Exercises for *Introduction to eQTL Analysis*

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Contents

Prerequisites

Using the Docker image

All exercises assume the use of the docker container `humburg/eqtl-intro` to provide the required data as well as the necessary software environment. This requires a working Docker installation[1]. The docker image can be obtained from DockerHub via

```
docker pull humburg/eqtl-intro
```

To run the RStudio server run

```
docker run -p 8787:8787 humburg/eqtl-intro
```

RStudio is then accessible at `localhost:8787` or, when using boot2docker via the IP address indicated by `boot2docker ip`.

Included data

The image includes a number of simulated and real data sets used for these exercises. All data are provided as tab-separated files (typically with a column header). Files are located in directories below `/data`. All simulated data are located in `/data/simulated`. Real data can be found in `/data/genotyping`, `/data/expression` and `/data/annotation` for genotyping, gene expression and annotation data respectively.

Associations between SNPs and gene expression - A simple example

We will investigate the properties of a small simulated data set consisting of genotypes for 10 SNPs and expression values for 10 genes from 300 individuals. Genotypes are encoded as 0, 1, and 2, indicating the number of copies of the second allele.

Genotypes are located in the file `/data/simulated/sim_genotypes.tab` and gene expression values can be found in `/data/simulated/expression1.tab`.

Questions

1. What are the minor allele frequencies of the different SNPs in the data set?
2. Consider pairs of SNPs and genes such that *snp_1* is paired with *gene_1*, *snp_2* with *gene_2* and so on.
   i. Create a plot showing gene expression by genotype for one of the SNP/gene pairs.
   ii. For each SNP/gene pair fit a linear regression model to obtain an estimate of the genotype effect on gene expression and compute the 95% confidence intervals for the ten SNP effects.
   iii. Create a plot to compare the estimated coefficients and their 95% confidence intervals to 1.5, the true value of β. What do you observe?

Solution for *Associations between SNPs and gene expression - A simple example*

We start by loading the data. This can be done using RStudio's data import functionality or manually through the command-line.

```
geno <- readr::read_tsv("/data/simulated/sim_genotypes.tab")
expr <- readr::read_tsv("/data/simulated/sim_expression1.tab")
```

Note that the first column contains the sample names.

[1] installation instructions are available from the Docker website.

Computing minor allele frequencies

The genotypes are encoded as the number of copies of the second allele carried by each individual. For eQTL analyses it is useful to ensure the second allele corresponds to the minor allele. This helps with the interpretation of genotype effects obtained from the analysis. In this case alleles have already been arranged in a suitable manner[2].

With the given encoding it is straightforward to obtain the frequency of the second allele.

```
maf <- colMeans(geno[-1])/2
maf
```

```
##       snp_1       snp_2       snp_3       snp_4       snp_5       snp_6
## 0.006666667 0.030000000 0.020000000 0.065000000 0.046666667 0.126666667
##       snp_7       snp_8       snp_9      snp_10
## 0.171666667 0.298333333 0.390000000 0.511666667
```

As it turns out the second allele for *snp_10* is actually the major allele. To ensure we actually get the MAF this needs to be inverted.

```
maf <- pmin(maf, 1-maf)
maf
```

```
##       snp_1       snp_2       snp_3       snp_4       snp_5       snp_6
## 0.006666667 0.030000000 0.020000000 0.065000000 0.046666667 0.126666667
##       snp_7       snp_8       snp_9      snp_10
## 0.171666667 0.298333333 0.390000000 0.488333333
```

Plotting gene expression by genotype

A convenient way to display gene expression values by genotype is as box plots. These provide a good, non-parametric, indication of the distributions. To convey a sense of the frequency of each genotype in the sample it is useful to also add points for each individual to the plot. Below is an example of how this might look for each of the ten SNP/gene pairs.

```
library(ggplot2)
```

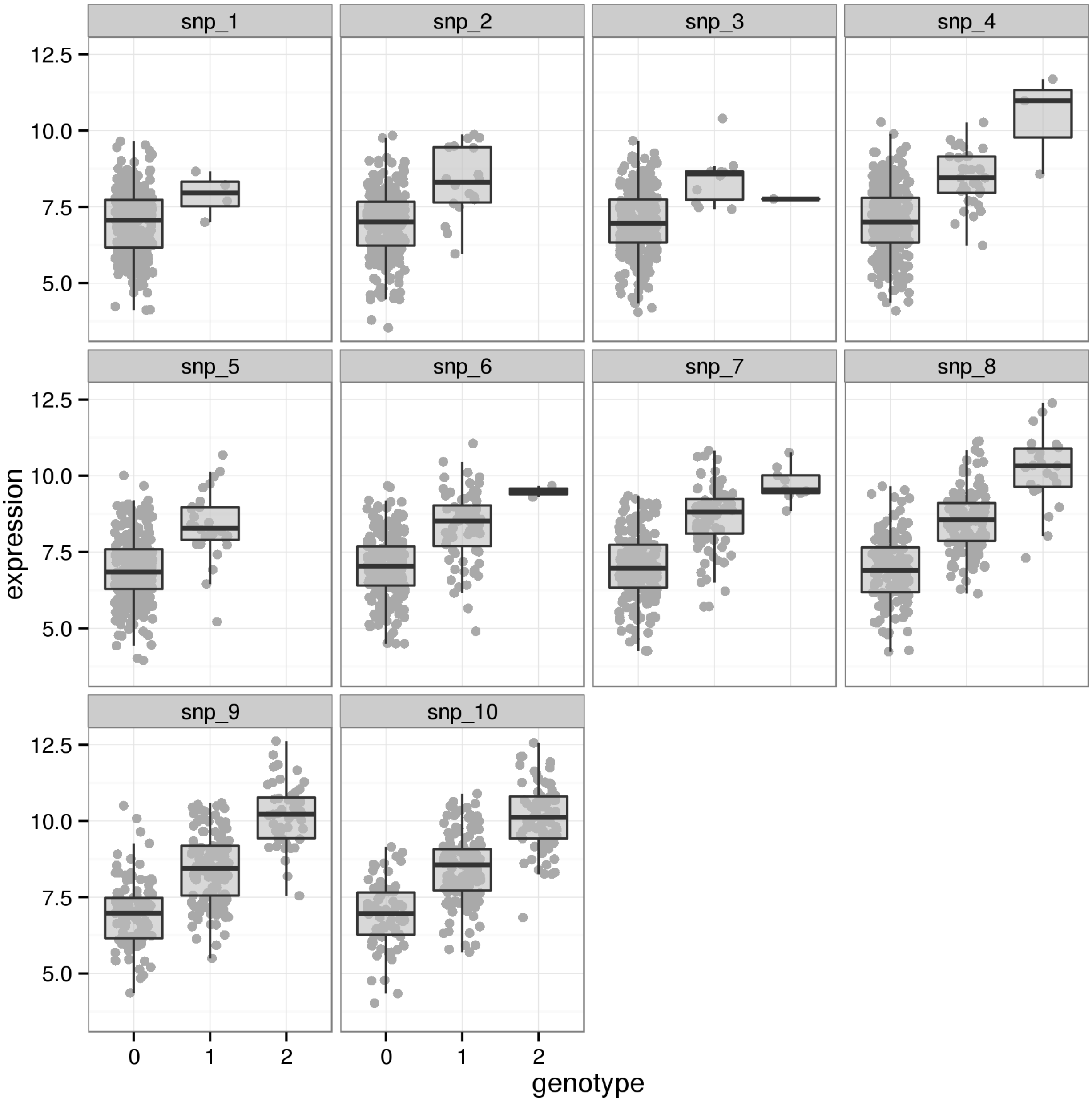
```
## Loading required package: methods
```

```
genoLong <- tidyr::gather(geno, snp, genotype, -sample)
exprLong <- tidyr::gather(expr, gene, expression, -sample)
dataLong <- cbind(genoLong, exprLong["expression"])
dataLong$genotype <- as.factor(dataLong$genotype)
ggplot(dataLong, aes(genotype, expression)) +
        geom_jitter(colour="darkgrey", position=position_jitter(width=0.25)) +
        geom_boxplot(outlier.size=0, alpha=0.6, fill="grey") +
        facet_wrap(~snp) + theme_bw()
```

