R for bioinformatics, data summarisation and statistics

HUST Bioinformatics course series

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section 1: TOC

前情提要

- basic plot functions
- basic ggplot2
- special letters
- equations
- advanced ggplot2

本次提要

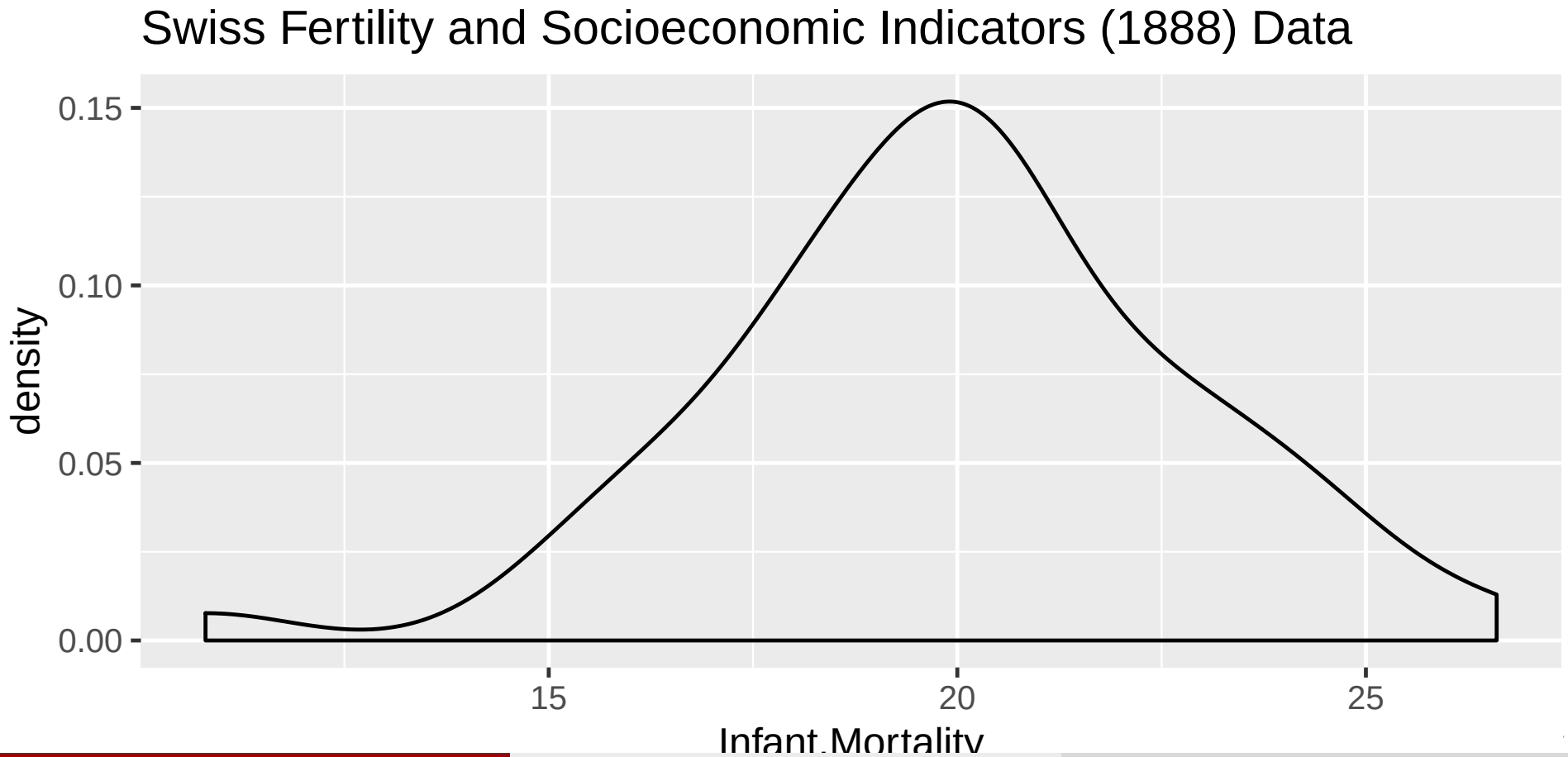
- data summarisation functions (vector data)
  - median, mean, sd, quantile, summary
- 图形化的 data summarisation (two-D data/ tibble/ table)
  - dot plot
  - smooth
  - linear regression
  - correlation & variance explained
  - groupping & bar/ box/ plots
- statistics
  - parametric tests
    - t-test
    - one way ANNOVA
    - two way ANNOVA
    - linear regression
    - model / prediction / coefficients
  - non-parametric comparison

section 2: vector summarisation

vector data

1. distribution

```
library(tidyverse);
ggplot( swiss, aes( x = Infant.Mortality ) ) + geom_density() +
   ggtitle("Swiss Fertility and Socioeconomic Indicators (1888) Data")
```



describe normal distributions

可以用 mean 和 sd 来描述

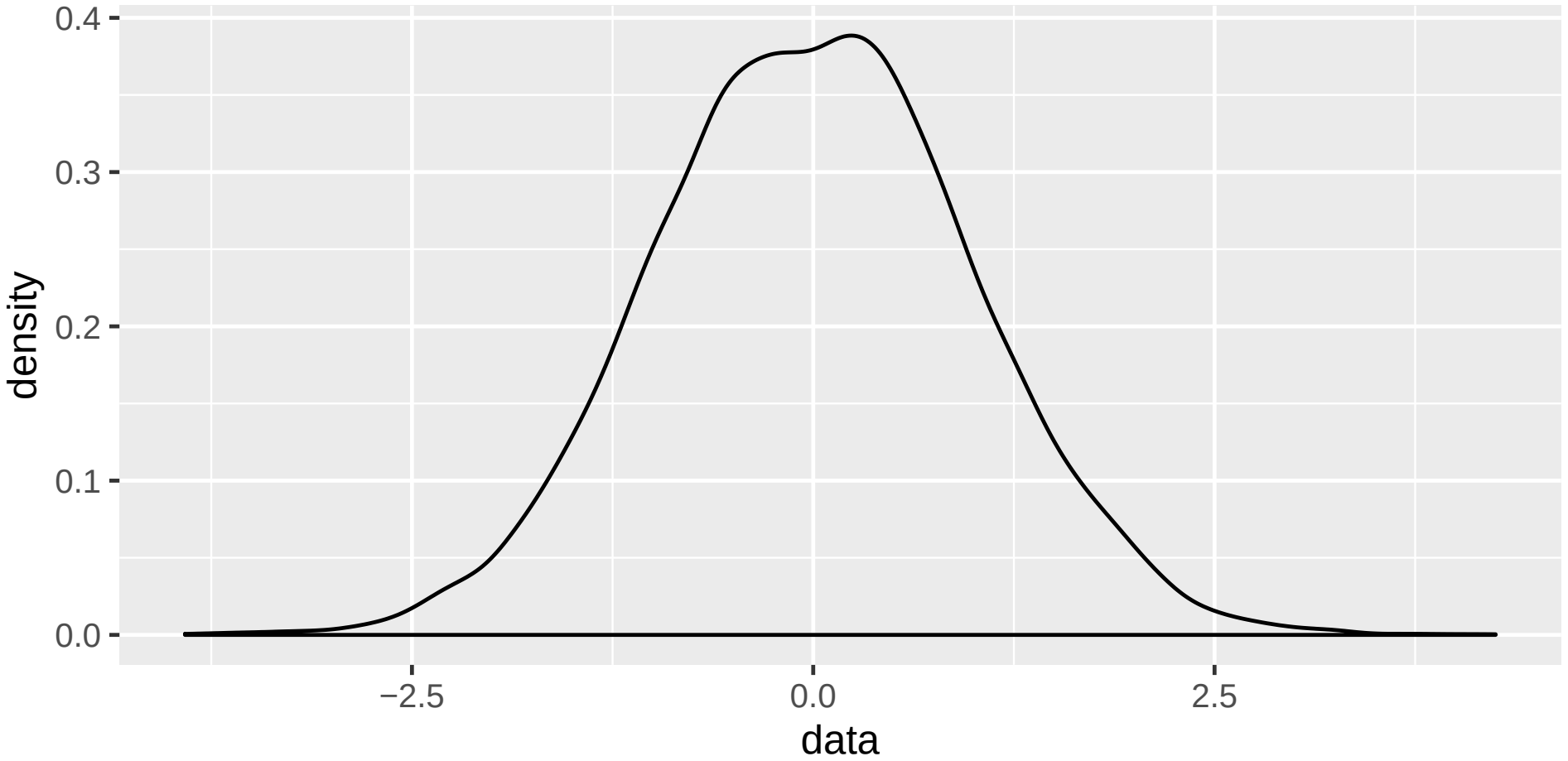
A. It's symmetrical.
B. Mean and median are the same.
C. Most common values are near the mean; less common values are farther from it.
D. Standard deviation marks the distance from the mean to the inflection point.

