



5. Simulation and Permutation

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Seattle, July 2013

Overview

- Simulation
- Permutation tests
- A mean
- Smallest p-value across multiple models
- Cautionary notes, which we won't get to.

Testing

In testing a null hypothesis we need a test statistic that will have different values under the null hypothesis and the alternatives we care about (eg a relative risk of diabetes)

The **p-value** is the probability that the test statistic would be *at least as extreme* as the data we observed, if the null hypothesis is true.

— *need to compute the sampling distribution of the test statistic when the null hypothesis is true.*

The **power** of a test is the probability that the p -value meets our threshold for interestingness ($p = 0.05$ or $p = 10^{-\text{exciting}}$)

— *need the sampling distribution when an alternative hypothesis is true.*

Simulation

In some examples, can work out the sampling distribution mathematically.

More generally, let a computer work out the sampling distribution by creating thousands of possible datasets and doing the analysis.

- Testing: create a data set where the **null hypothesis** is true, work out the test statistic, repeat many times
- Power: create a data set where an **alternative hypothesis** is true, work out the p -value, repeat many times.

Tools

- Generating random numbers: `rnorm()`, `rbinom()`, `rpois()`, and others
- Taking random samples with equal or unequal probability: `sample()`
- `t.test()`, `chisq.test()`, `lm()`, `glm()` for doing computations
- `replicate()` for repeating computations multiple times

Permutations

A permutation test computes the sampling distribution for any test statistic, under the 'strong null hypothesis' that a set of genetic variants has absolutely no effect on the outcome.

To estimate the sampling distribution of the test statistic we need many samples generated under the strong null hypothesis.

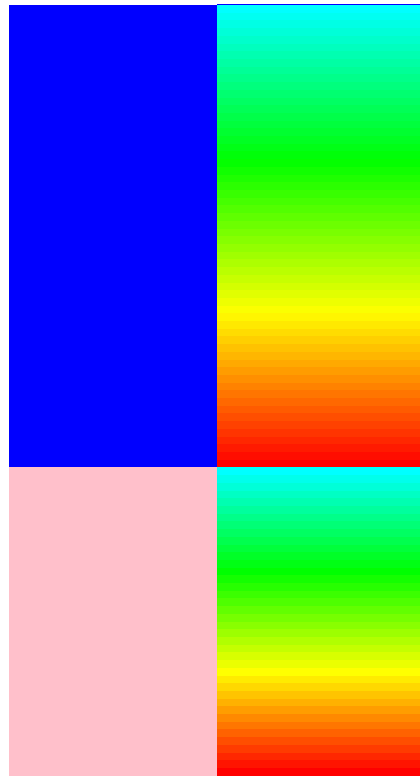
If the null hypothesis is true, changing the exposure would have no effect on the outcome. By randomly shuffling the exposures we can make up as many 'null' data sets as we like.

If the null hypothesis is true, the shuffled data sets should look like the real data. Otherwise, they should look different from the real data.

Comparing the shuffled test statistics to the real test statistic gives a p -value