Estimating SNP effects

To obtain estimates of the genotypic contribution to gene expression we fit a simple linear regression model of the form $E_i = \beta_0 + \beta G_i + \varepsilon$, where E_i is the vector of gene expression values for gene i and G_i is the genotype vector for SNP i. We are interested in the estimate for β which indicates the change in gene expression for each copy of the second allele.

[2] at least for the most part, see below



plot of chunk simple_exprPlot

```
fit <- mapply(function(e, g) lm(e ~ g), expr[-1], geno[-1], SIMPLIFY=FALSE)
betaHat <- sapply(fit, coef)[2,]
betaHat
```

```
##    gene_1    gene_2    gene_3    gene_4    gene_5    gene_6    gene_7
## 0.9416734 1.3518024 1.1324493 1.5005237 1.4329068 1.2928385 1.5106722
##    gene_8    gene_9   gene_10
## 1.6050215 1.6038507 1.6162730
```

We use the function `confint` to obtain 95% confidence intervals of the estimated SNP effects.

```
ci <- sapply(fit, confint, "g")
rownames(ci) <- c("lower", "upper")
ci
```

```
##           gene_1    gene_2    gene_3   gene_4   gene_5   gene_6   gene_7
## lower -0.1043169 0.8457378 0.5723167 1.156449 1.017273 1.026215 1.286452
## upper  1.9876638 1.8578670 1.6925819 1.844598 1.848541 1.559462 1.734892
##         gene_8   gene_9  gene_10
## lower 1.412641 1.426662 1.443334
## upper 1.797402 1.781040 1.789212
```

Plotting results

```
estimates <- data.frame(estimate=betaHat, t(ci), maf=maf)
ggplot(estimates, aes(x=maf)) + geom_hline(yintercept=1.5) +
        geom_hline(yintercept=0, linetype="longdash") +
        geom_errorbar(aes(ymin=lower, ymax=upper)) +
        geom_point(aes(y=estimate))  + theme_bw()
```

In this example all resulting confidence intervals include the true value[3] but intervals for small minor allele frequencies are large (and in one case this means that 0 is included in the CI). As one would expect the uncertainty in the estimate, as measured by the length of the confidence interval, decreases with increasing minor allele frequency. However, even at high MAF considerable uncertainty remains and point estimates are somewhat lacking in accuracy, overestimating the true effect.

Associations between SNPs and gene expression - Confounding variation

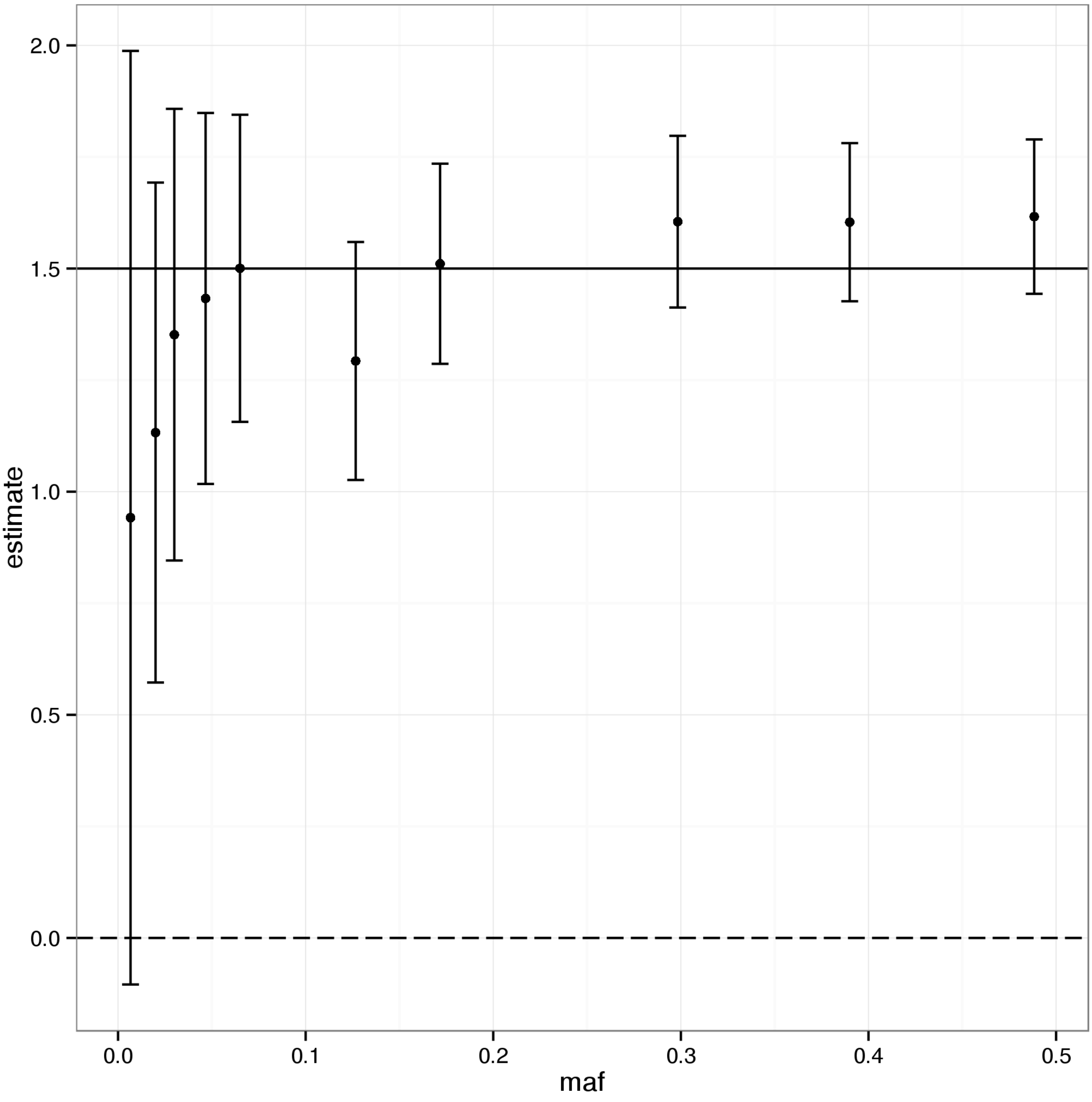
In this example we investigate the effect that the presence of other sources of variation has on our ability to detect the genotypic effects of interest.

