GWAS/GS exercise using GAPIT

BIO373

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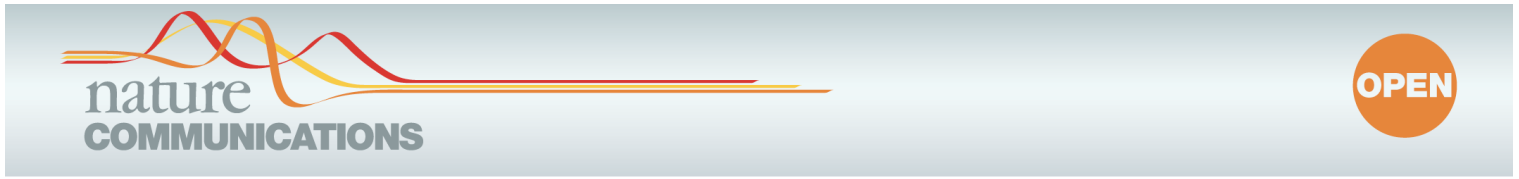
Dataset: Rice 44k genomes

- Data from Zhao et al. (2011) Nature Communications 2:467
- Data available at http://www.ricediversity.org/data/
- 34 agronomic traits were examined for 413 rice accessions



ARTICLE

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Genome-wide association mapping reveals a rich genetic architecture of complex traits in *Oryza sativa*

Keyan Zhao[1,2], Chih-Wei Tung[3], Georgia C. Eizenga[4], Mark H. Wright[1], M. Liakat Ali[5], Adam H. Price[6], Gareth J. Norton[6], M. Rafiqul Islam[7], Andy Reynolds[1], Jason Mezey[1], Anna M. McClung[4], Carlos D. Bustamante[1,2] & Susan R. McCouch[3]

What to do in this exercise

- GWAS example: Find genomic region associated with the seed length
- GS example: Predict flowering time between different study years
- Finally, you will be able to try GWAS/GS for traits of your interests!

Source codes and input data

- RiceDiversity_44K_Genotypes_PLINK_imputed.txt.gz
- RiceDiversity_44K_Genotypes_PLINK_info.txt
- This instruction PDF

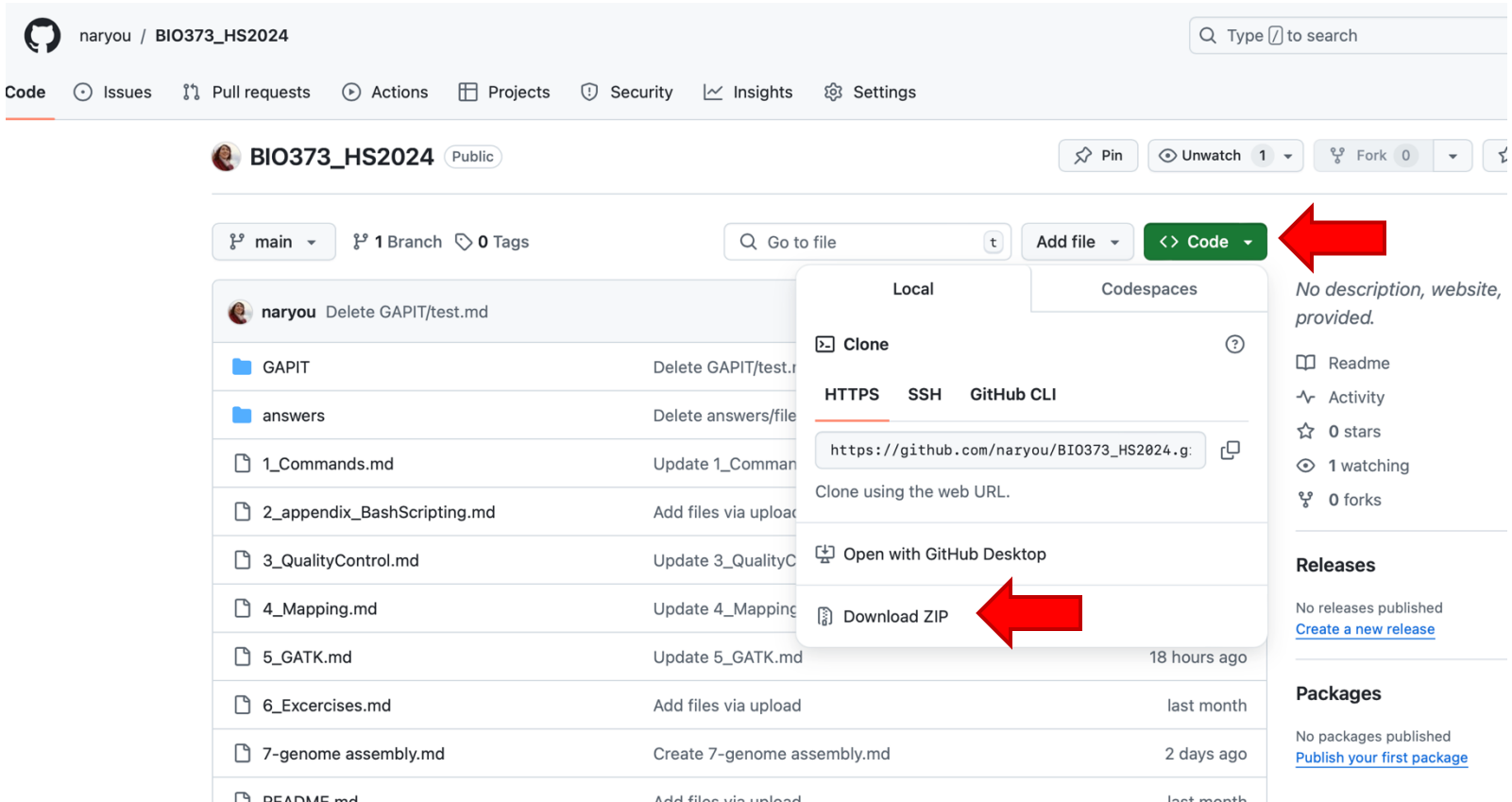
- All available at:

https://github.com/naryou/BIO373_HS2024/tree/main/GAPIT

Download materials

- Download .zip from:

https://github.com/naryou/BIO373_HS2024/tree/main/GAPIT



Set up the working directory

- Note: No support will be provided for your local environment (e.g., laptop)

- 1. Access to RStudio server (https://fgcz-genomics.uzh.ch) via terminal and log-in with your B-fabric username and Password