Example: null is true

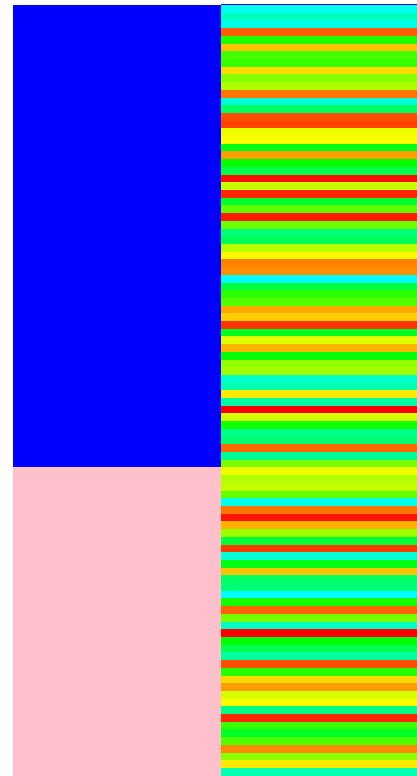
Data



gender

outcome

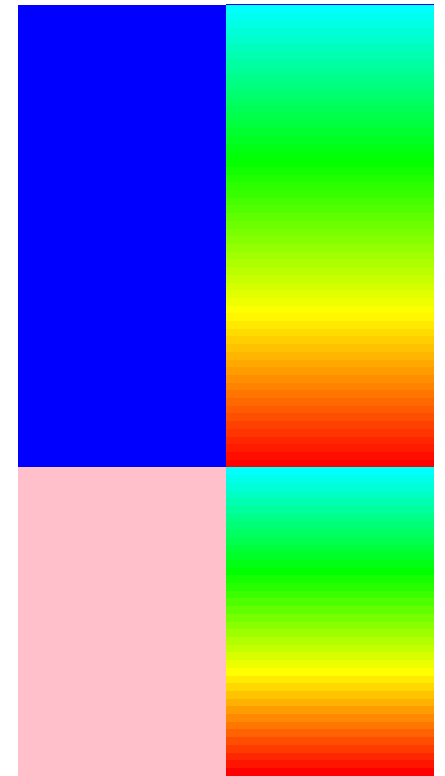
Shuffling outcomes



gender

outcome

Shuffling outcomes (ordered)

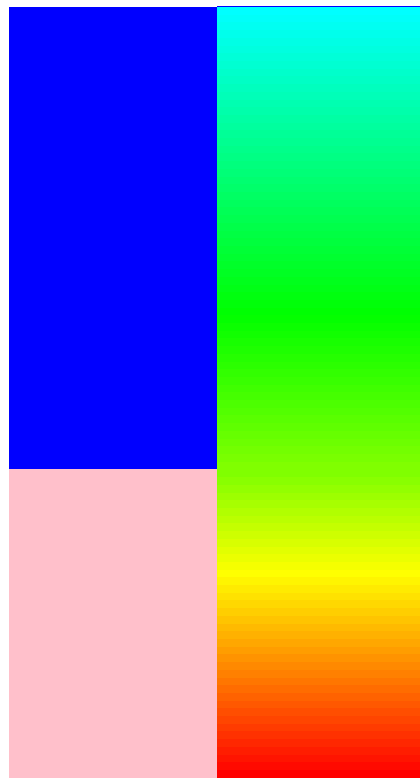


gender

outcome

Example: null is false

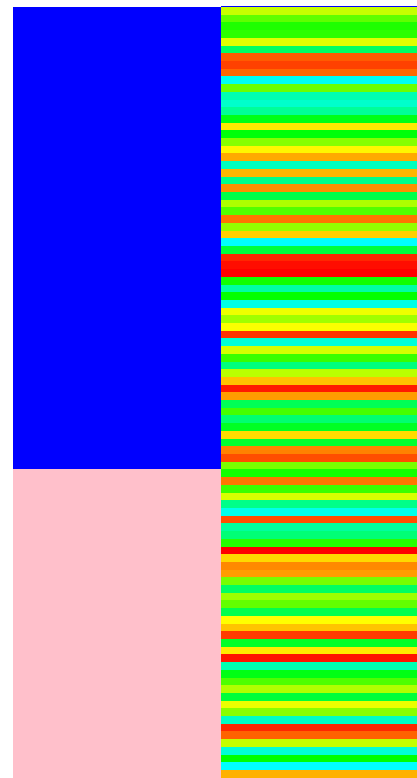
Data



gender

outcome

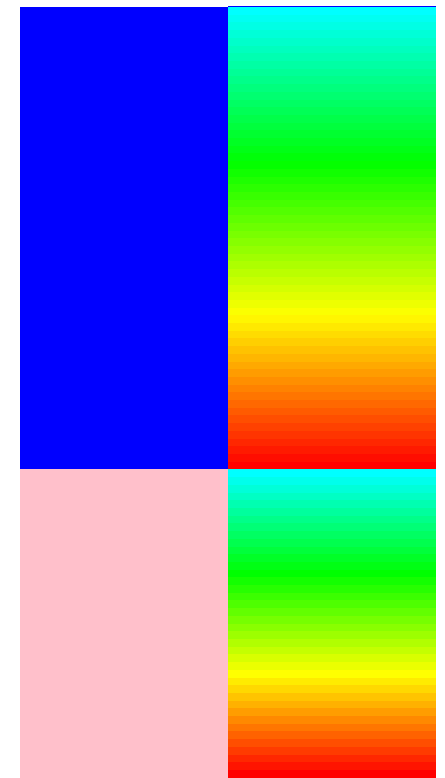
Shuffling outcomes



gender

outcome

Shuffling outcomes (ordered)



gender

outcome

Testing: Mean difference (v1)

Our first example is a difference in mean outcome in a dominant model for a single SNP

```
## make up some 'true' data  
carrier<-rep(c(0,1), c(100,200))  
alt.y<-rnorm(300, mean=carrier/2)
```

There is a real effect; carriers have higher y -values, on average.