This exercise uses the same simulated genotypes as the previous one (`/data/simulated/sim_genotypes.tab`). The gene expression data is located in `/data/simulated/sim_expression2.tab`. The later parts of the exercise also requires a number of covariates located in `/data/simulated/sim_covariates.tab`

Questions

1. Create a plot of gene expression by genotype for one of the SNP/gene pairs. How does this compare to the plot from the previous exercise?
2. Carry out a simple eQTL analysis for the matched SNP/gene pairs.
    i. For each SNP/gene pair fit a linear regression model to obtain an estimate of the genotype effect on gene expression and compute the 95% confidence intervals for the ten SNP effects.

[3] although sometimes only just



plot of chunk simple_plot

   ii. Create a plot that compares the estimates of effect size obtained above to the true value of 1.5. How does this compare to the results from the previous example?

3. Using the additional variables contained in the covariates file, fit another set of models.
   i. For each gene fit a model that incorporates the corresponding SNP as well as the first five variables from the covariates file.
   ii. Create the same plot of effect size estimates as before for this extended model. How do they compare?
   iii. Repeat the above analysis with all covariates included in the model.
   iv. Create a plot of gene expression by genotype illustrating the effect.

Solution for *Associations between SNPs and gene expression - Confounding variation*

We start by loading and plotting the data.

```
geno <- readr::read_tsv("/data/simulated/sim_genotypes.tab")
expr <- readr::read_tsv("/data/simulated/sim_expression2.tab")
```

Note that the first column contains the sample names.

```
library(ggplot2)
genoLong <- tidyr::gather(geno, snp, genotype, -sample)
exprLong <- tidyr::gather(expr, gene, expression, -sample)
dataLong <- cbind(genoLong, exprLong["expression"])
dataLong$genotype <- as.factor(dataLong$genotype)
ggplot(dataLong, aes(genotype, expression)) +
        geom_jitter(colour="darkgrey", position=position_jitter(width=0.25)) +
        geom_boxplot(outlier.size=0, alpha=0.6, fill="grey") +
        facet_wrap(~snp) + theme_bw()
```

These data show very little evidence of a SNP effect on gene expression.

Simple linear regression

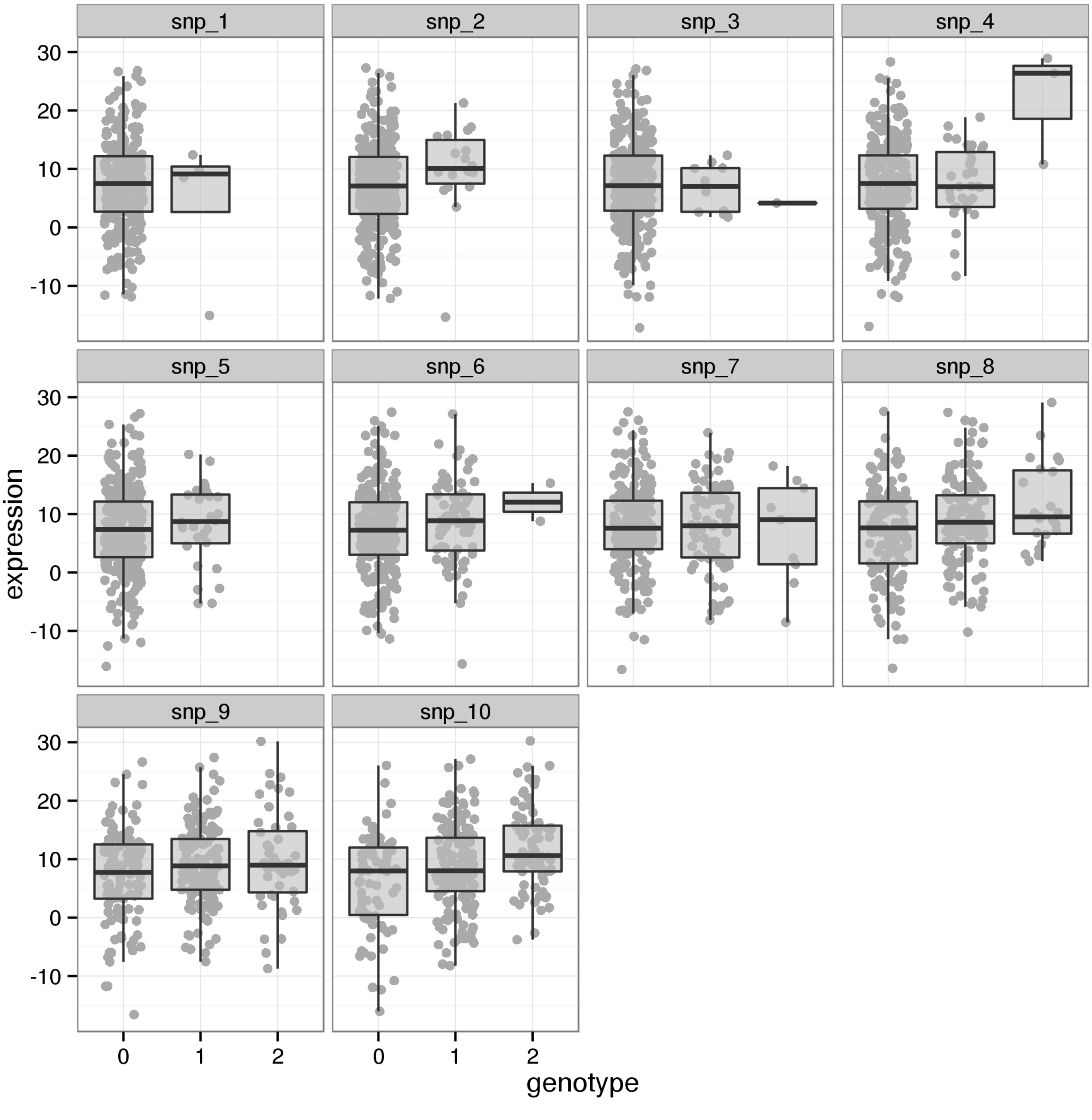
We fit a simple linear regression and compute confidence intervals for the SNP effects as before.

```
simpleFit <- mapply(function(e, g) lm(e ~ g), expr[-1], geno[-1], SIMPLIFY=FALSE)
simpleBetaHat <- sapply(simpleFit, coef)[2,]
simpleBetaHat
```

```
##     gene_1     gene_2     gene_3     gene_4     gene_5     gene_6 
## -3.4013977  2.8772877 -0.9171664  2.0536036  0.9620831  1.5380368 
##     gene_7     gene_8     gene_9    gene_10 
## -0.1677433  2.3152640  1.3726739  2.9264897
```