- 2. Make and change your working directory with mkdir GAPIT from Terminal; and then setwd("./GAPIT") from R Console

- 3. Upload RiceDiversity_44K_Genotypes_PLINK_imputed.txt.gz and RiceDiversity_44K_Genotypes_PLINK_info.txt to the directory you made

First of all, load Genomic Association and Prediction Integrated Tool (GAPIT)

- Now access to RStudio server (https://fgcz-genomics.uzh.ch) in a browser
- Install GAPIT source code and its dependency
- Wait ca. 15 min. to install everything
- Some packages are not installed but they are negligible
- Try twice when it fails

```
# clean up your workplace
rm(list=ls())
# select "1:All" if this asks something about dependent packages
install.packages("devtools")
BiocManager::install("snpStats")
devtools::install_github("SFUStatgen/LDheatmap")
devtools::install_github("jiabowang/GAPIT3@078fe28",force=TRUE)
# load GAPIT3 package
```

Load and see phenotype data

```
url <-
"http://www.ricediversity.org/data/sets/44kgwas/RiceDiversity_44K_Phenotypes_34traits_PLINK.txt"
p <- read.table(url, sep="\t", header=TRUE)
nrow(p) # no. of plants
## [1] 413
head(p)
```

```
## HybID NSFTVID Flowering.time.at.Arkansas Flowering.time.at.Faridpur
## 1 081215-A05 1 75.08333 64
## 2 081215-A06 3 89.50000 66
## 3 081215-A07 4 94.50000 67
## 4 081215-A08 5 87.50000 70
## 5 090414-A09 6 89.08333 73
## 6 090414-A10 7 105.00000 NA
## Flowering.time.at.Aberdeen FT.ratio.of.Arkansas.Aberdeen
## 1 81 0.9269547
## 2 83 1.0783133
## 3 93 1.0161290
## 4 108 0.8101852
## 5 101 0.8820132
## 6 158 0.6645570
## FT.ratio.of.Faridpur.Aberdeen Culm.habit Leaf.pubescence Flag.leaf.length
## 1 0.7901235 4.0 1 28.37500
## 2 0.7951807 7.5 0 39.00833
## 3 0.7204301 6.0 1 27.68333
## 4 0.6481481 3.5 1 30.41667
## 5 0.7227723 6.0 1 36.90833
```

Read genotype data and marker information

```
g <- read.table("RiceDiversity_44K_Genotypes_PLINK_imputed.txt.gz",
header=TRUE, sep="\t")
gm <- read.table("RiceDiversity_44K_Genotypes_PLINK_info.txt.gz",
header=TRUE, sep="\t")
nrow(g) # no. of plants
```

```
## [1] 413
```

```
ncol(g[,-1]) # no. of SNPs
```

```
## [1] 36901
```

```
head(gm) # marker info
```

```
## ID CHROM POS
## 1 id1000001 1 13147
## 2 id1000003 1 73192
## 3 id1000005 1 74969
## 4 id1000007 1 75852
## 5 id1000008 1 75953
## 6 id1000011 1 91016
```

(1) Genome-wide association study (GWAS)

Aim: Looking for genomic region underlying the length of rice grains

- Indica cultivars have long grains, while Japonica have round-shaped grains
- Grain Size 3 (GS3) is known to regulate the seed length in rice (Wang et al. 2011)
- Can we detect the known loci with GWAS?

Run GWAS with a general linear model (GLM) or mixed linear model (MLM)

- It takes several minutes. Wait.
- When finished, output files appear in the current directory
- Warning messages occur but the program still works
- Note: When you run GAPIT twice, the second run may not work. In such a case, log-out once and retry from data loading.

```
myGAPIT <- GAPIT( # warnings occur but it still works
Y=p[,c("HybID","Seed.length")],
GD=g,
GM=gm,
SNP.MAF=0.05, # cut-off minor alleles at 0.05
Inter.Plot=TRUE, # option to make interactive plots
model=c("GLM", "MLM"),
kinship.algorithm="VanRaden",
Multiple_analysis=TRUE)
```