(In this case we know from theory the distribution of a difference in means and could just do a t -test)

Means: permutation test

To perform the permutation test...

```
alt.diff<-mean(alt.y[carrier==1])-mean(alt.y[carrier==0])
```

```
one.diff <- function(x,y) {  
  xstar<-sample(x)  
  mean(y[xstar==1])-mean(y[xstar==0])  
}
```

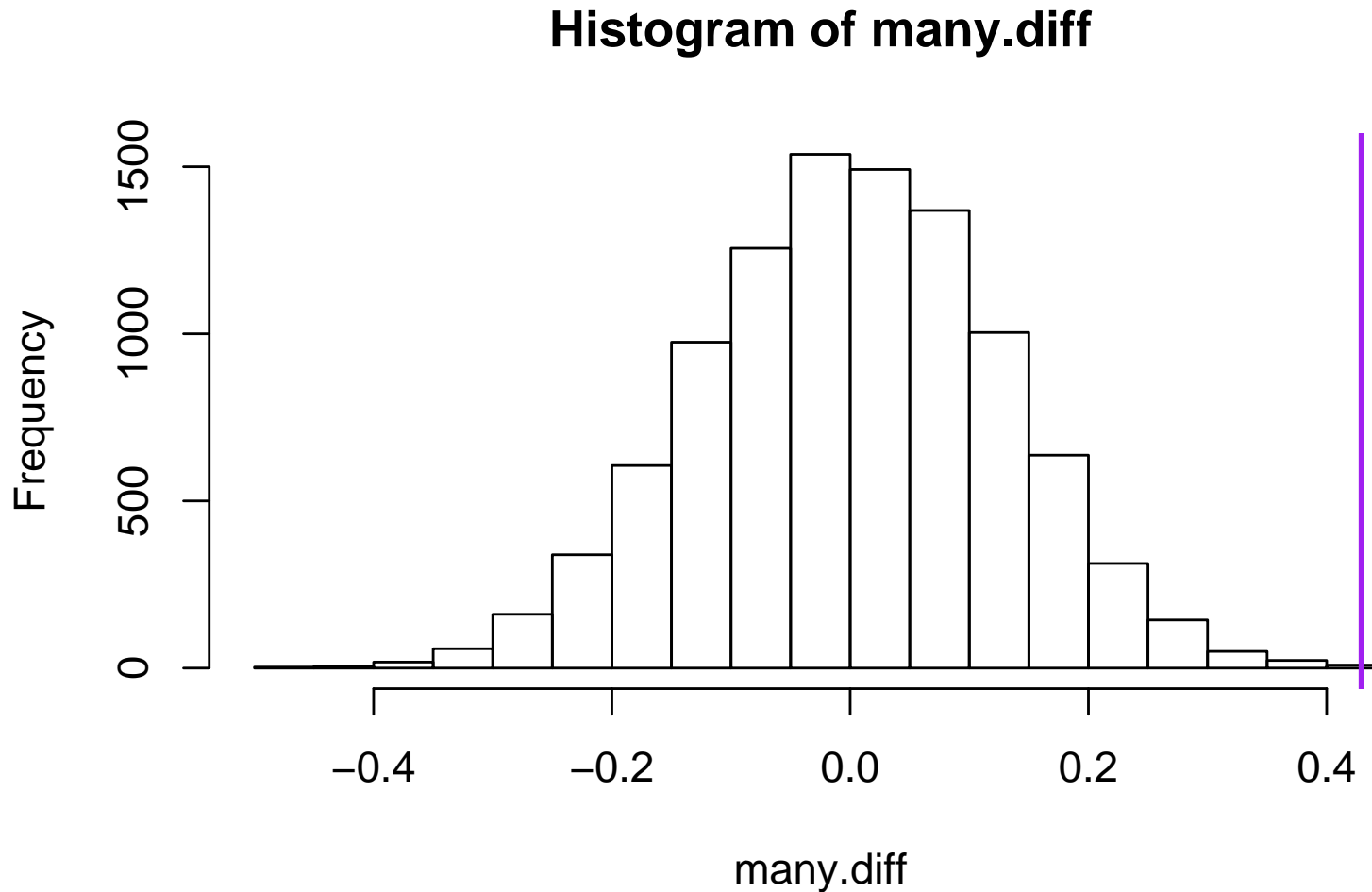
```
many.diff <- replicate(10000, one.diff(x=carrier, y=alt.y))
```

```
hist(many.diff)
```

```
abline(v=alt.diff, lwd=2, col="purple")
```

```
mean(abs(many.diff) > abs(alt.diff))
```

Example: null is false



9 out of 10,000 shuffled difference exceeds observed difference:
 $p \approx 0.0009$.