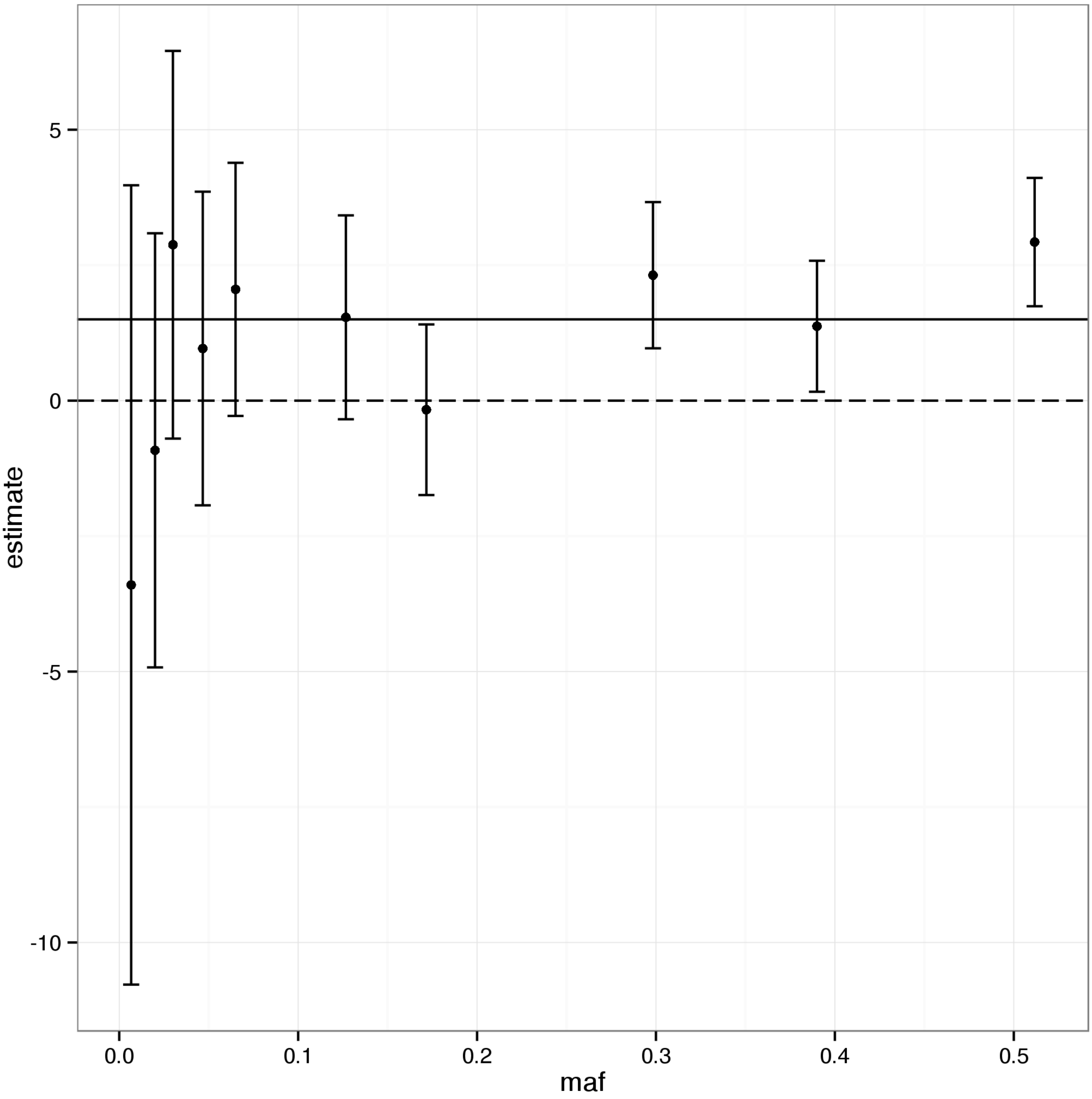
```
simpleCI <- sapply(simpleFit, confint, "g")
rownames(simpleCI) <- c("lower", "upper")
simpleCI
```

```
##           gene_1     gene_2    gene_3     gene_4    gene_5     gene_6
## lower -10.777348 -0.7001765 -4.923182 -0.2827436 -1.932525 -0.3434186
## upper   3.974552  6.4547520  3.088849  4.3899507  3.856691  3.4194923
##          gene_7    gene_8    gene_9  gene_10
## lower -1.742395 0.9660378 0.1639046 1.742243
## upper  1.406908 3.6644902 2.5814431 4.110736
```



plot of chunk covar_exprPlot

```
maf <- colMeans(geno[-1])/2
estimates <- data.frame(estimate=simpleBetaHat, t(simpleCI), maf=maf)
ggplot(estimates, aes(x=maf)) + geom_hline(yintercept=1.5) +
        geom_hline(yintercept=0, linetype="longdash") +
        geom_errorbar(aes(ymin=lower, ymax=upper)) +
        geom_point(aes(y=estimate))  + theme_bw()
```



plot of chunk covar_plot_simple

The confidence intervals obtained from this analysis are much wider than previously. Unlike before they frequently contain 0 and although most of them still contain the true value this is not always the case. Also note that the most pronounced estimate is a clear over estimation of the real effect.

Incorporating covariates

We first load the additional variables:

```
covar <- readr::read_tsv("/data/simulated/sim_covariates.tab")
```

and then proceed to fit the extended model.

```
covarFit <- mapply(function(e, g, var) lm(e ~ g + var), expr[-1], geno[-1],
            MoreArgs=list(as.matrix(covar[2:6])), SIMPLIFY=FALSE)
covarBetaHat <- sapply(covarFit, coef)[2,]
covarCI <- sapply(covarFit, confint, "g")
rownames(covarCI) <- c("lower", "upper")
covarBetaHat
```

```
##      gene_1      gene_2      gene_3      gene_4      gene_5      gene_6
## -0.92692418  2.58287646  0.05357444  2.33807018  0.02783755  2.38987947
##      gene_7      gene_8      gene_9     gene_10
##  0.12701684  1.51168513  1.63007198  1.89560493
```

```
covarCI
```

```
##          gene_1      gene_2    gene_3   gene_4    gene_5   gene_6
## lower -6.345923 -0.04080591 -2.896184 0.590358 -2.096652 1.008258
## upper  4.492075  5.20655884  3.003333 4.085782  2.152327 3.771501
##          gene_7    gene_8    gene_9   gene_10
## lower -1.036165 0.5142636 0.7415275 0.9973932
## upper  1.290199 2.5091067 2.5186165 2.7938167
```

```
estimates <- data.frame(estimate=covarBetaHat, t(covarCI), maf=maf)
ggplot(estimates, aes(x=maf)) + geom_hline(yintercept=1.5) +
        geom_hline(yintercept=0, linetype="longdash") +
        geom_errorbar(aes(ymin=lower, ymax=upper)) +
        geom_point(aes(y=estimate))  + theme_bw()
```

The inclusion of the covariates leads to a tighter set of confidence intervals. While it remains difficult to detect any meaningful genotypic effect at low minor allele frequencies the estimates appear to be more reliable at higher frequencies.