GWAS is done. Let us see a trait diagnosis first

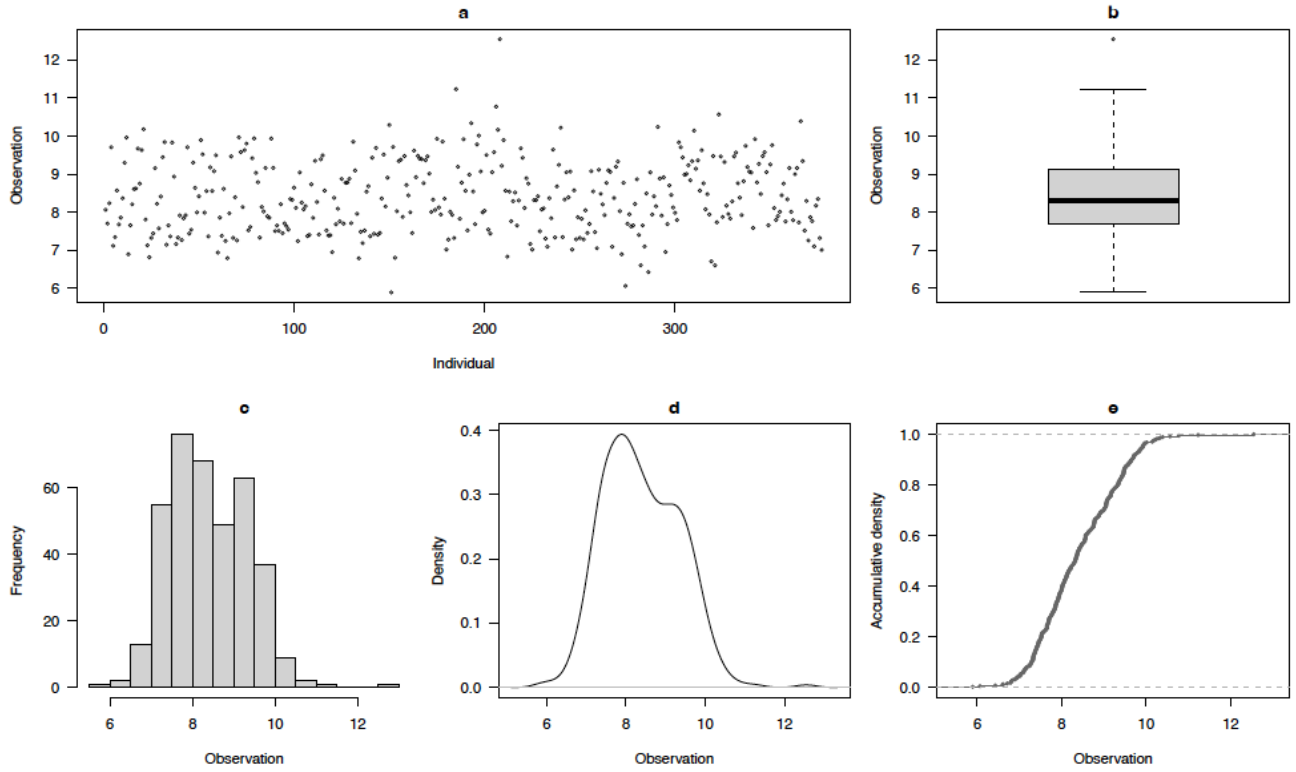


Figure 1: “GAPIT.Phenotype.View.Seed.length.pdf”

- The seed length looks normally distributed

. . . and also check heritability in the seed length

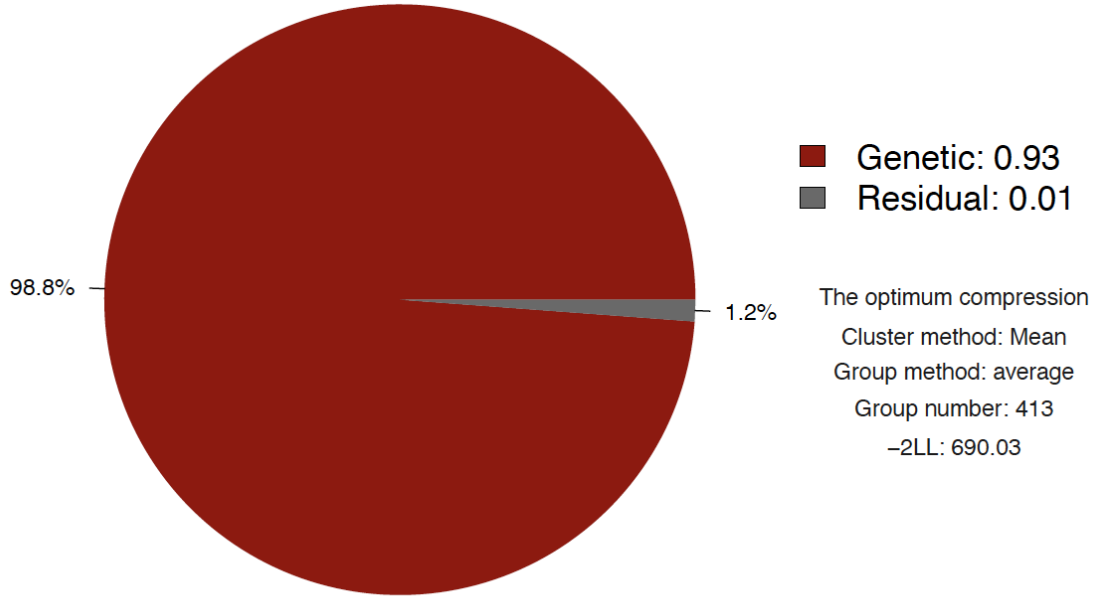


Figure 2: "GAPIT.Association.Optimum.MLM.Seed.length.pdf"

Heritability, $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2)$,
where σ_g^2: genetic variance; σ_e^2: residual variance
h^2 (%) is calculated as
$100 * (\sigma_g^2/(\sigma_g^2 + \sigma_e^2)) = 100 * (0.93/(0.93 + 0.01)) = 98.8\%$

Check LD to see what kbp we should refer around the SNPs.