Testing: Mean difference (v2)

Now we generate some y where the null holds, i.e. mean is unrelated to being a carrier;

```
carrier<-rep(c(0,1), c(100,200))  
null.y<-rnorm(300, mean=0)
```

(what should happen?)

Testing: Mean difference (v2)

The rest of the commands work exactly as before...

```
null.diff<-mean(null.y[carrier==1])-mean(null.y[carrier==0])
```

```
one.diff <- function(x,y) {  
  xstar<-sample(x)  
  mean(y[xstar==1])-mean(y[xstar==0])  
} # same function as before
```

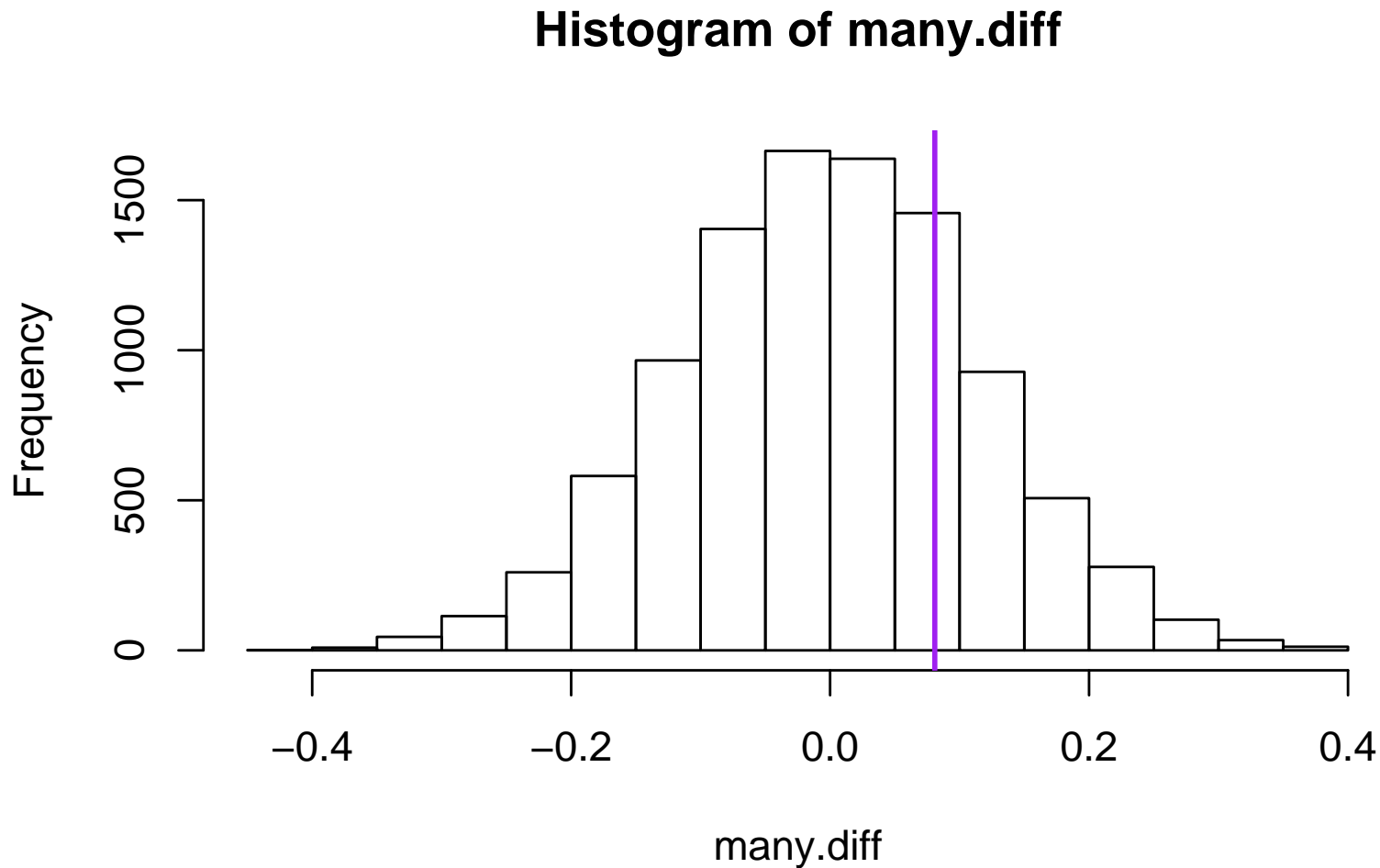
```
many.diff <- replicate(10000, one.diff(x=carrier, y=null.y))
```

```
hist(many.diff)
```

```
abline(v=null.diff, lwd=2, col="purple")
```

```
mean(abs(many.diff) > abs(null.diff))
```