(mean + 1 * sd) >= 68%

(mean + 2 * sd) >= 95% 的数据

functions to generate random normal distrubions

```r
# 生成 10000 个随机数字, 使其 mean = 0, sd = 1, 且为 normal distribution ...
x <- rnorm(10000, mean = 0, sd = 1);
ggplot( data.frame( data = x ), aes( data ) ) + geom_density(  );
```



More to read: http://uc-r.github.io/generating_random_numbers/

other functions to generate random normal distributions

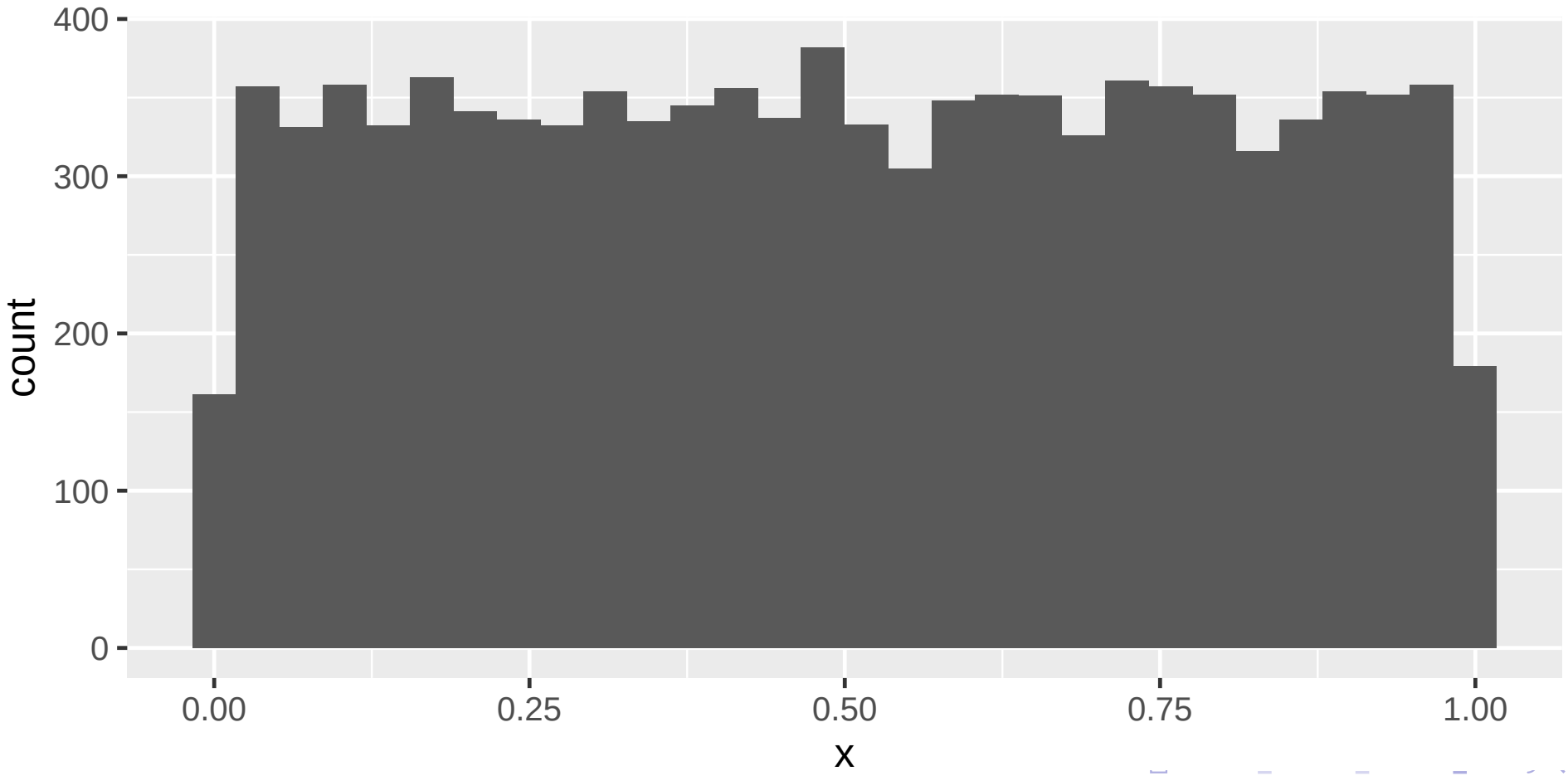
注意，以下函数中的 q, p, x 需要自行提供

```
# generate CDF probabilities for value(s) in vector q
pnorm(q, mean = 0, sd = 1)

# generate quantile for probabilities in vector p
qnorm(p, mean = 0, sd = 1)

# generate density function probabilites for value(s) in vector x
dnorm(x, mean = 0, sd = 1)
```

其它规律的 distributions

1. uniform distributions

```
x <- runif( 10000 ); ## random numbers of uniform distributions between 0 and 1
ggplot( data.frame( dat = x ), aes( x ) ) + geom_histogram();
```



uniform distribution 的各种函数

注：以下函数中的 n 需要自行决定

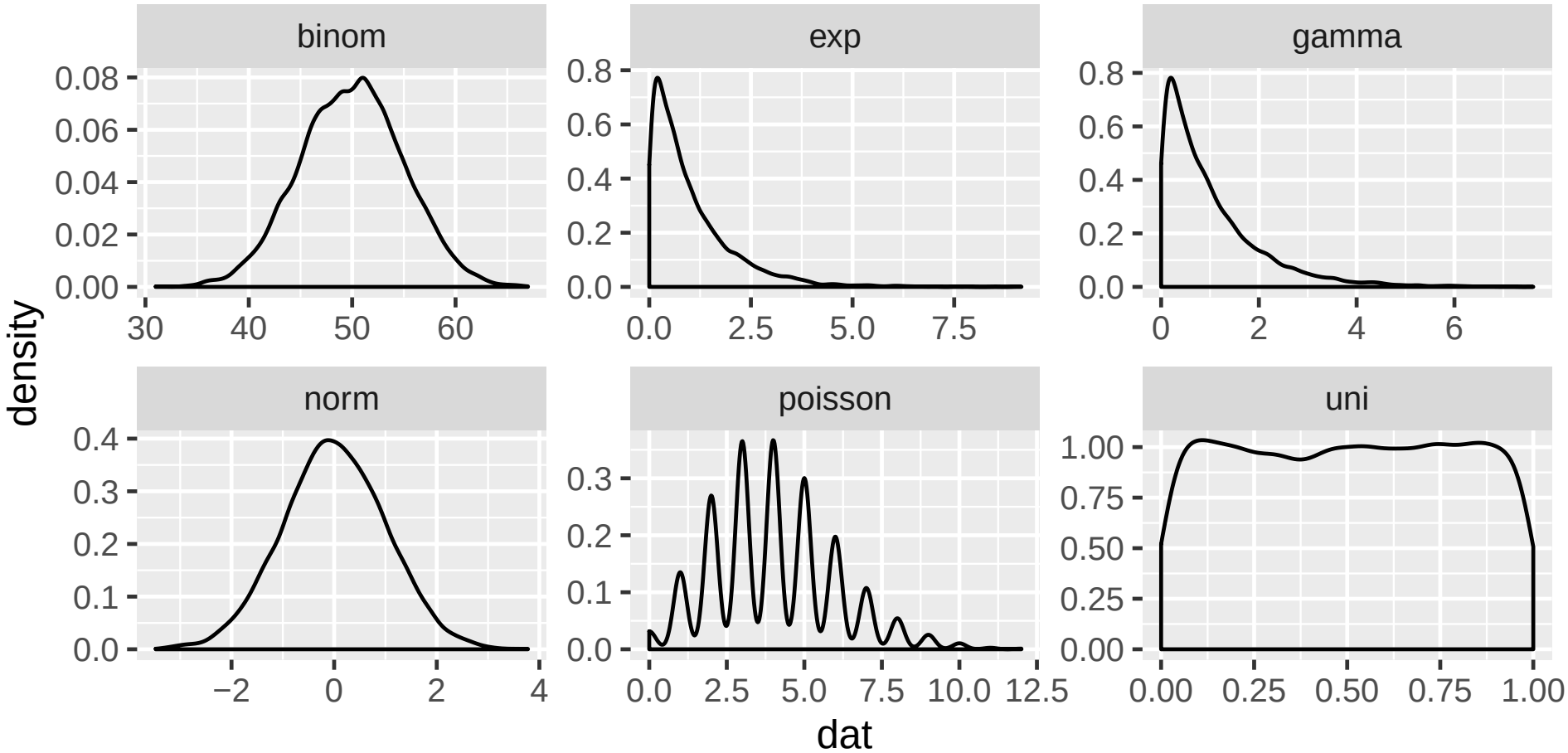
```
# generate n random numbers between 0 and 25
runif(n, min = 0, max = 25)

# generate n random numbers between 0 and 25 (with replacement)
sample(0:25, n, replace = TRUE)

# generate n random numbers between 0 and 25 (without replacement)
sample(0:25, n, replace = FALSE)
```

other distributions, cont.

```
n <- 10000;
uni <- tibble( dat = runif(n), type = "uni" );
norm <- tibble( dat = rnorm(n), type = "norm" );
binom <- tibble( dat = rbinom(n, size = 100, prob = 0.5), type = "binom" );
poisson <- tibble( dat = rpois(n, lambda = 4), type = "poisson" );
exp <- tibble( dat = rexp(n, rate = 1) , type = "exp");
gamma <- tibble( dat = rgamma(n, shape = 1) , type = "gamma");

combined <- bind_rows( uni, norm, binom, poisson, exp, gamma );

plot1 <-
   ggplot( combined , aes( dat ) ) + geom_density() +
      facet_wrap( ~type, ncol = 3, scales = "free");
```