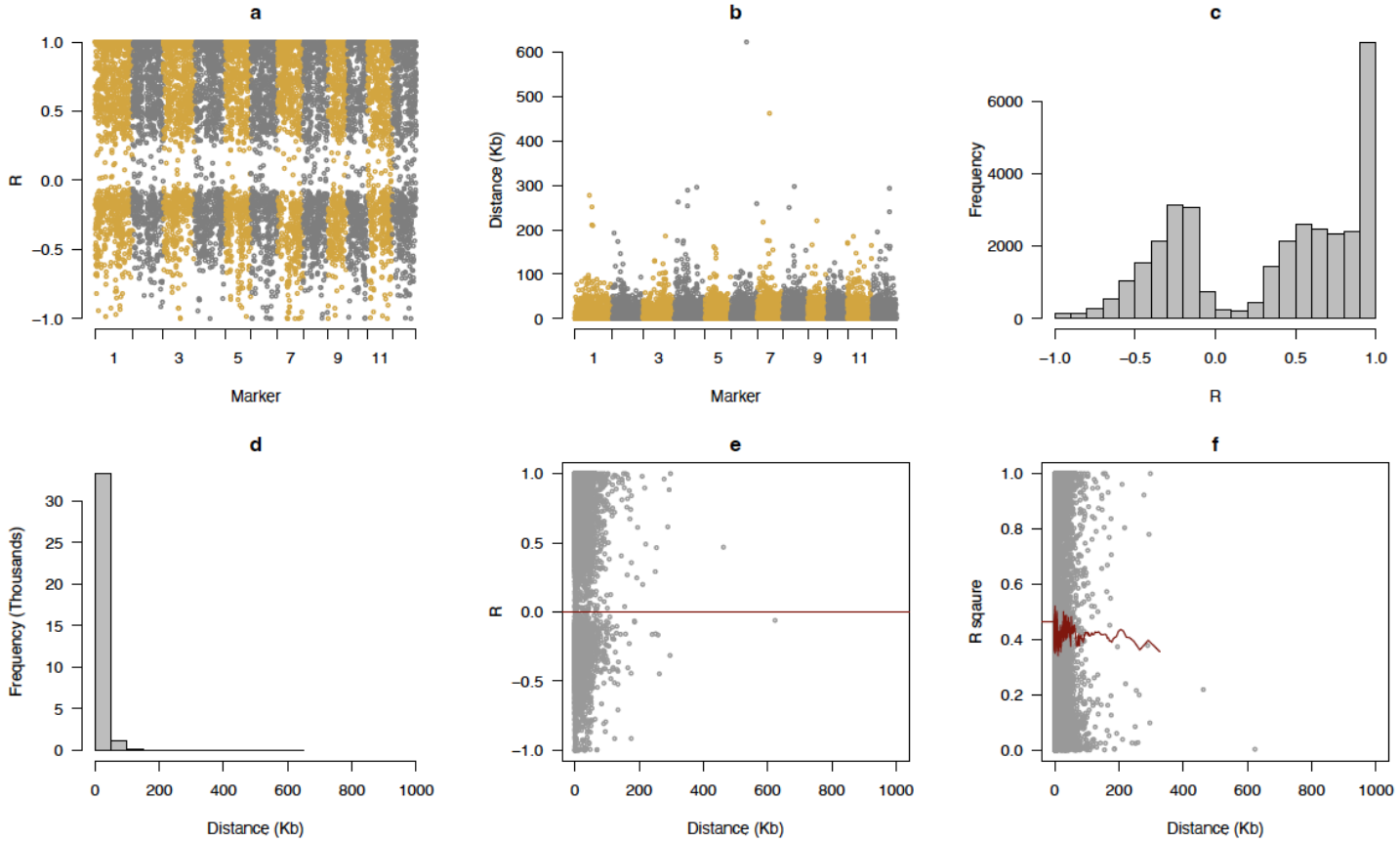


Figure 3: “GAPIT.Genotype.Density_R_sqaure.pdf”

(b) The length of linkage disequilibrium is at most 600 kbp.

Compare the marker density with the LD length

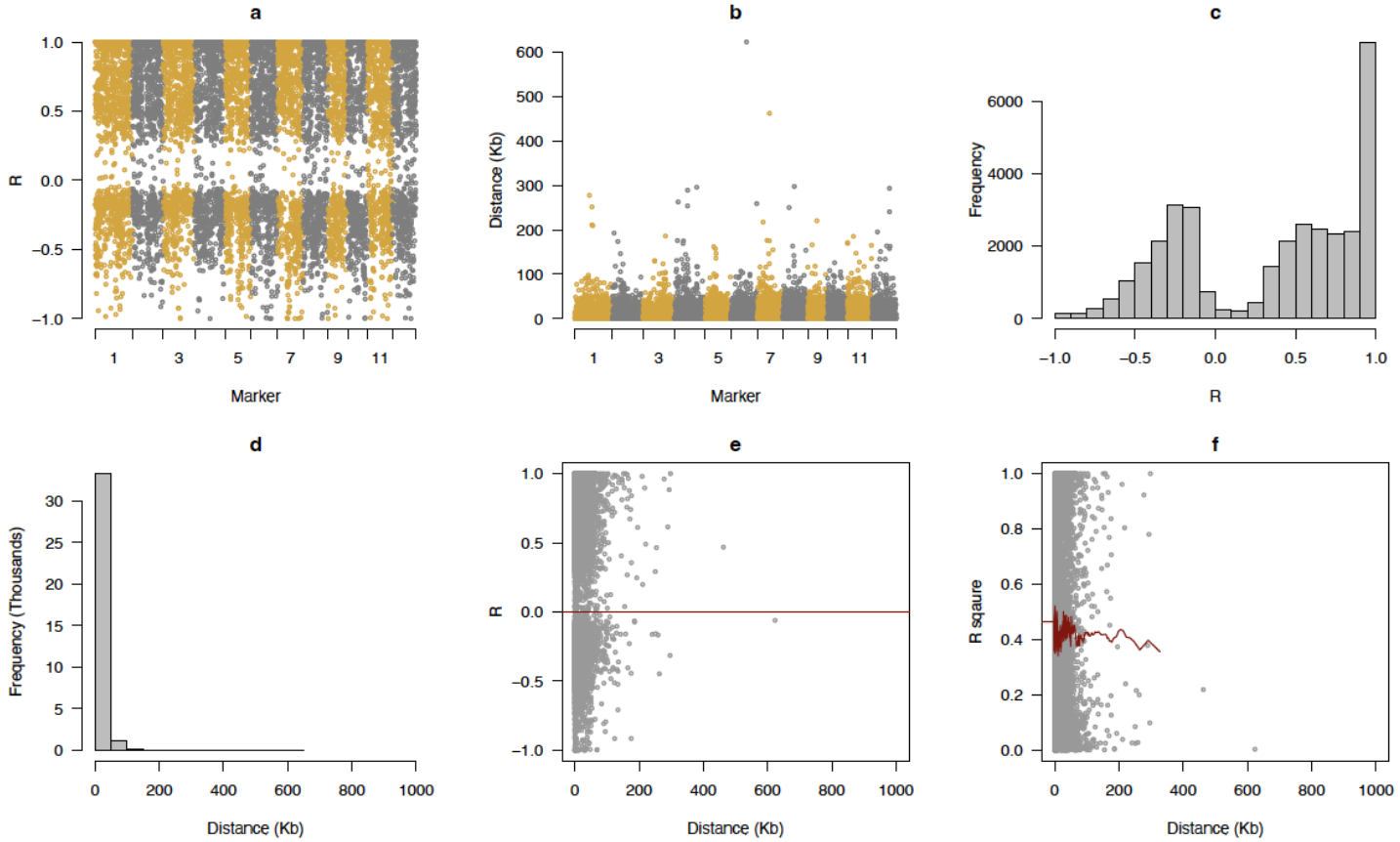


Figure 4: “GAPIT.Genotype.Density_R_sqaure.pdf”

(d) The marker intervals are much shorter than the length of LD,

- indicating that marker density was enough

Manhattan plot of the general linear model (GLM)