Example: null is false



4815/10000 difference exceeds observed difference: $p \approx 0.48$.

Means: t -test

How do our permutations compare to classical t -tests?

```
> t.test(alt.y~carrier, var.equal=T)
      Two Sample t-test
data:  alt.y by carrier
t = -3.4696, df = 298, p-value = 0.0005983
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -0.6723963 -0.1856865
sample estimates:
mean in group 0 mean in group 1
 0.1416750      0.5707164
> t.test(null.y~carrier, var.equal=T)
      Two Sample t-test
data:  null.y by carrier
t = -0.6945, df = 298, p-value = 0.4879
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -0.3099976  0.1482715
sample estimates:
mean in group 0 mean in group 1
-0.09224281    -0.01137975
```

How many permutations?

With 10000 permutations the smallest possible p -value is 0.0001, and the uncertainty near $p = 0.05$ is about $\pm 0.4\%$

If we have multiple testing we may need **much** more precision.

Using 100,000 permutations reduces the uncertainty near $p = 0.05$ to $\pm 0.1\%$ and allows accurate p -values as small as 0.00001.

A useful strategy is to start with 1000 permutations and continue to larger numbers only if p is small enough to be interesting, eg $p < 0.1$.

Parallel computing of permutations is easy: just run R on multiple computers.

Debugging

R error messages are sometimes opaque, because the error occurs in a low-level function.

`traceback()` reports the entire **call stack**, which is useful for seeing where the error really happened.

Suppose our outcome variable were actually a data frame with one column rather than a vector:

```
> one.diff(x=carrier, y=ywrong)
Error in '[.data.frame'(y, xstar == 1) : undefined columns selected
```

We didn't know we were calling `'[.data.frame'`, so we don't understand the message

Debugging

```
> traceback()
5: stop("undefined columns selected")
4: `[.data.frame`(y, xstar == 1)
3: y[xstar == 1]
2: mean(y[xstar == 1])
1: one.diff(x=carrier, y=ywrong)
```

so the problem happens in our line of code `mean(y[xstar==1])` and is a problem with computing `y[xstar==1]`.

We might want to have a look at `y` and `xstar`

Debugging

The post-mortem debugger lets you look inside the code where the error occurred.

```
> options(error=recover)
> one.diff(x=carrier, y=ywrong)
Error in '[.data.frame'(y, xstar == 1) : undefined columns selected
```

Enter a frame number, or 0 to exit

```
1: one.diff(x=carrier, y=ywrong)
2: mean(y[xstar == 1])
3: y[xstar == 1]
4: '[.data.frame'(y, xstar == 1)
```

Selection: 1

Called from: eval(expr, envir, enclos)

Debugging

```
Browse[1]> str(xstar)
```

```
num [1:300] 1 1 1 1 1 1 1 0 1 1 ...
```

```
Browse[1]> str(y)
```

```
'data.frame': 300 obs. of 1 variable:
```

```
$ null.y: num 1.265 0.590 -0.722 0.676 -0.431 ...
```

Turn the post-mortem debugger off with

```
options(error=NULL)
```

Minimum p -value

Little point in permutation test for the mean: same result as t -test

Permutation test is useful when we do not know how to compute the distribution of a test statistic.

Suppose we test additive effects of 8 SNPs, one at a time, and we want to know if the most significant association is real.

For any one SNP the z -statistic from a logistic regression model has a Normal distribution.