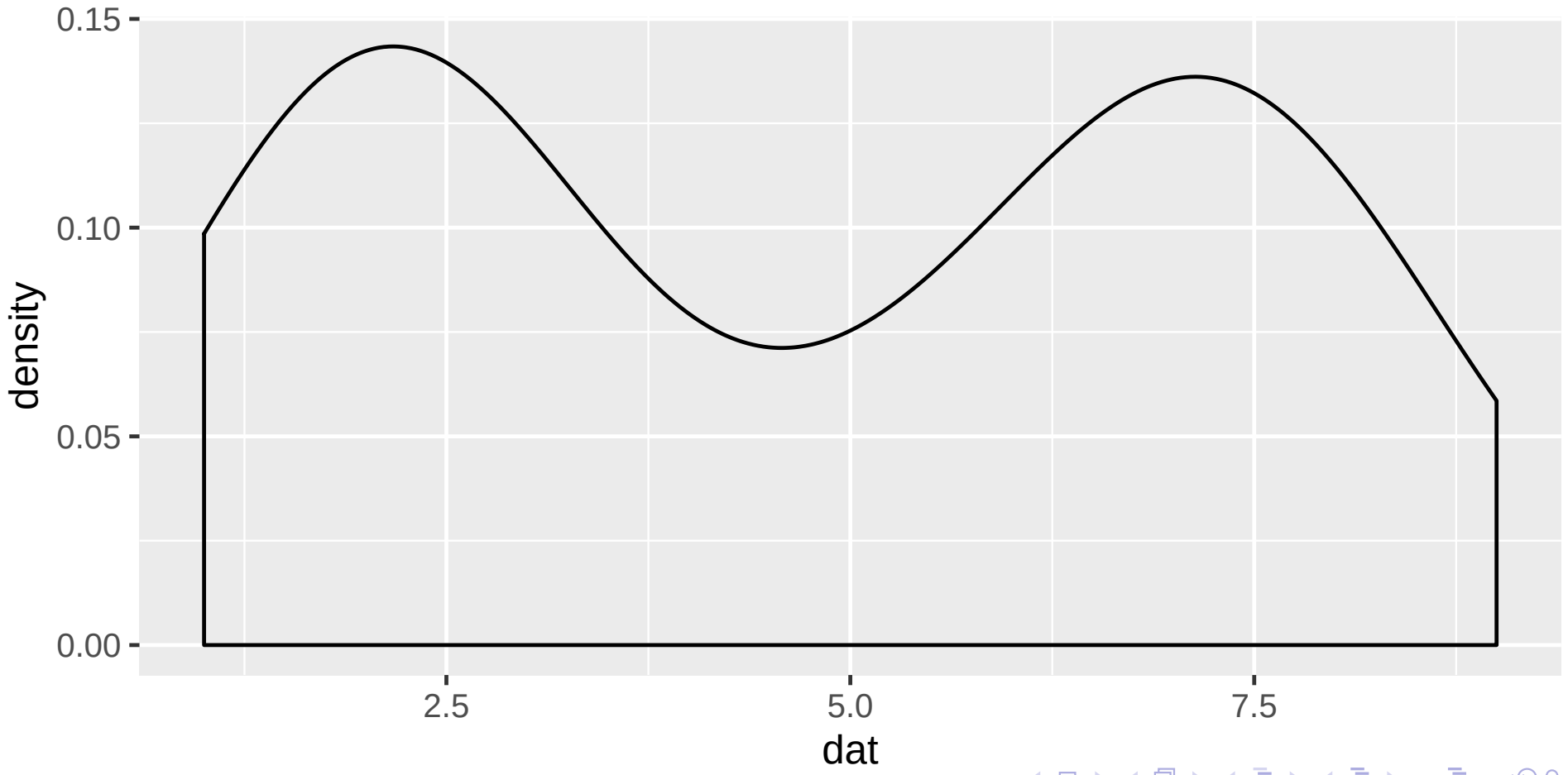
other distributions, plot

```
plot1;
```



non-parametric distribution

```
## votes on people's desire to visit
bi <- c(7, 3, 2, 1, 7, 3, 4, 5, 7, 6, 2, 2, 1, 3, 7, 2, 6, 8, 2, 7, 2, 2, 1,
3, 5, 8, 2, 6, 7, 8, 6, 2, 8, 7, 9, 2, 7, 5, 1, 8, 8, 2, 3, 7, 3, 8);
ggplot( data.frame( dat = bi ), aes(dat)) + geom_density();
```



量化描述数据

使用以下同名函数

mean: aka average, is the sum of all of the numbers in the data set divided by the size of the data set.

median: The median is the value that is in the middle when the numbers in a data set are sorted in increasing order.

sd: standard deviation

var: measures how far a set of numbers are spread out

range: 取值范围

note: from: `https://www.ai-therapy.com/psychology-statistics/descriptive/mean-mode-median`

量化描述函数

```
mean( norm$dat );
median( norm$dat );
## mode( norm$dat ); ## ???

sd(norm$dat);
var(norm$dat);
range(norm$dat);
```

quantile and summary

```
quantile( norm$dat );
```

```
##           0%          25%          50%          75%         100%
## -3.477057e+00 -6.630663e-01  7.636815e-05  6.881583e-01  3.777296e+00
```

```
## quantile 还接受其它参数
quantile( norm$dat, probs = seq(0, 1, length = 11));
```

```
##           0%          10%          20%          30%          40%
## -3.477057e+00 -1.290048e+00 -8.363559e-01 -5.200171e-01 -2.500945e-01
##          50%          60%          70%          80%          90%
##  7.636815e-05  2.546565e-01  5.338732e-01  8.579359e-01  1.312853e+00
##         100%
##  3.777296e+00
```

```
## summary ...
summary( norm$dat );
```

```
##      Min.   1st Qu.    Median      Mean   3rd Qu.      Max.
## -3.477057 -0.663066  0.000076  0.005601  0.688158  3.777296
```

```
## summary 也可应用于非数值
summary( combined$type );
```

```
##    Length     Class      Mode
##     60000 character character
```

summary, cont.

```
## summary 可应用于整个表格; 相当于对每列进行 summary ...
summary( combined );
```

```
##       dat                type
##  Min.   :-3.4771   Length:60000
##  1st Qu.: 0.3157   Class :character
##  Median : 0.9422   Mode  :character
##  Mean   : 9.4104
##  3rd Qu.: 4.0000
##  Max.   :67.0000
```

table 函数

返回 vector 当中 unique 值和它们的出现次数

```
table( combined$type );
```

```
##
##   binom     exp   gamma    norm poisson     uni
##   10000   10000   10000   10000   10000   10000
```

** 注 ** : table 还接受 data.frame 作为输入，比如 table(combined)。请自行尝试并理解结果

section 3: two column data: part 1

数据介绍: a numeric vector and a facterial vector

此类数据，通常一列是数值，另一列是分组信息，如下例:

```
data.fig3a <- read_csv( file = "data/talk10/nc2015_data_for_fig3a.csv" );

head( data.fig3a[ c("tai", "trans.at") ] ); ## 只显示有用的两列
```

```
## # A tibble: 6 x 2
##     tai trans.at
##   <dbl>    <dbl>
## 1     1    0.195
## 2     1   -0.320
## 3     1   -0.327
## 4     1   -0.304
## 5     1   -0.275
## 6     1   -0.167
```

数据介绍，cont.

tai: 表达量的一种计算方式，1 == lowest, 5 == highest

trans.at：A - T 碱基使用偏好；

假说：

(a) de novo synthesis cost of A is higher than T,
(b) therefore highly expressed genes will tend to use T than A when possible.
(c) 因此，在高表达的基因当中，A - T 的差值会变大（更负）。