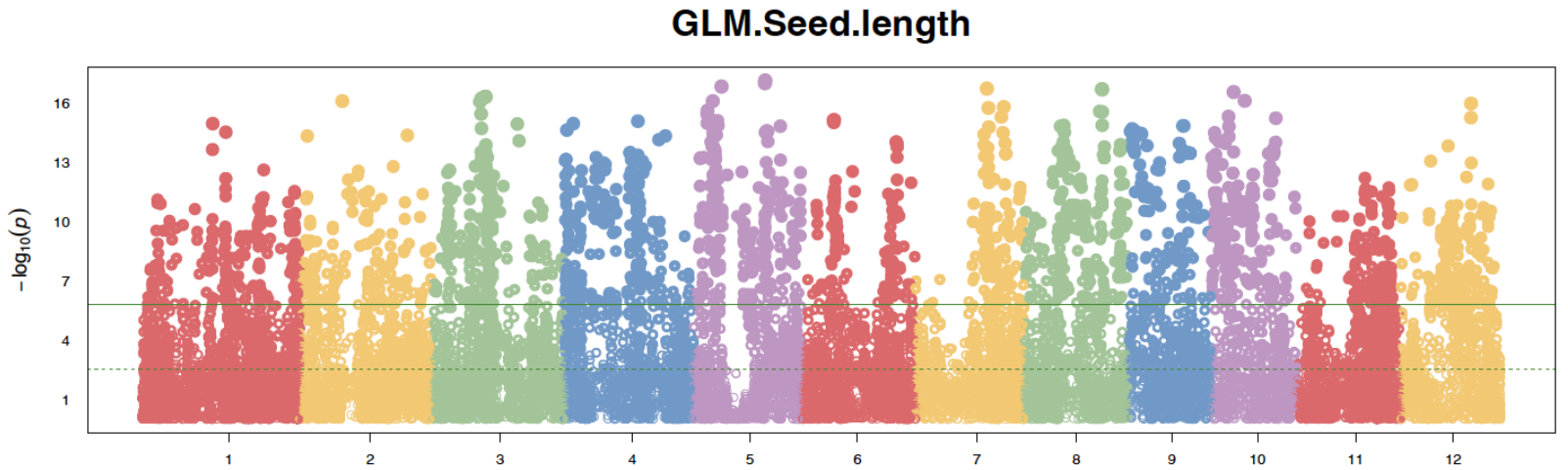


Figure 5: "GAPIT.Association.Manhattan_Geno.GLM.Seed.length.pdf"

- Is everything significant?? Very difficult to find key variants. . .

Quantile-quantile (QQ) plot also shows inflated p-values

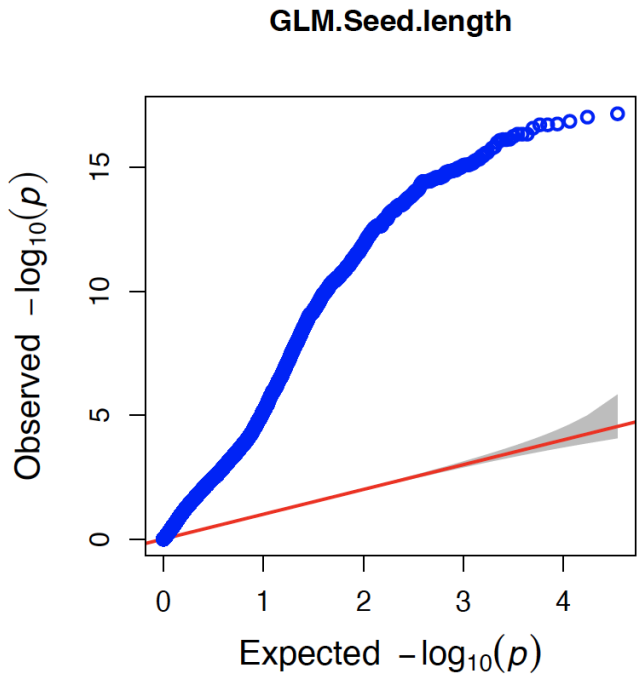


Figure 6: "GAPIT.Association.QQ.GLM.Seed.length.pdf"

- Blue dots: Observed -log10(p-values)
- Red line: Expected -log10(p-values) when they are random

Heatmap of the kinship matrix shows two clusters

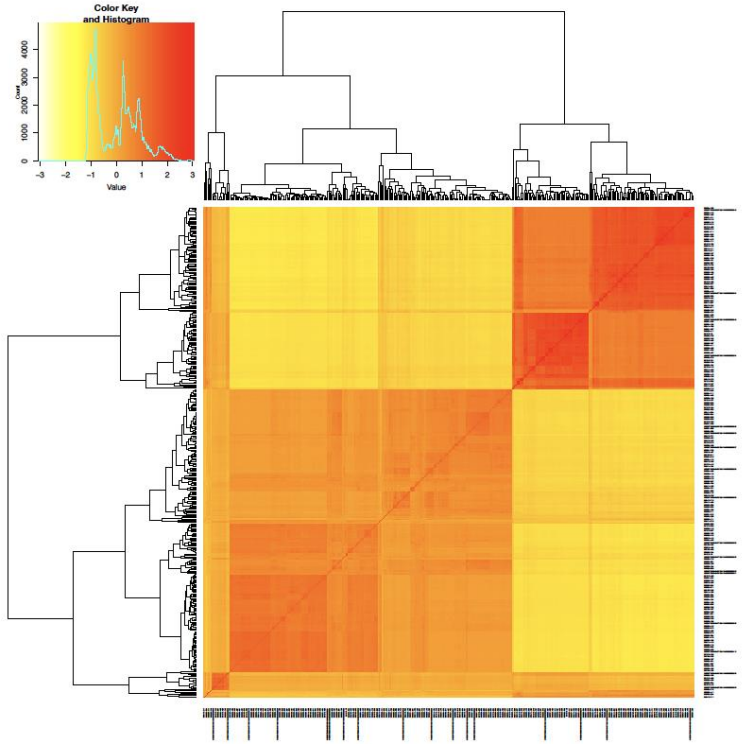


Figure 7: “GAPIT.Genotype.Kin_VanRaden.pdf”

- Here we see a complex kinship structure
- A mixed linear model is worth trying to correct it

Manhattan plot of the mixed linear model (MLM)