Full model

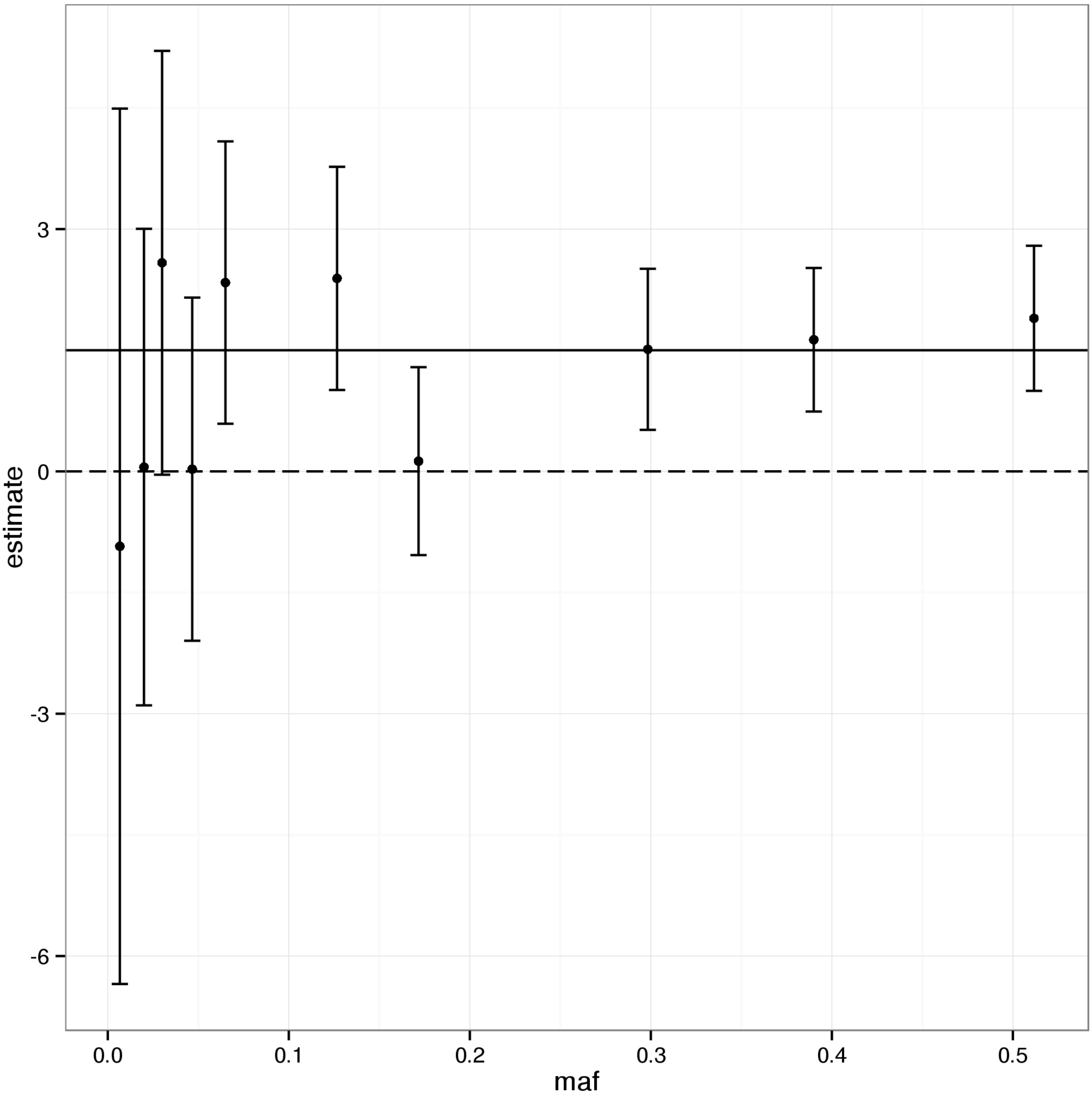
The computations for this are essentially the same as before with several additional variables in the model.

```
fullFit <- mapply(function(e, g, var) lm(e ~ g + var), expr[-1], geno[-1],
            MoreArgs=list(as.matrix(covar[-1])), SIMPLIFY=FALSE)
fullBetaHat <- sapply(fullFit, coef)[2,]
fullCI <- sapply(fullFit, confint, "g")
rownames(fullCI) <- c("lower", "upper")
fullBetaHat
```

```
##    gene_1    gene_2    gene_3    gene_4    gene_5    gene_6    gene_7
## 0.9770184 1.3352627 1.1639125 1.4780764 1.4690379 1.2627829 1.5150835
##    gene_8    gene_9   gene_10
## 1.5612290 1.5650634 1.6577558
```

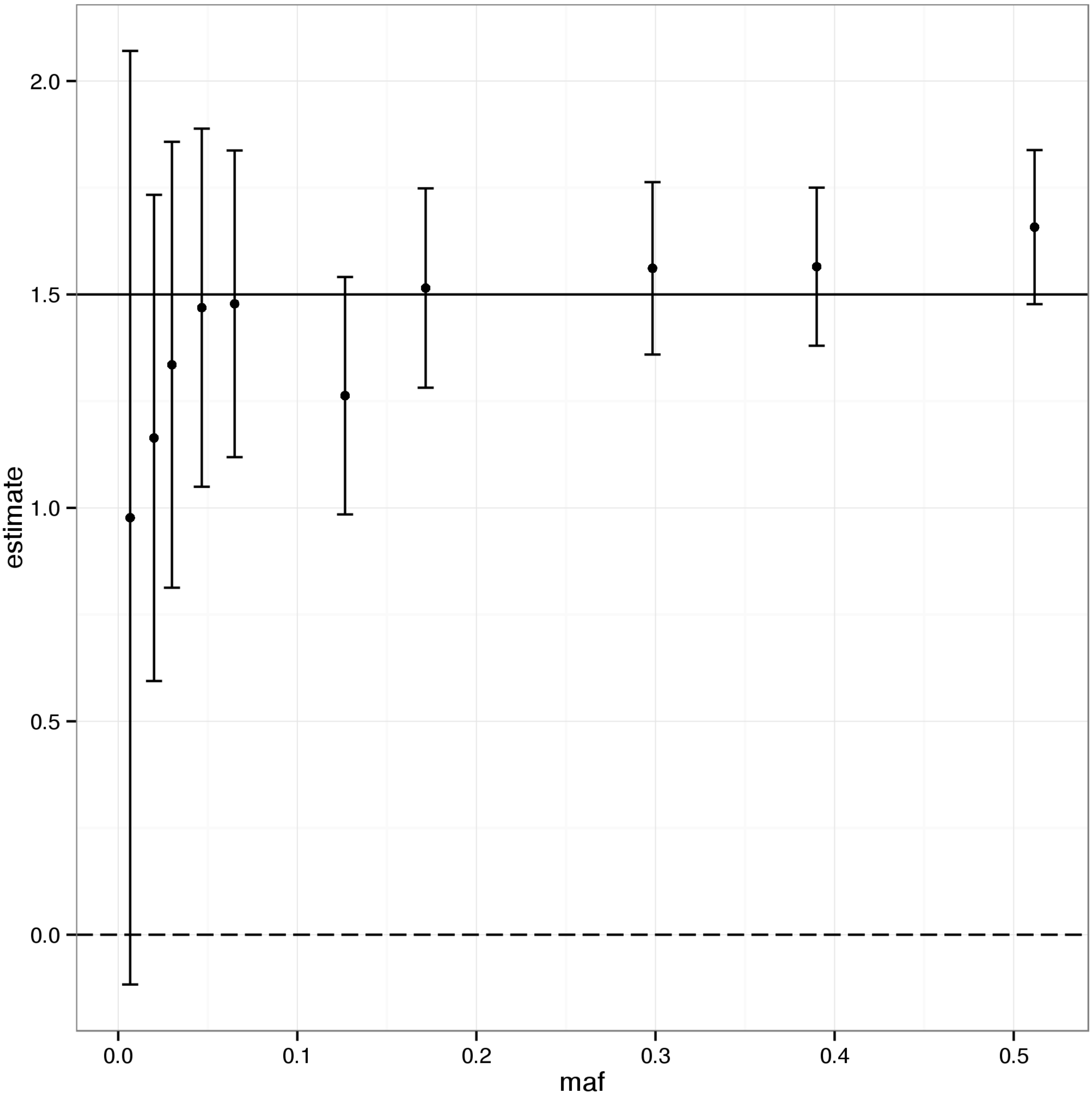
```
fullCI
```

```
##           gene_1   gene_2   gene_3   gene_4   gene_5    gene_6   gene_7
## lower -0.1165821 0.812906 0.594418 1.118809 1.049537 0.9846741 1.281600
## upper  2.0706189 1.857619 1.733407 1.837344 1.888539 1.5408917 1.748568
##         gene_8   gene_9  gene_10
## lower 1.359286 1.379866 1.477203
## upper 1.763172 1.750261 1.838309
```