We need to know the distribution of the most extreme of eight z -statistics. This is not a standard distribution, but a permutation test is still straightforward.

Minimum p -value

```
dat <- data.frame(y=rep(0:1,each=100),  
SNP1=rbinom(200,size=2,prob=.1), SNP2=rbinom(200,size=2,prob=.2),  
SNP3=rbinom(200,size=2,prob=.2), SNP4=rbinom(200,size=2,prob=.4),  
SNP5=rbinom(200,size=2,prob=.1), SNP6=rbinom(200,size=2,prob=.2),  
SNP7=rbinom(200,size=2, prob=.2), SNP8=rbinom(200,size=2,prob=.4))
```

```
> head(dat)
```

	y	SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8
1	0	0	0	0	0	0	1	0	0
2	0	0	1	0	1	0	1	0	2
3	0	0	1	0	1	1	0	0	0
4	0	0	0	1	1	0	0	0	0
5	0	0	1	0	1	1	0	0	0
6	0	0	0	0	1	0	1	0	1

Minimum p -value

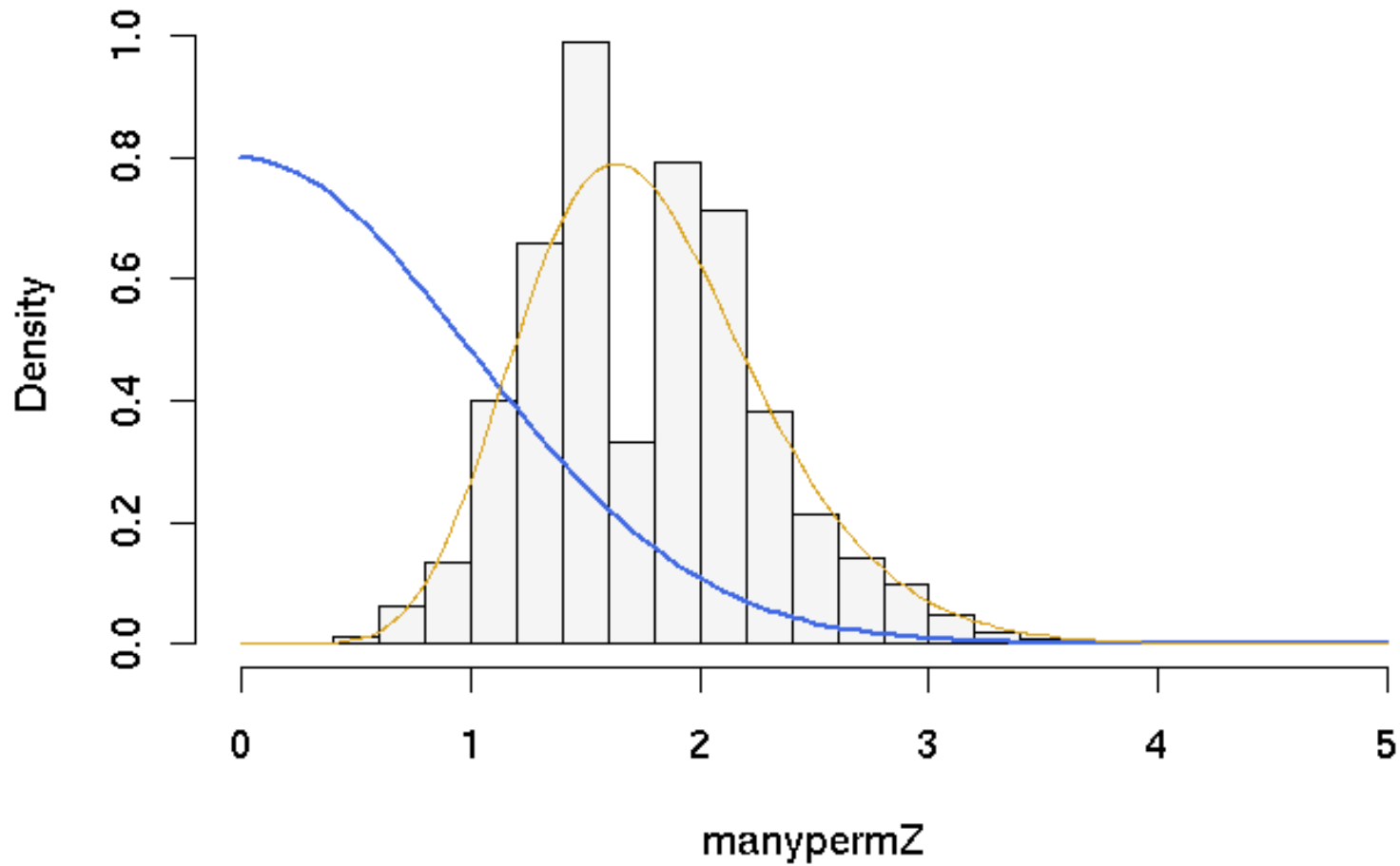
```
oneZ<-function(outcome, snp){
  model <- glm(outcome~snp, family=binomial)
  coef(summary(model))["snp","z value"]
}
maxZ<-function(outcome, snps){
  allZs <- sapply(snps,
    function(this.snp){ oneZ(outcome, snp=this.snp) })
  max(abs(allZs))
}

true.maxZ<-maxZ(outcome=dat$y, snps=dat[, -1])

manypermZ<-replicate(10000,
  maxZ(outcome=sample(dat$y), snps=dat[, -1]))
```