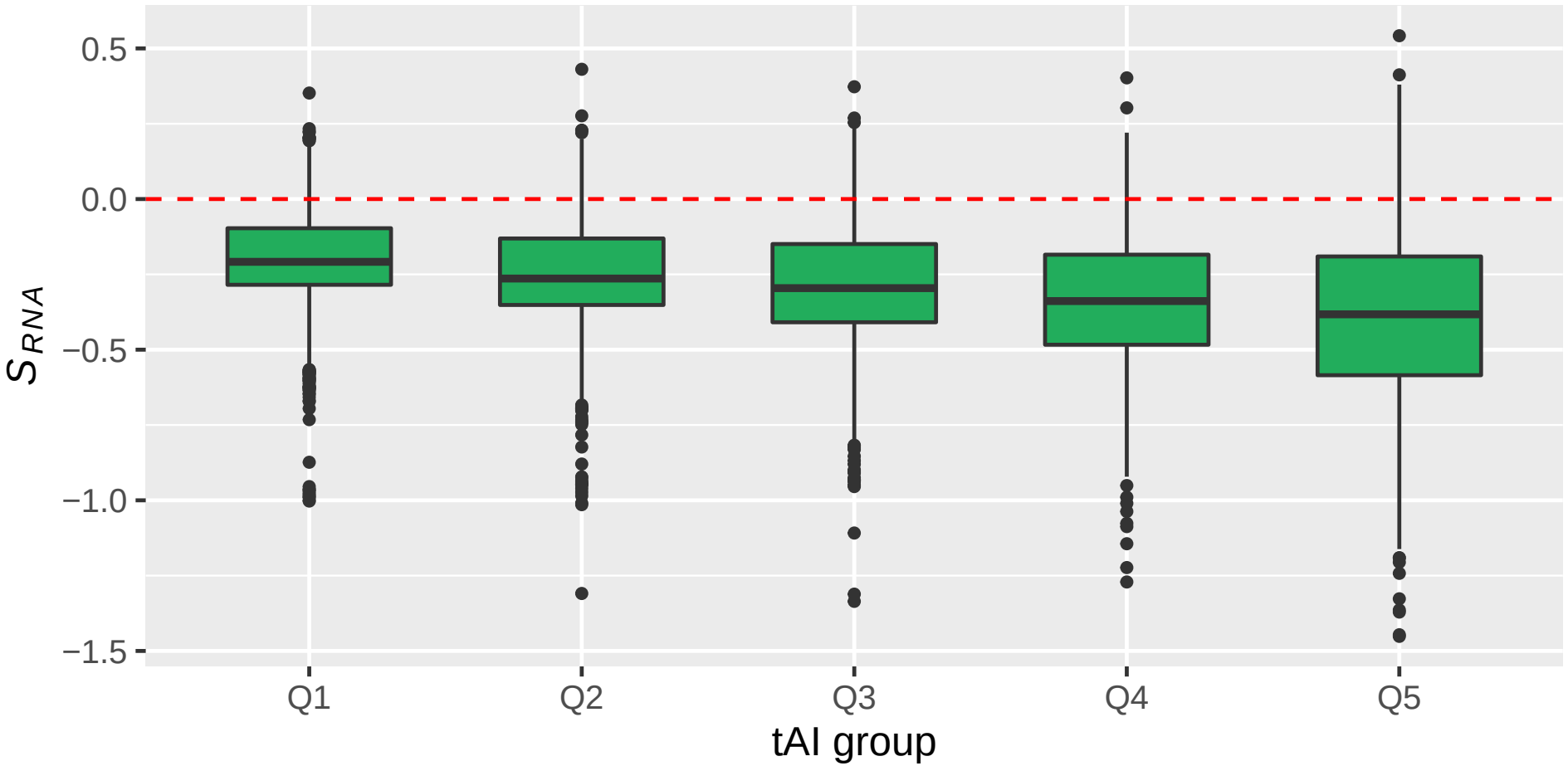
data source: Chen et al, Nature Communications, 2016

boxplot

```
fig3a <-
   ggplot( data.fig3a, aes( factor(tai), trans.at ) ) +
      geom_boxplot( fill = "#22AD5C", linetype = 1 ,outlier.size = 1, width = 0.6) +
      xlab( "tAI group" ) +
      ylab( expression( paste( italic(S[RNA]) ) ) ) +
      scale_x_discrete(breaks= 1:5 , labels= paste("Q", 1:5, sep = "") ) +
      geom_hline( yintercept = 0, colour = "red", linetype = 2);
```

show the plot

```
fig3a;
```



说明:

1. 此种情况下，我们通常只看 median 值的趋势；
2. 也可增加 Q1 与 Q5 之间的差异分析 (wilcoxon Rank sum test)；p-value = 3.243538e-87

another example plot

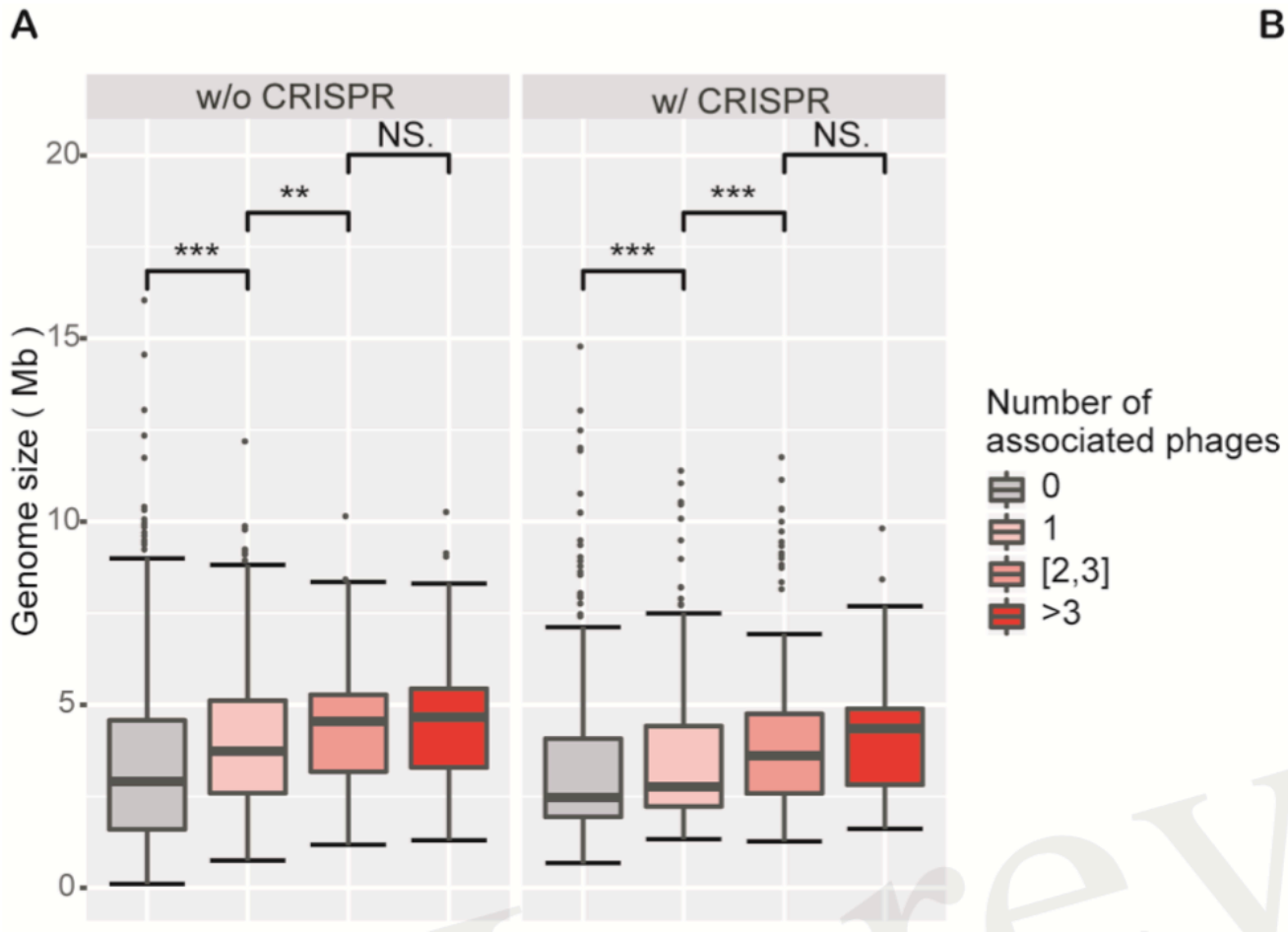
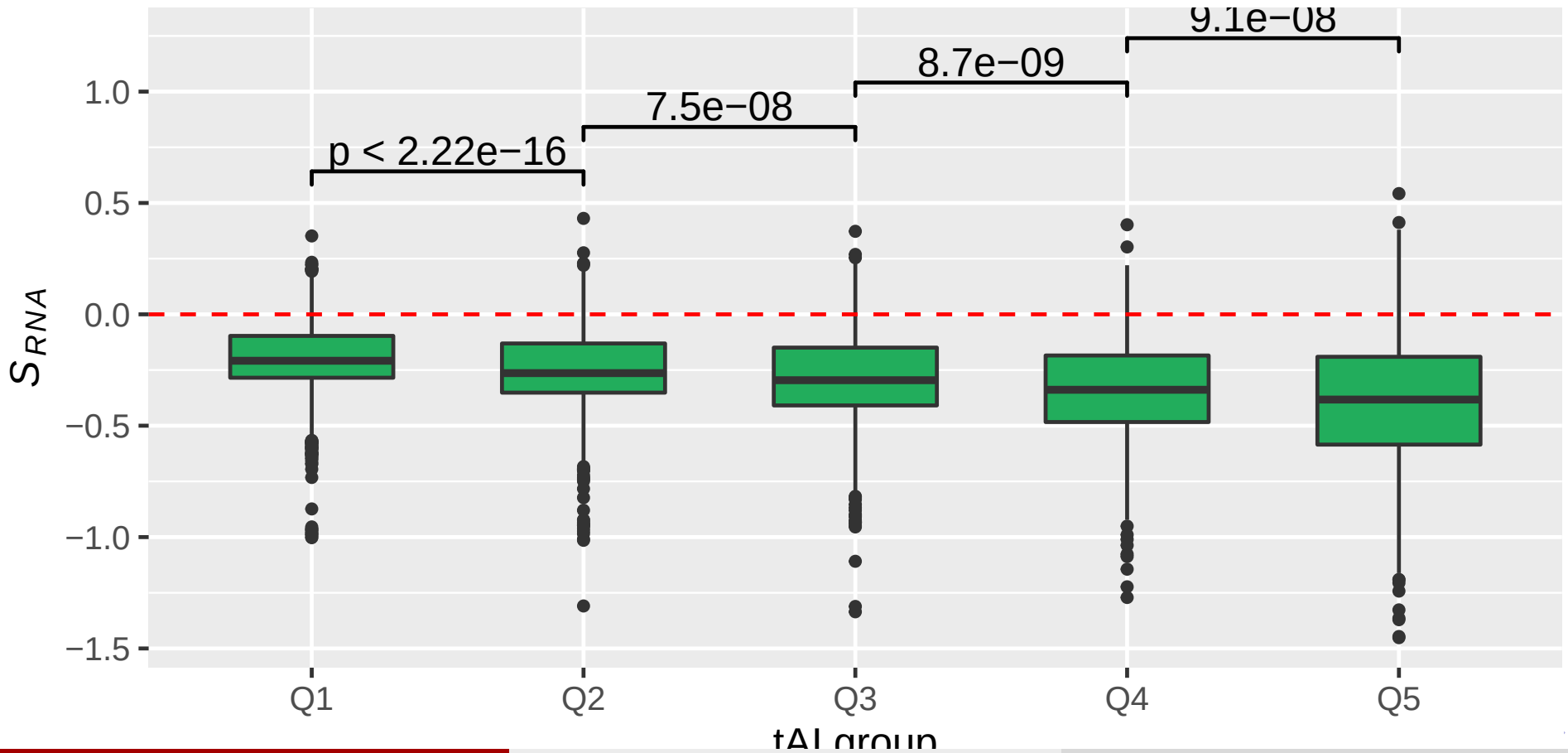