plot of chunk covar_plot_5cv

```
estimates <- data.frame(estimate=fullBetaHat, t(fullCI), maf=maf)
ggplot(estimates, aes(x=maf)) + geom_hline(yintercept=1.5) +
        geom_hline(yintercept=0, linetype="longdash") +
        geom_errorbar(aes(ymin=lower, ymax=upper)) +
        geom_point(aes(y=estimate))  + theme_bw()
```



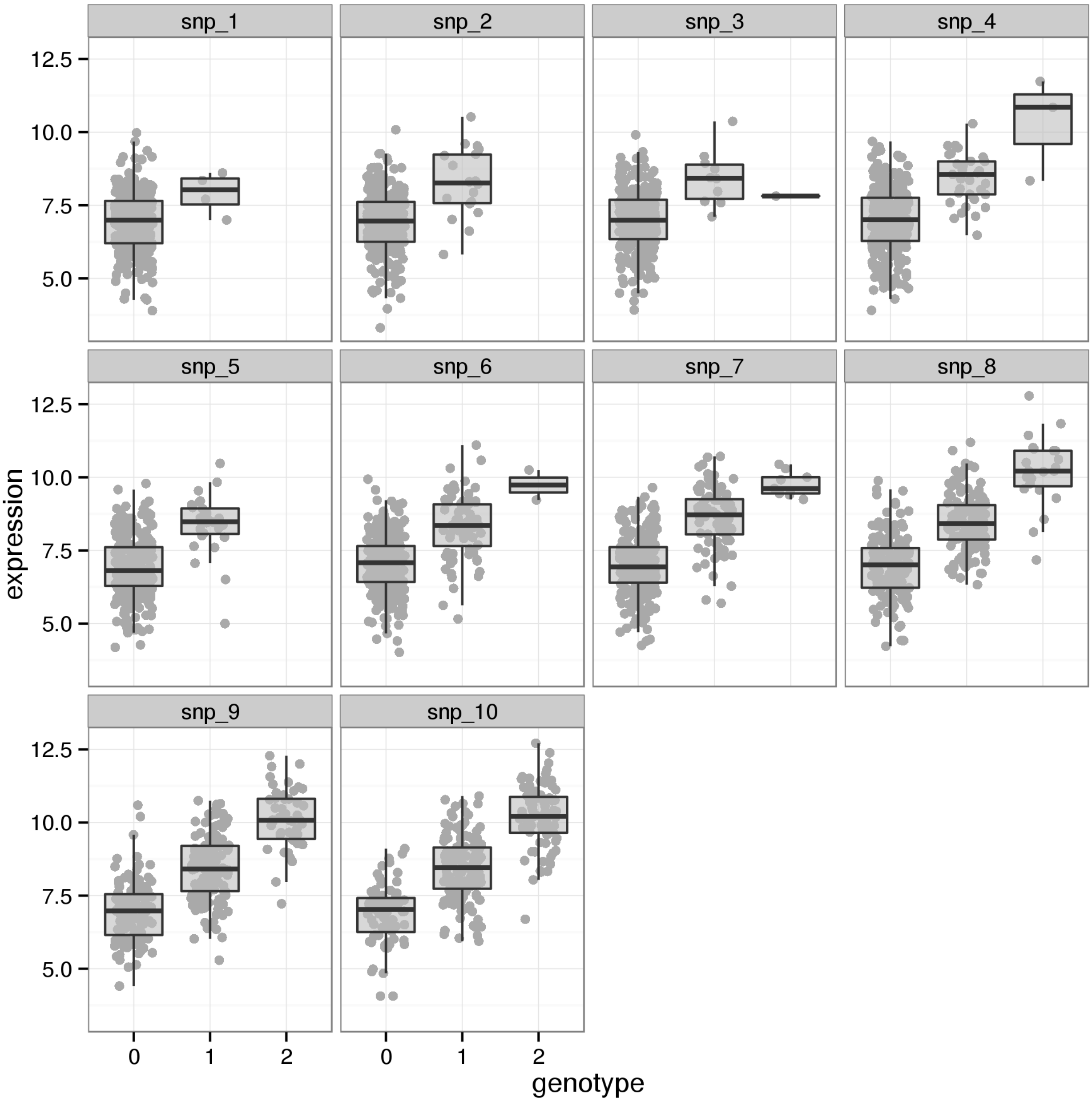
plot of chunk covar_plot_full

Including the full set of covariates in the model produces results similar to the ones from the initial, simple example. This shows that genotypic effects can be recovered if all confounders are accounted for.

Visualising SNP effects on gene expression in the presence of other covariates

When the effect of a SNP on gene expression is obscured by confounding variation this can be accounted for during the analysis by including appropriate variables in the model (assuming that they are known or can be otherwise captured). However, when plotting the gene expression values by genotype the effect still appears diminished, if it is visible at all. To obtain a plot that matches the result of the analysis the gene expression data has to be corrected for the effects attributed to the other covariates used in the model.

```
corrected <- mapply(function(f, x, var) x - colSums(coef(f)[-(1:2)]*t(var)),
        fullFit, expr[-1], MoreArgs=list(covar[-1]))
correctedLong <- tidyr::gather(data.frame(corrected), gene, expression)
combLong <- cbind(genoLong, correctedLong["expression"])
combLong$genotype <- as.factor(dataLong$genotype)
ggplot(combLong, aes(genotype, expression)) +
        geom_jitter(colour="darkgrey", position=position_jitter(width=0.25)) +
        geom_boxplot(outlier.size=0, alpha=0.6, fill="grey") +
        facet_wrap(~snp) + theme_bw()
```



plot of chunk covar_plot_corrected

Using principle components as covariates

We will explore the use of principle components as covariates in linear models of gene expression to account for unknown sources of variation.