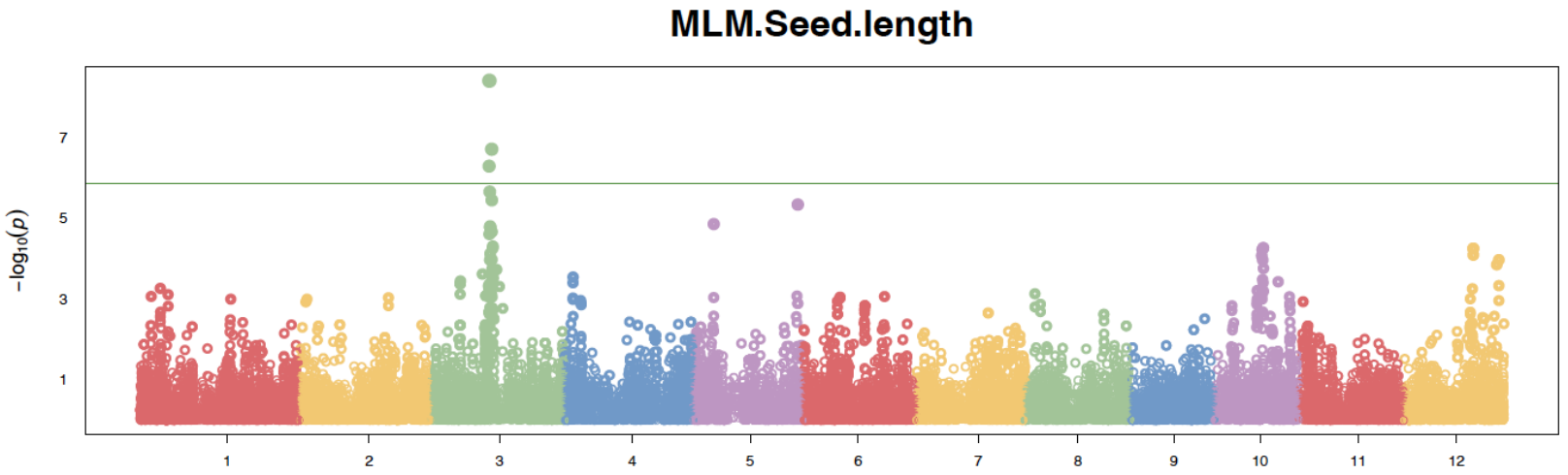


Figure 8: “GAPIT.Association.Manhattan_Geno.MLM.Seed.length.pdf”

- We can find a peak on the chromosome 3!

QQ plot of the mixed linear model

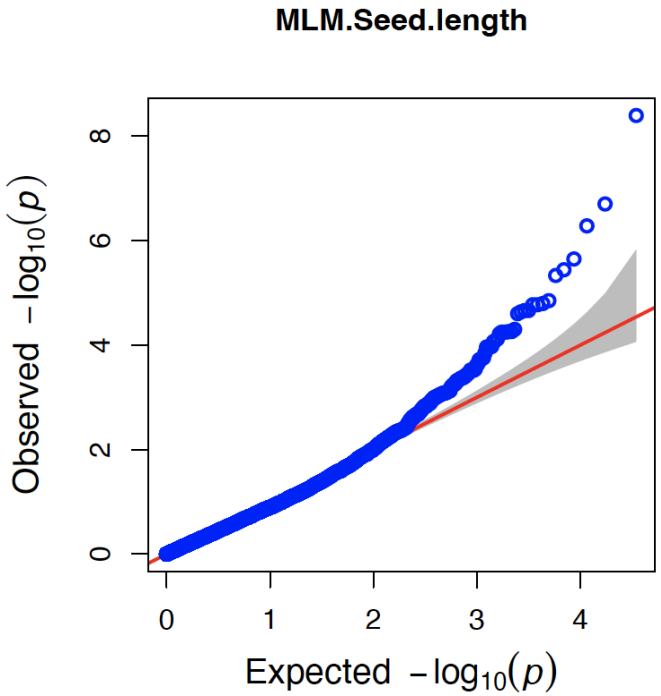


Figure 9: "GAPIT.Association.QQ.MLM.Seed.length.pdf"

- Only for top-scoring SNPs, -log10(p-values) are higher than expected

GWAS works well. Check the position of top-scoring SNPs

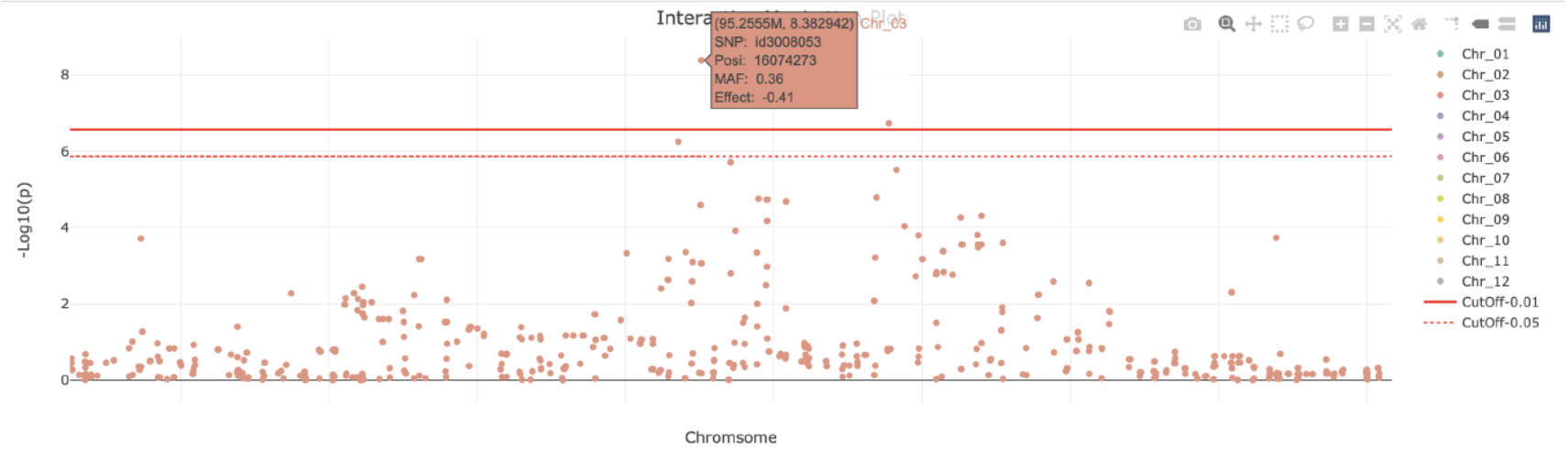
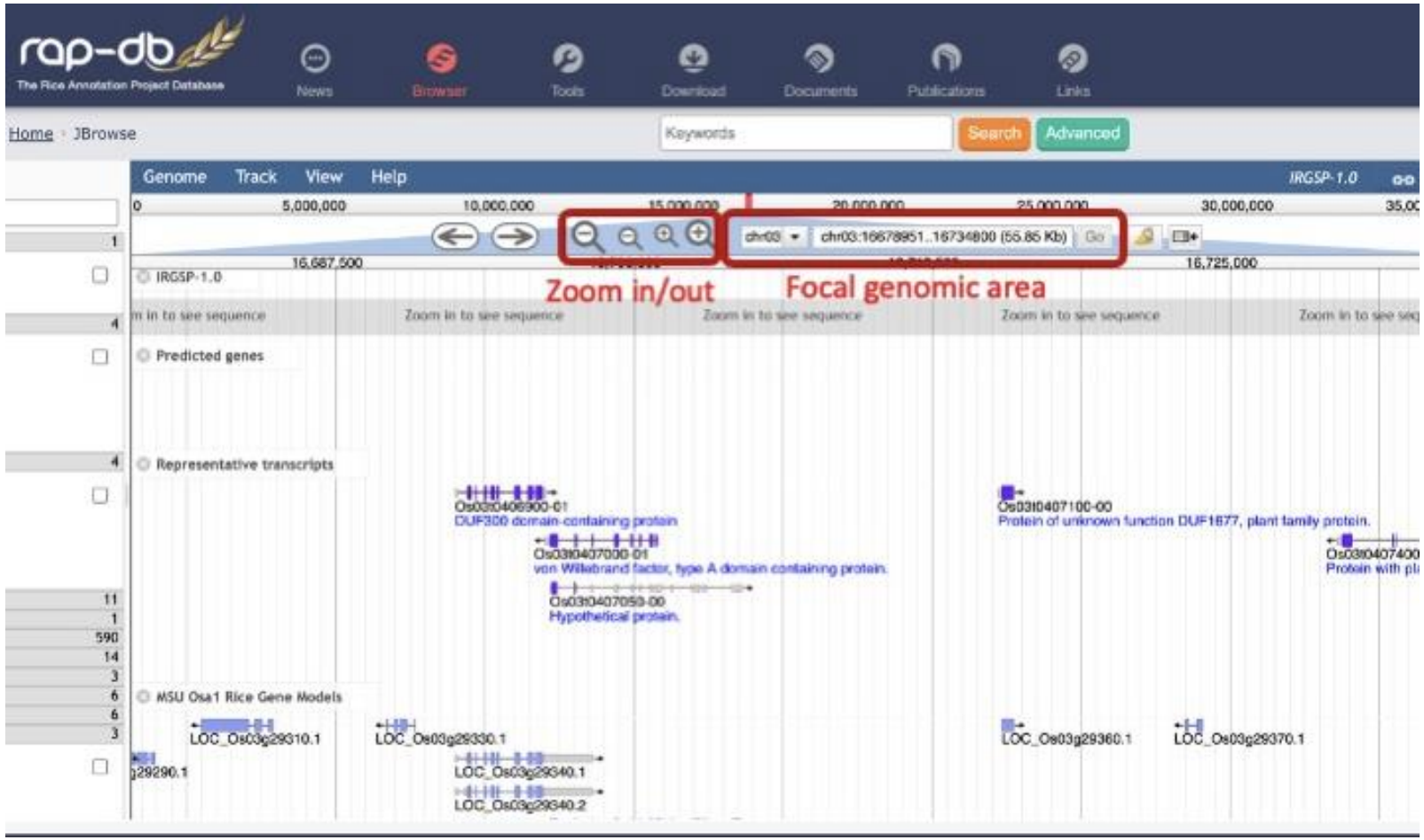