Figure 1: a figure from an in-press manuscript

how to add significance indicators to plot??

ggplot2 的扩展包，geom_signif；如果第一次使用，请先安装。

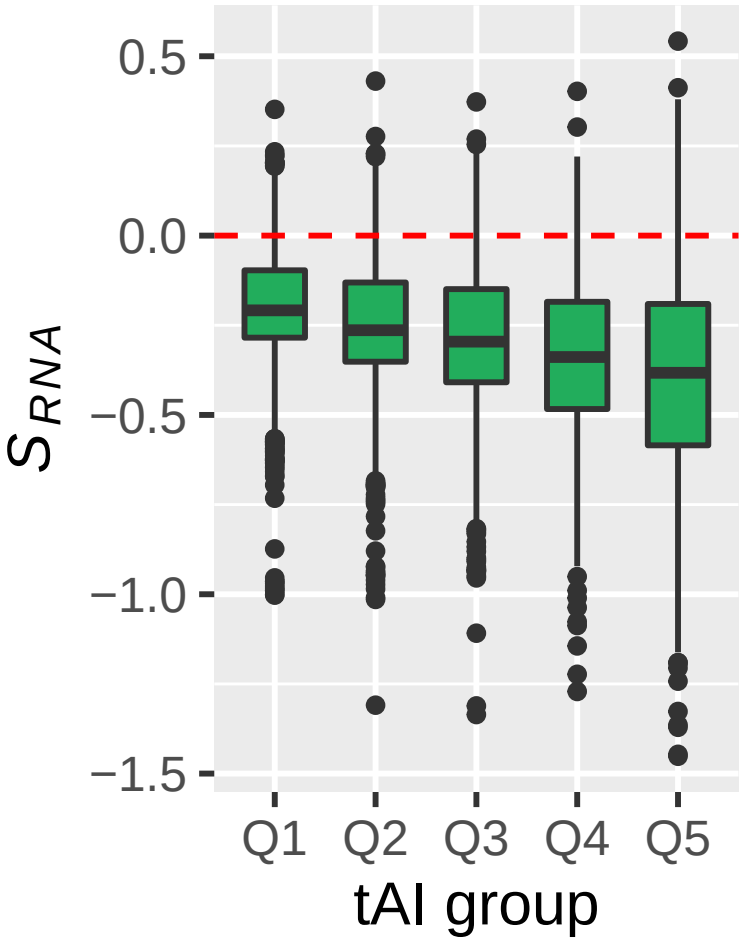
```
library(ggsignif);
fig3a + geom_signif( comparisons = list(1:2, 2:3, 3:4, 4:5), test = wilcox.test,
                     step_increase = 0.1 );
```



boxplot 画图注意事项

正确的画法为：要高瘦，不要矮胖!!!!

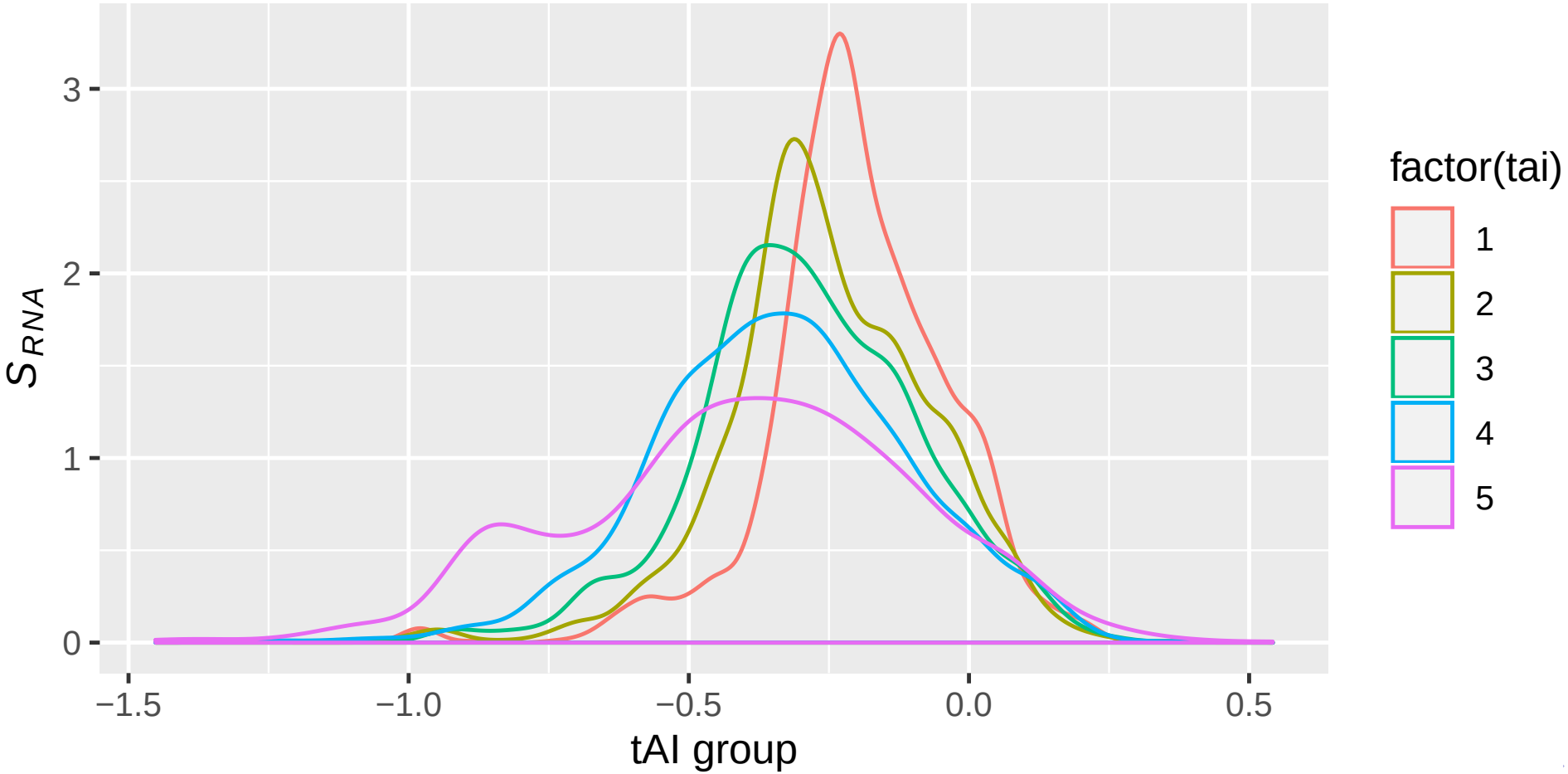
```
fig3a;
```



此类数据的另一种可视化方式

density plot; 但在此例中，不如 boxplot 好。

```
ggplot( data.fig3a, aes( trans.at, colour = factor(tai) ) ) + geom_density( ) +
   xlab( "tAI group" ) + ylab( expression( paste( italic(S[RNA]) ) ) );
```