Minimum p -value

Histogram of manypermZ



Minimum p -value

The histogram shows the permutation distribution for the maximum Z -statistic.

The blue curve is the theoretical distribution for one Z -statistic

The yellow curve is the theoretical distribution for the maximum of eight independent Z -statistics.

Clearly the multiple testing is important: a Z of 2.5 gives $p = 0.012$ for a single test but $p = 0.075$ for the permutation test.

The theoretical distribution for the maximum has the right range but the permutation distribution is quite discrete. The discreteness is more serious with small sample size and rare SNPs.

[The theoretical distribution is not easy to compute except when the tests are independent.]

More debugging

Permutation tests on other people's code might reveal a lack of robustness.

For example, a permutation might result in all controls being homozygous for one of the SNPs and this might give an error message

We can work around this with `tryCatch()`

```
oneZ<-function(outcome, snp){
  tryCatch({model <- glm(outcome~snp, family=binomial())
            coef(summary(model))["snp","z value"]},
    error=function(e) NA
  )
}
```

Now `oneZ()` will return `NA` if there is an error in the model fitting.

Caution: wrong null

Permutation tests cannot solve all problems: they are valid only when the null hypothesis is 'no association'

Suppose we are studying a set of SNPs that each have some effect on outcome and we want to test for interactions (epistasis).

Permuting the genotype data would break the links between genotype and outcome and created shuffled data with no main effects of SNPs.

Even if there are no interactions the shuffled data will look different from the real data.

Caution: weak null hypothesis

A polymorphism could increase the variability of an outcome but not change the mean.

In this case the strong null hypothesis is false, but the hypothesis of equal means is still true.

- If we want to detect this difference the permutation test is unsuitable because it has low power
- If we do not want to detect this difference the permutation test is invalid, because it does not have the correct Type I error rate.

When groups are the same size the Type I error rate is typically close to the nominal level, otherwise it can be too high or too low.

To illustrate this we need many replications of a permutation test. We will do 1000 permutation tests for a mean, each with 1000 permutations.

```
meandiff<-function(x,trt){
mean(x[trt==1])-mean(x[trt==2])
}
meanpermtest<-function(x,trt,n=1000){
observed<-meandiff(x,trt)
perms<-replicate(n, meandiff(x, sample(trt)))
mean(abs(observed)>abs(perms))
}

trt1<-rep(c(1,2),c(10,90))

perm.p<-replicate(1000, {
  x1<-rnorm(100, 0, s=trt1)
  meanpermtest(x1,trt1)})

table(cut(perm.p,c(0,.05,.1,.5,.9,.95,1)))/1000
```

(0,0.05]	(0.05,0.1]	(0.1,0.5]	(0.5,0.9]	(0.9,0.95]	(0.95,1]
86	99	564	244	6	0

The p -values are too small, relative to a uniform distribution. If we reverse the standard errors we get

(0,0.05]	(0.05,0.1]	(0.1,0.5]	(0.5,0.9]	(0.9,0.95]	(0.95,1]
27	28	275	354	67	249

If the two groups each have 50 observations we get

(0,0.05]	(0.05,0.1]	(0.1,0.5]	(0.5,0.9]	(0.9,0.95]	(0.95,1]
50	45	403	407	52	43

which is much better.