Figure 10: “GAPIT.Association.Interactive_Manhattan.MLM.Seed.length.html”

- Open “Interactive.Manhattan.MLM.Seed.length.html”
- You can find 2 significant SNPs on the chromosome 3
- The position info appears when you put your cursor on a SNP

What genes are located nearby? Check the database

- Access RAP-DB website at https://rapdb.dna.affrc.go.jp/
- Look for and click “JBrowse”



Short exercise: Let's find the GS3 locus.

- By using RAP-DB,
- 1. Input significant SNP positions ± 3 bp as a focal genomic area and "Go"
  - e.g., chr03:16706777..16706779
- 2. Zoom in/out ± the average LD length near the SNP
- 3. Find the locus ID "Os03t0407400-01" (= GS3) and click!
- Point of (biological) interpretation
- Which SNPs can you find GS3 locus nearby?
- How far is the GS3 from the significant SNP?
- What family of proteins does the GS3 encode?

(2) Genomic selection (GS)

Aim: Prediction of the flowering time in rice cultivars

- Flowering time, or heading date in rice, was recorded at Arkansas on 2006 and 2007 (Zhao et al. 2011)
- Genotypes were same but environment should be different between years
- The flowering time of some accessions were unavailable
- Can we predict the flowering time only on the basis of genotypes?

Estimate a trait value of each plant with gBLUP

- When finished, results are stored in “myGAPIT_BLUP” object.

```
# gBLUP for the flowering time 2006 at Arkansas
myGAPIT_BLUP <- GAPIT( # warnings occur but it still works
Y=p[,c("HybID","Year06Flowering.time.at.Arkansas")],
GD=g,
GM=gm,
SNP.MAF=0.05,
model="gBLUP",
kinship.algorithm="VanRaden",
file.output=FALSE)
```

```
## [1] "--------------------- Welcome to GAPIT ---------------------------"
## [1] "gBLUP"
## [1] "--------------------Processing traits----------------------------------"
## [1] "Phenotype provided!"
## [1] "The 1 model in all."
## [1] "MLM"
## [1] "GAPIT.DP in process..."
## [1] "GAPIT will filter marker with MAF setting !!"
## [1] "The markers will be filtered by SNP.MAF: 0.05"
## maf_index
## FALSE TRUE
## 2150 34751
## [1] "Calculating kinship..."
## [1] "Number of individuals and SNPs are 413 and 34751"
## [1] "Calculating kinship with VanRaden method..."
## [1] "substracting P..."
```

We get BLUP, PEV, BLUE, and predicted trait values

- BLUP: Best Linear Unbiased Predictor shows trait variance around mean
- PEV: prediction error variance of BLUP
- BLUE: Best Linear Unbiased Estimator shows mean differences of traits
- BLUP + BLUE = Prediction

```
# load results of genomic prediction
pred <- myGAPIT_BLUP$Pred
head(pred)
```

```
## Taxa Group RefInf ID BLUP PEV BLUE Prediction
## 1 081215-A05 1 1 1 -10.3304078 11.01517 89.3738566605352 79.04345
## 2 081215-A06 2 1 2 0.7679973 21.42044 89.3738566605352 90.14185
## 3 081215-A07 3 1 3 -1.9286126 20.80244 89.3738566605352 87.44524
## 4 081215-A08 4 1 4 0.4137206 15.23512 89.3738566605352 89.78758
## 5 090414-A09 5 1 5 1.8817708 18.49218 89.3738566605352 91.25563
## 6 090105-A02 7 1 6 8.8639442 20.66431 89.3738566605352 98.23780
```