section 4: two column data: part 2

数据介绍：two numerical vectors

多用于描述两组（量化）数据之间的关系；

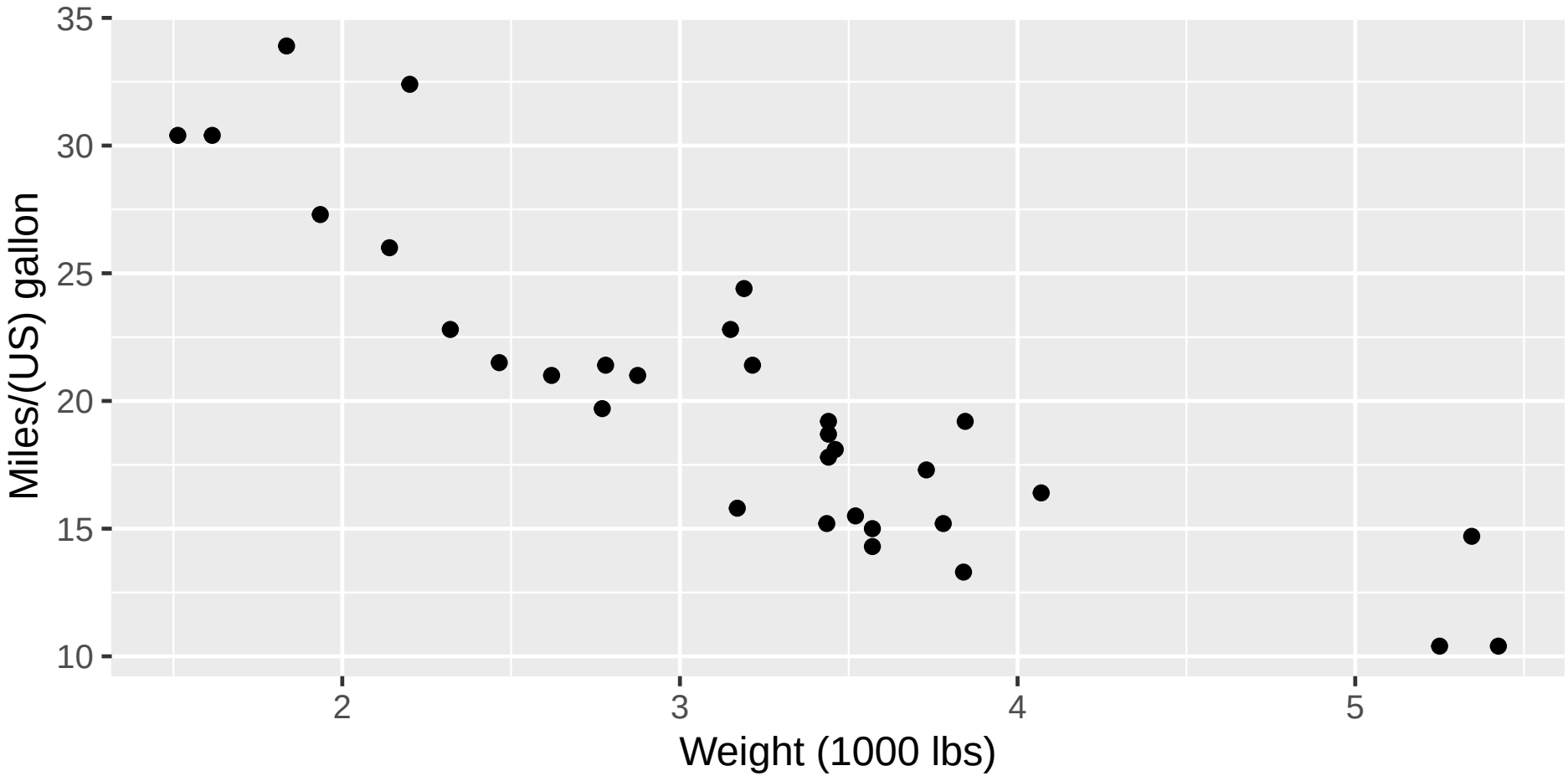
以 mtcars 为例：

```
head(mtcars);
```

```
##                   mpg cyl disp  hp drat    wt  qsec vs am gear carb
## Mazda RX4         21.0   6  160 110 3.90 2.620 16.46  0  1    4    4
## Mazda RX4 Wag     21.0   6  160 110 3.90 2.875 17.02  0  1    4    4
## Datsun 710        22.8   4  108  93 3.85 2.320 18.61  1  1    4    1
## Hornet 4 Drive    21.4   6  258 110 3.08 3.215 19.44  1  0    3    1
## Hornet Sportabout 18.7   8  360 175 3.15 3.440 17.02  0  0    3    2
## Valiant           18.1   6  225 105 2.76 3.460 20.22  1  0    3    1
```

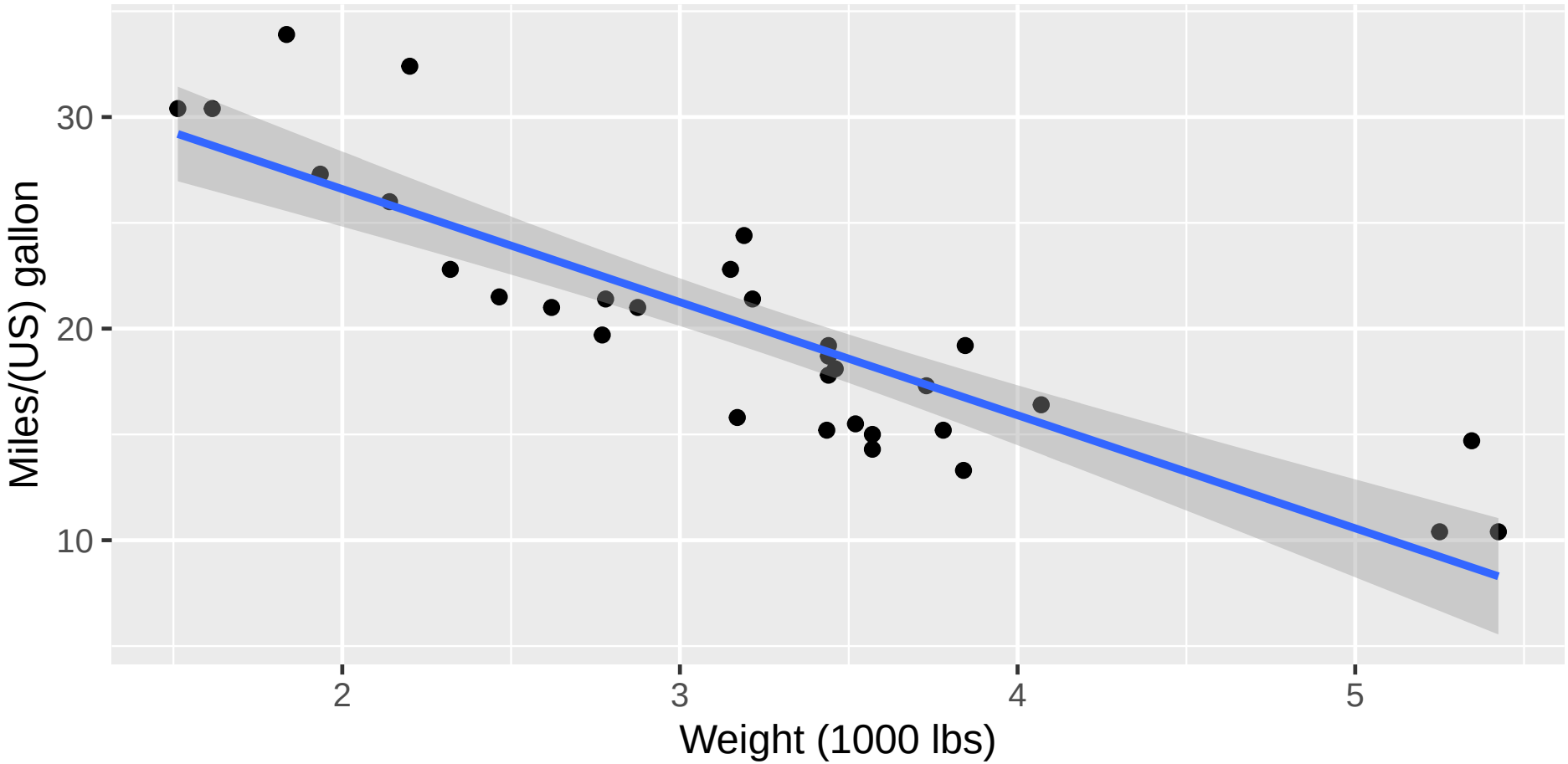
查看重量与燃油效率之间的关系

```
plotcars <-
   ggplot( mtcars, aes( x = wt, y = mpg ) ) +
      geom_point() + xlab( "Weight (1000 lbs)" ) + ylab( "Miles/(US) gallon" );
plotcars;
```



smooth, 减少噪音

```
plotcars + geom_smooth( method = "lm" ); ## default is lowess
```



作业：计算 correlation 并做图

用 talk09 中介绍的方法添加类似于第 43 页的两个公式

expression(R^2) variance of x explained by y ...

```
( r <- with( mtcars, cor.test( mpg, wt )$estimate ) );
```

```
##        cor
## -0.8676594
```

```
## variance of mpg can be explained by weight
r^2;
```

```
##       cor
## 0.7528328
```