Gene expression data are located in */data/monocytes/expression/ifn_expression.tab.gz* Genotypes are located in */data/genotypes/genotypes.tab.gz* (provided during course)

These data are part of the dataset published in Fairfax, Humburg, Makino, et al. Innate Immune Activity Conditions the Effect of Regulatory Variants upon Monocyte Gene Expression. Science (2014). doi:10.1126/science.1246949.

In addition to the primary datasets a few files with annotations for SNPs and genes is available in the */data/monocytes/annotation* directory:

snp_loc_hg19.tab Genomic location of SNPs.
probe_loc_hg19.tab Genomic location of gene expression probes.
probeAnnotations.tab Further annotations for gene expression probes, including associated gene symbols.

All coordinates refer to the hg19 reference build.

Exercises

1. Determine the dimensions of this dataset. How many genes, SNPs and samples are included?
2. Principle components of the expression data.
   i. Compute the principle components.
   ii. Create a plot of the variances for the first 20 PCs.
   iii. How much of the total variance is explained by the first 20 PCs?
3. Using PCs in eQTL analysis.
   i. Model the expression measured by probe 3710685 as a function of SNP rs4077515 and the first 10 PCs.
   ii. Create a plot of gene expression by genotype with the effect of the PCs removed.
   iii. How does this compare to the simple linear regression model for this SNP/gene pair.

Solution for *Using principle components as covariates*

We start by loading all relevant data.

```
geno <- readr::read_tsv(file("/data/genotypes/genotypes.tab.gz"))
expr <- readr::read_tsv(file("/data/monocytes/expression/ifn_expression.tab.gz"))

probePos <- readr::read_tsv("/data/monocytes/annotation/probe_loc_hg19.tab")
snpPos <- readr::read_tsv("/data/monocytes/annotation/snp_loc_hg19.tab")
probeAnno <- readr::read_tsv("/data/monocytes/annotation/probeAnnotations.tab")
```

Size of dataset

```
dim(expr)
```

```
## [1] 382 368
```

```
dim(geno)
```

```
## [1] 28307   368
```

Note that these files have samples in columns and variables in rows. So the data consists of 367 samples with measurements for 382 gene expression probes and 28307 SNPs.

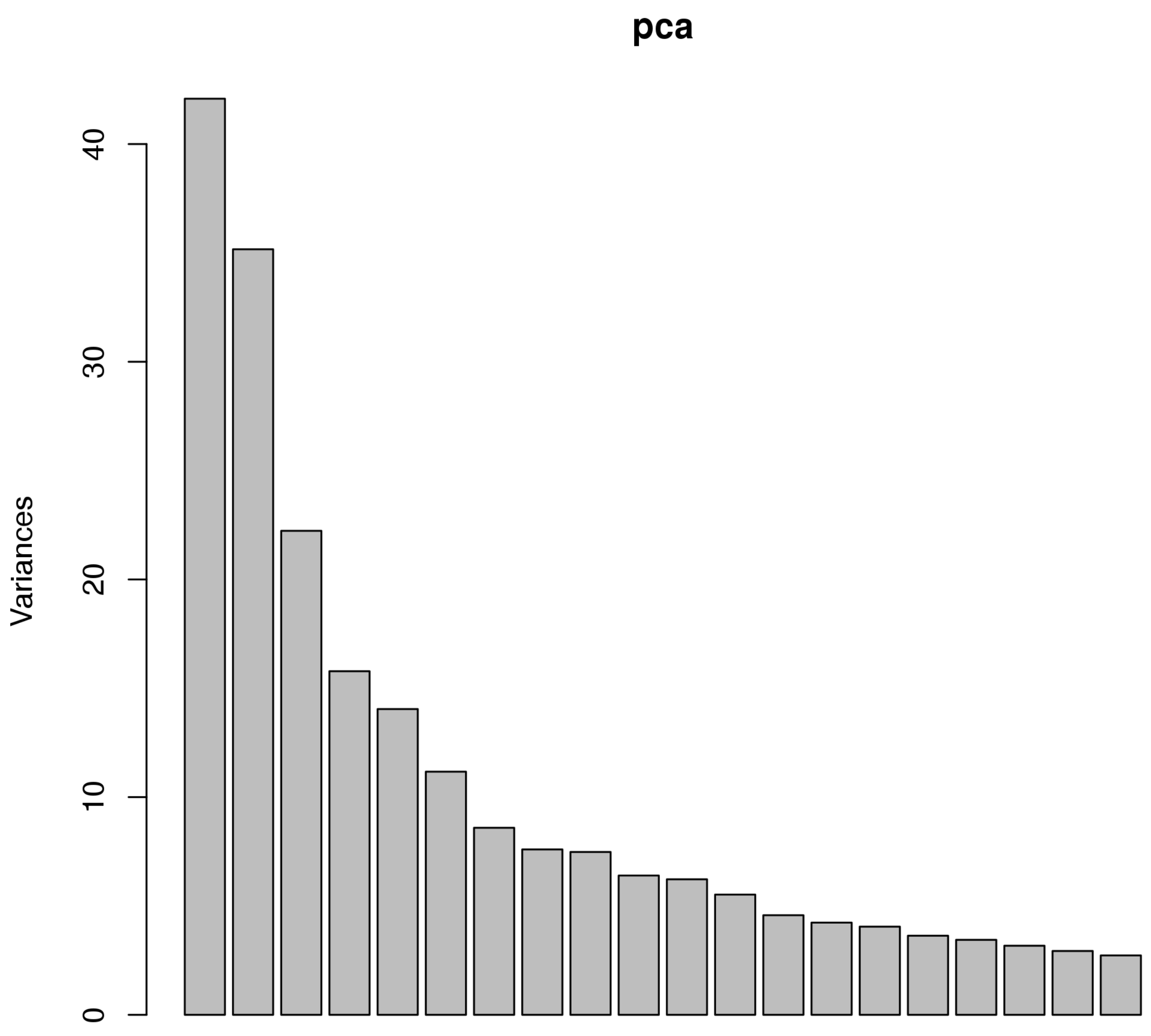
Computing principle components

R provides the function `prcomp` for this task. Like most standard R functions it expects data to be laid out with variables in columns and samples in rows. We therefore have to transpose the data, compute and extract the principle components (stored in the `x` element of the return value).

```
pca <- prcomp(t(expr[-1]), center=TRUE, scale = TRUE)
pc <- pca$x
```