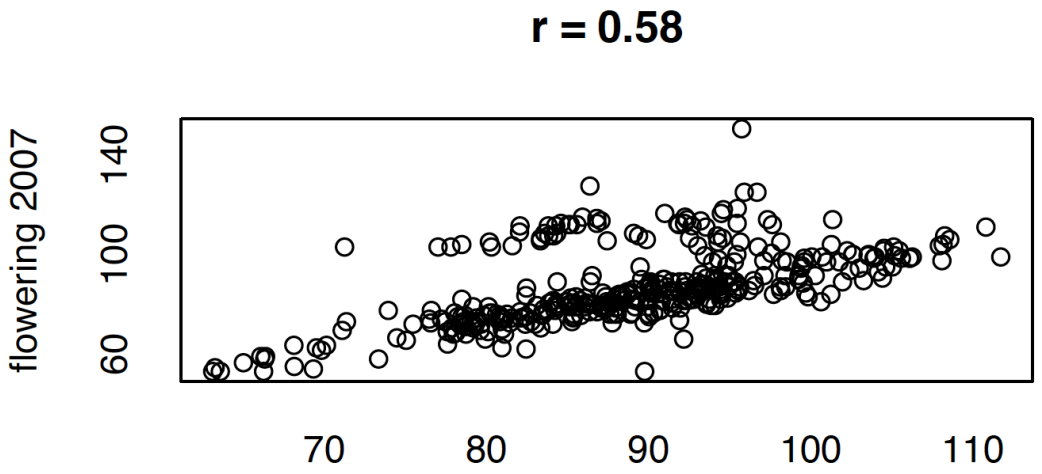
Of course, predicted flowering time is well correlated with observed values

```
# align predicted and observed traits following the taxa name
pred <- pred[order(pred$Taxa),]
y <- p[order(p$HybID),]
# calculate Pearson s correlation between predicted and observed flowering
cor.test(pred$Prediction, y$Year06Flowering.time.at.Arkansas , method =
'pearson')
```

```
##
## Pearson's product-moment correlation
##
## data: pred$Prediction and y$Year06Flowering.time.at.Arkansas
## t = 49.431, df = 335, p-value < 2.2e-16
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
## 0.9234637 0.9494873
## sample estimates:
## cor
## 0.9377791
```

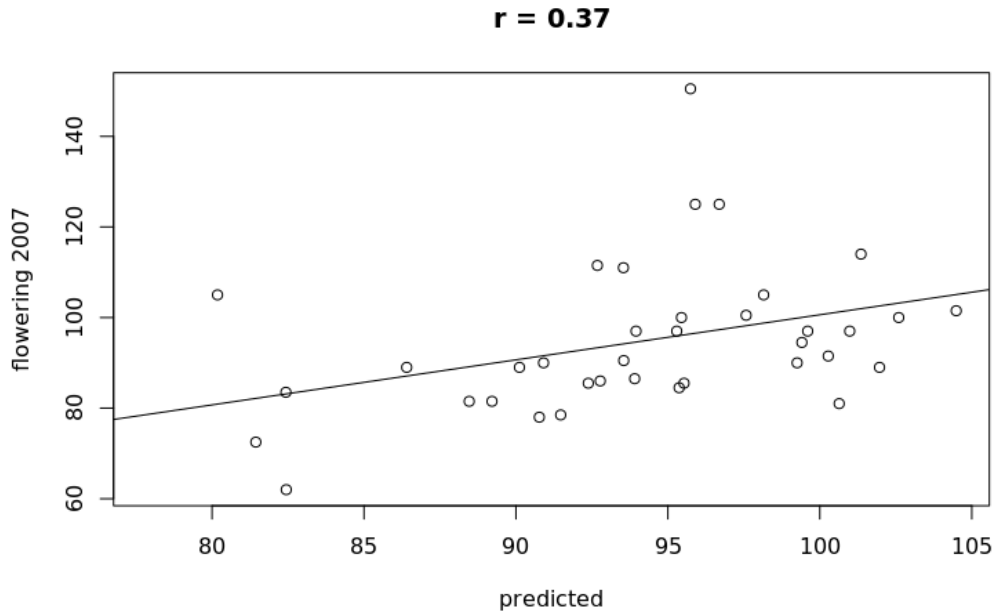
Predicted flowering time is correlated with those observed on 2007

```
# perform a linear regression to estimate the slope and intercept
res <- lm(y$Year07Flowering.time.at.Arkansas~pred$Prediction)
# plot the results
plot(pred$Prediction,
y$Year07Flowering.time.at.Arkansas,
ylab="flowering 2007", xlab="predicted",
main=paste("r =",round(sqrt(summary(res)$r.squared),2)))
abline(res)
```



Predicted flowering time is correlated with those of missing accessions

```
NA06 <- is.na(y$Year06Flowering.time.at.Arkansas)
# perform a linear regression to estimate the slope and intercept
res <- lm(y$Year07Flowering.time.at.Arkansas[NA06]~pred$Prediction[NA06])
# plot the results
plot(pred$Prediction[NA06],
y$Year07Flowering.time.at.Arkansas[NA06],
ylab="flowering 2007", xlab="predicted",
main=paste("r =",round(sqrt(summary(res)$r.squared),2)))
abline(res)
```



(3) Exercise

- Q1. Try GWAS of the flowering time at Aberdeen. How high is the heritability of this trait? At which chromosome can you find a peak?

- Q2. Find HEADING DATE1 (Hd1: locus ID “Os06g0275000”). How distant is this gene from the top-scoring SNP? What is the ortholog of Hd1 in Arabidopsis thaliana?

- Q3. Try gBLUP of the flowering time at Aberdeen. How large is the correlation between the predicted flowering time and observed one at Arkansas?

- Q4. More? You can test any traits of your interests!

(4) GWAS group work (30 min. incl. a break)

- Select 1 interesting trait for 1 group
- 1. Report its heritability,
- 2. perform MLM, and report –log10(p) of the most significant SNP,
- 3. and list up 2 interesting candidate genes (specified by the code like Os03txxxxx) <200 kb near the most significant SNP.
- Send the results to me (narjes.yousefi2@uzh.ch).
- One email from a representative is ok. Please add your group no. to the email title.

References

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