当趋势不明显时，可以按另一组数据分组

这里还以 mtcars 为例。

两种分组（binning）方法 equal-distance, equal-size binning

举例：

```
mtcars2 <- mtcars %>%
   mutate( group1 = ntile( wt, 4 ), ## equal-size bining
           group2 = cut( wt,
                         breaks = seq( from = min(wt), to = max(wt),
                                       by = (max(wt) - min(wt)) / 4 ),
                         include.lowest = T ) ## equal-distance ...
           ) ;
```

ntile 函数的参数

... *tile 函数都是 equal size

```
## ntile 的结果
table( mtcars2$group1 );
```

```
##
## 1 2 3 4
## 8 8 8 8
```

cut 函数

按指定的间隔 (breaks) 对数据进行分割。

```
table( mtcars2$group2);
```

```
##
## [1.51,2.49] (2.49,3.47] (3.47,4.45] (4.45,5.42]
##           8          13           8           3
```

使用方法:

```
cut(x, ...)
# S3 method for default
cut(x, breaks, labels = NULL,
    include.lowest = FALSE, right = TRUE, dig.lab = 3,
    ordered_result = FALSE, ...)
```

cut 示例

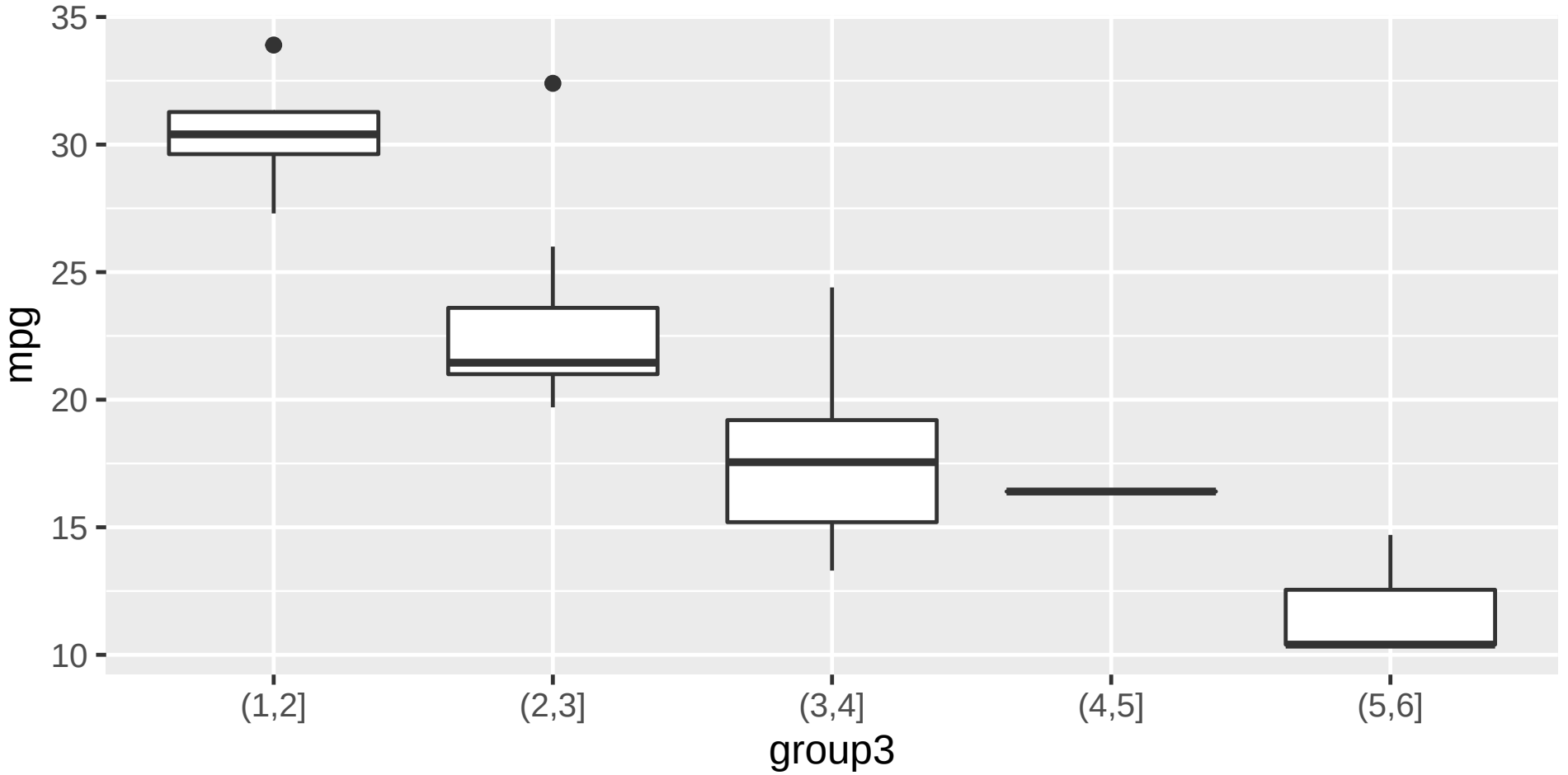
不仅可用于 equal distance，还可以用于任意间距

```
mtcars3 <- mtcars2 %>%
   mutate( group3 = cut( mtcars$wt, breaks = c(0,1,2,3,4,5,6) ) )   ;
table(mtcars3$group3);
```

```
##
## (0,1] (1,2] (2,3] (3,4] (4,5] (5,6]
##     0     4     8    16     1     3
```

分组后的数据适合用 boxplot

```
ggplot( mtcars3, aes( group3, mpg ) ) +
   geom_boxplot();
```



小结

目前讲述了以下内容：

一维数据

`table`, `summary`, `range`, `quantile`, `mean`, `median` ...

二维数据

- boxplot
- point plot
- correlation
- 分组：equal distance, equal size binning ...

section 5: parametric tests

parametric tests

1. 包括：
   - t-test
   - analysis of variance
   - linear regression
2. 数据有较明确的分布（e.g. normal distribution），或假设数据有明确的分布；当假设不成立时，检测会无效；
3. 更灵敏（相比 nonparametric test），p-value 更低

more to read: `http://rcompanion.org/handbook/I_01.html`

适用性

适用于

- 数量化性状，比如：身高、体重、产量、污染值
- 整数值：成绩、年龄、每天步数

不适用于

- 其它 count data 或者 discrete data；
- 或者有太多趋向于 min 或 max 的值
- 百分比或比例

详见：`http://rcompanion.org/handbook/index.html`

需要的 packages

需要的 packages

```
## chooseCRANmirror()
if(!require(psych)) {
   install.packages("psych");
}
if( !require(rcompanion) ) {
   install.packages("rcompanion");
}

library(psych);
library(rcompanion)
```

数据

注意 source() 函数的用法

```
source("data/talk10/input_data1.R"); ## 装入 Data data.frame ...

str(Data);
```

```
## 'data.frame':    26 obs. of  5 variables:
##  $ Student: Factor w/ 26 levels "a","b","c","d",..: 1 2 3 4 5 6 7 8 9 10 ...
##  $ Sex    : Factor w/ 2 levels "female","male": 1 1 1 1 1 1 2 2 2 2 ...
##  $ Teacher: Factor w/ 3 levels "Catbus","Satsuki",..: 1 1 1 1 1 1 1 1 1 1 ...
##  $ Steps  : int  8000 9000 10000 7000 6000 8000 7000 5000 9000 7000 ...
##  $ Rating : int  7 10 9 5 4 8 6 5 10 8 ...
```

检查数据

```
library(psych)
```

```
##
## Attaching package: 'psych'
```

```
## The following objects are masked from 'package:ggplot2':
##
##     %+%, alpha
```

```
headTail(Data); ## psych 包提供的函数
```

```
##     Student    Sex Teacher Steps Rating
## 1         a female  Catbus  8000      7
## 2         b female  Catbus  9000     10
## 3         c female  Catbus 10000      9
## 4         d female  Catbus  7000      5
## ...    <NA>   <NA>    <NA>   ...    ...
## 23        w   male  Totoro  6000      8
## 24        x   male  Totoro  8000     10
## 25        y   male  Totoro  7000      7
## 26        z   male  Totoro  7000      7
```

查看数据, cont.

```
## 其它常用函数
str(Data)
```

```
## 'data.frame':    26 obs. of  5 variables:
##  $ Student: Factor w/ 26 levels "a","b","c","d",..: 1 2 3 4 5 6 7 8 9 10 ...
##  $ Sex    : Factor w/ 2 levels "female","male": 1 1 1 1 1 1 2 2 2 2 ...
##  $ Teacher: Factor w/ 3 levels "Catbus","Satsuki",..: 1 1 1 1 1 1 1 1 1 1 ...
##  $ Steps  : int  8000 9000 10000 7000 6000 8000 7000 5000 9000 7000 ...
##  $ Rating : int  7 10 9 5 4 8 6 5 10 8 ...
```

```
summary(Data)
```

```
##     Student       Sex        Teacher       Steps           Rating
##  a      : 1   female:15   Catbus :10   Min.   : 5000   Min.   : 4.000
##  b      : 1   male  :11   Satsuki: 7   1st Qu.: 7000   1st Qu.: 7.000
##  c      : 1               Totoro : 9   Median : 8000   Median : 8.000
##  d      : 1                            Mean   : 7692   Mean   : 7.615
##  e      : 1                            3rd Qu.: 8750   3rd Qu.: 9.000
##  f      : 1                            Max.   :10000   Max.   :10.000
##  (Other):20
```