Plotting the variances for the first 20 PCs is then straightforward.

```
plot(pca, npcs=20)
```



plot of chunk pcPlot

Since the data were scaled prior to the PCA the total variance is the same as the number of probes. The variance accounted for by each component is available through the `sdev` field of the `prcomp` return value.

```
sum(pca$sdev[1:20]^2)/nrow(expr)
```

```
## [1] 0.5525595
```

Fitting a model with PC covariates

To make our life a bit easier we collect all the relevant data into a single *data.frame*.

```
data <- data.frame(probe=unlist(subset(expr, Probe=="3710685")[-1]),
        rs4077515=unlist(subset(geno, id=="rs4077515")[-1]), pc[,1:10])
```

Now we fit the model including the PCs:

```
pcFit <- lm(probe ~ ., data=data)
summary(pcFit)
```

```
##
## Call:
## lm(formula = probe ~ ., data = data)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.60608 -0.11507 -0.00226  0.11512  0.50406
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept) 11.1795963  0.0144326 774.605  < 2e-16 ***
## rs4077515    0.2007593  0.0132594  15.141  < 2e-16 ***
## PC1         -0.0056932  0.0014343  -3.969 8.72e-05 ***
## PC2         -0.0033494  0.0015755  -2.126 0.034199 *
## PC3          0.0360871  0.0019708  18.311  < 2e-16 ***
## PC4         -0.0008474  0.0023431  -0.362 0.717816
## PC5          0.0240226  0.0024796   9.688  < 2e-16 ***
## PC6          0.0121790  0.0027809   4.380 1.57e-05 ***
## PC7          0.0030278  0.0031813   0.952 0.341884
## PC8         -0.0115654  0.0033857  -3.416 0.000709 ***
## PC9         -0.0096879  0.0034202  -2.833 0.004881 **
## PC10         0.0164822  0.0036735   4.487 9.78e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1777 on 355 degrees of freedom
## Multiple R-squared:  0.6834, Adjusted R-squared:  0.6736
## F-statistic: 69.67 on 11 and 355 DF,  p-value: < 2.2e-16
```

For comparison we also fit the simple model:

```
simpleFit <- lm(probe ~ rs4077515, data=data)
summary(simpleFit)
```

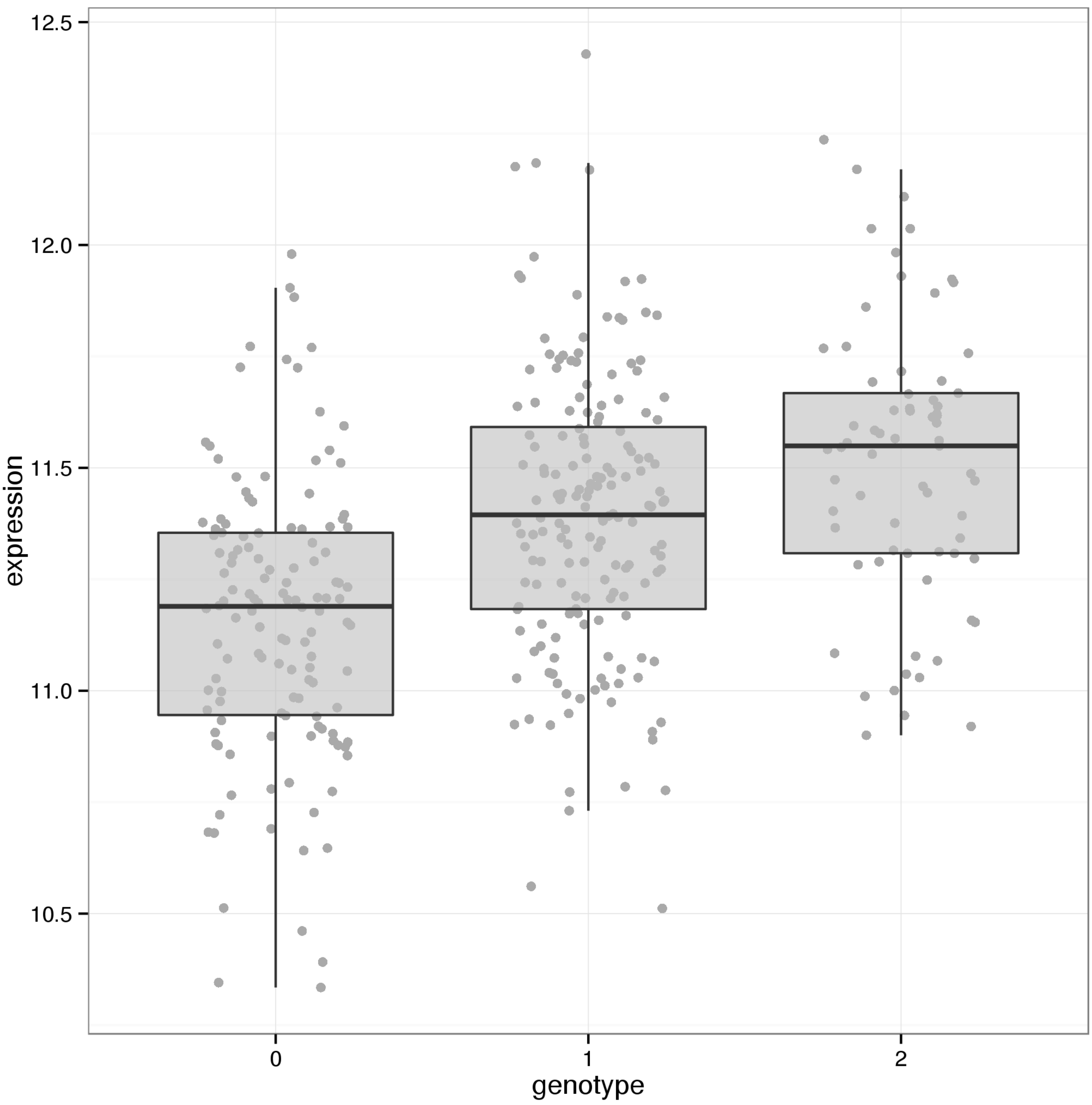
```
##
## Call:
## lm(formula = probe ~ rs4077515, data = data)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.80426 -0.17877  0.00095  0.19813  0.84187
```

```
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 11.17425    0.02190  510.32   <2e-16 ***
## rs4077515    0.20717    0.01991   10.41   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2736 on 365 degrees of freedom
## Multiple R-squared:  0.2288, Adjusted R-squared:  0.2267
## F-statistic: 108.3 on 1 and 365 DF,  p-value: < 2.2e-16
```

Visualising SNP effect on gene expression

As in the previous set of exercises we plot the gene expression with the effect of the non-genetic covariates removed.

```
library(ggplot2)
corrected <- data$probe - rowSums(coef(pcFit)[-(1:2)]*data[, 3:12])
corrected <- data.frame(expression=corrected, genotype=factor(data$rs4077515))
ggplot(corrected, aes(genotype, expression)) +
        geom_jitter(colour="darkgrey", position=position_jitter(width=0.25)) +
        geom_boxplot(outlier.size=0, alpha=0.6, fill="grey") + theme_bw()
```



plot of chunk rs4077515Plot