000e-01 0.9999692    0.5    0.5
Map1-005 1.000000e+00 5.000000e-01 5.00000e-01 0.9999995    0.5    0.5
            TP110_1 TP111_1      TP116_1   TP154_1
Map1-001 0.6151508     0.5 5.000000e-01 0.5000000
Map1-002 0.3309265     1.0 5.000000e-01 1.0000000
Map1-003 0.4375625     1.0 2.949491e-02 0.9922811
Map1-005 0.3732712     0.5 9.517981e-10 1.0000000
```

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
```