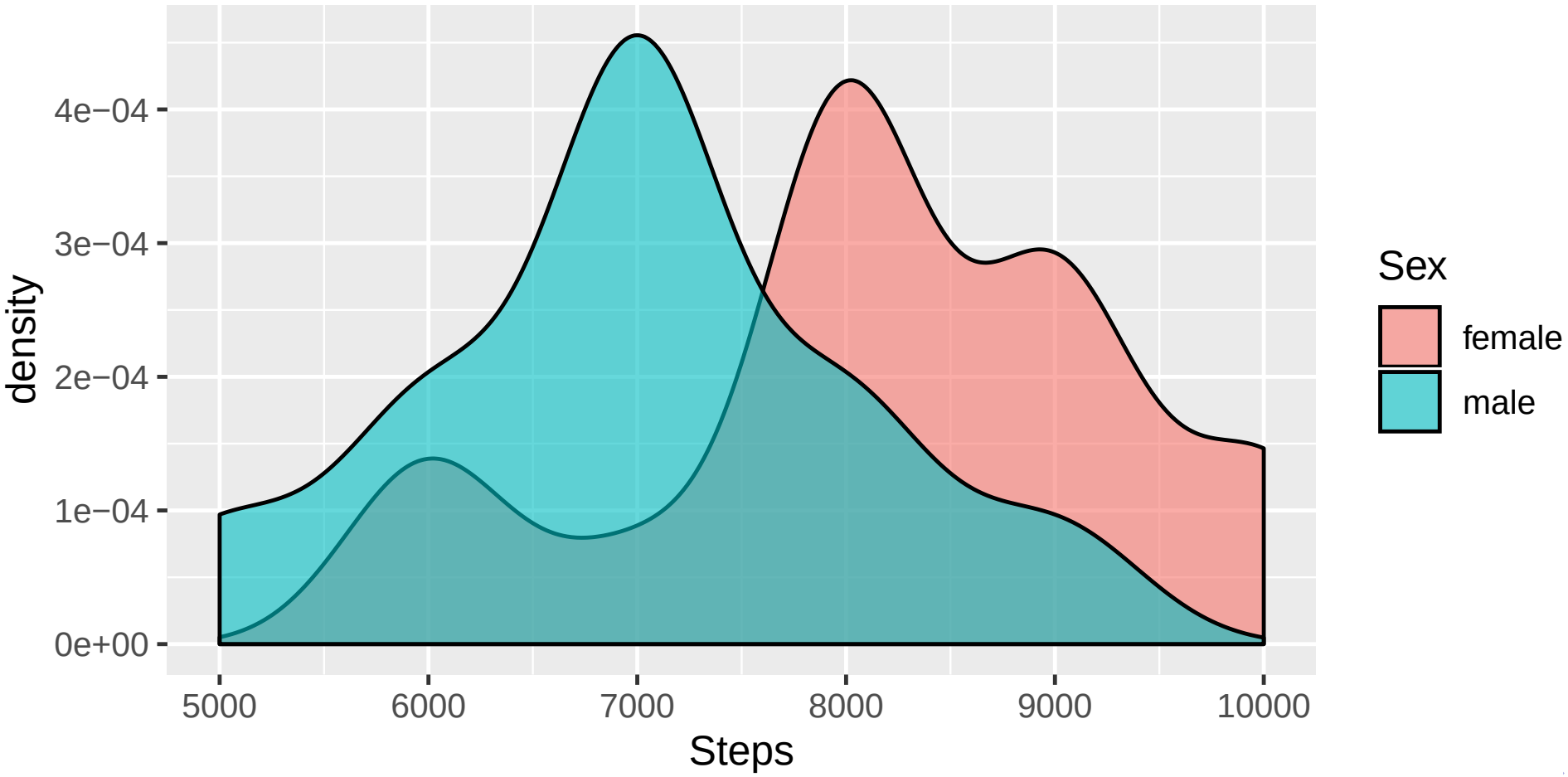
parametric test 的要求

1. 随机取样
2. 值或 residuals 为正态分布；residules 是指观察值与预测值 (mean) 之差

数据的分布

```
ggplot(Data, aes(Steps, fill = Sex)) +
       geom_density(position="dodge", alpha = 0.6)
```

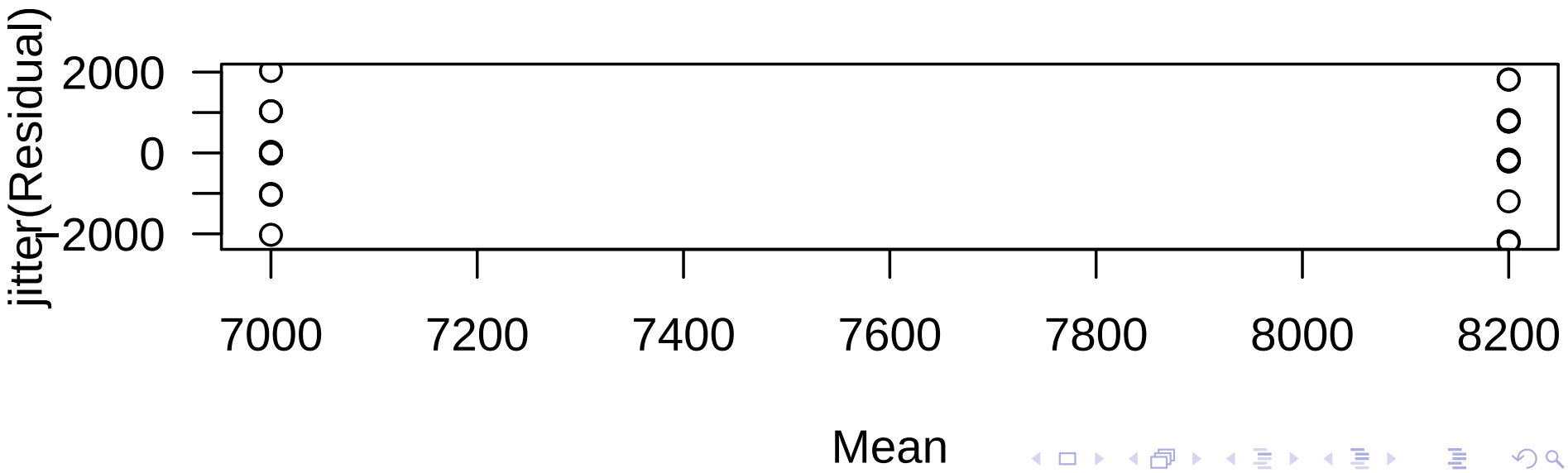
```
## Warning: Width not defined. Set with `position_dodge(width = ?)`
```



parametric test 的要求，cont.

3. 有相同的 variance

```
M1 = mean(Data$Steps[Data$Sex=="female"])
M2 = mean(Data$Steps[Data$Sex=="male"])

Data$Mean[Data$Sex=="female"] = M1
Data$Mean[Data$Sex=="male"]   = M2

Data$Residual = Data$Steps - Data$Mean

plot(jitter(Residual) ~ Mean, data = Data, las = 1);
```



how to detect outlier ??

一个很模糊的定义：Outliers are extreme values that fall a long way outside of the other observations. For example, in a normal distribution, outliers may be values on the tails of the distribution.

对于 normal distribution，通常 mean +- 2 or 3 * sd

对于 non-parametric distribution (注：IRQ 计算可使用同名函数：`IRQ`)：

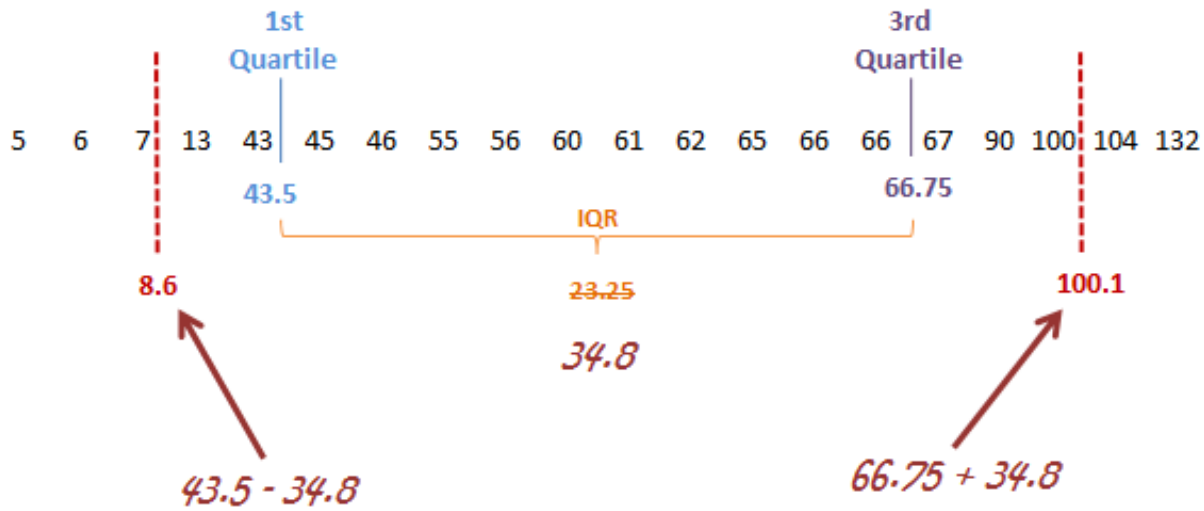
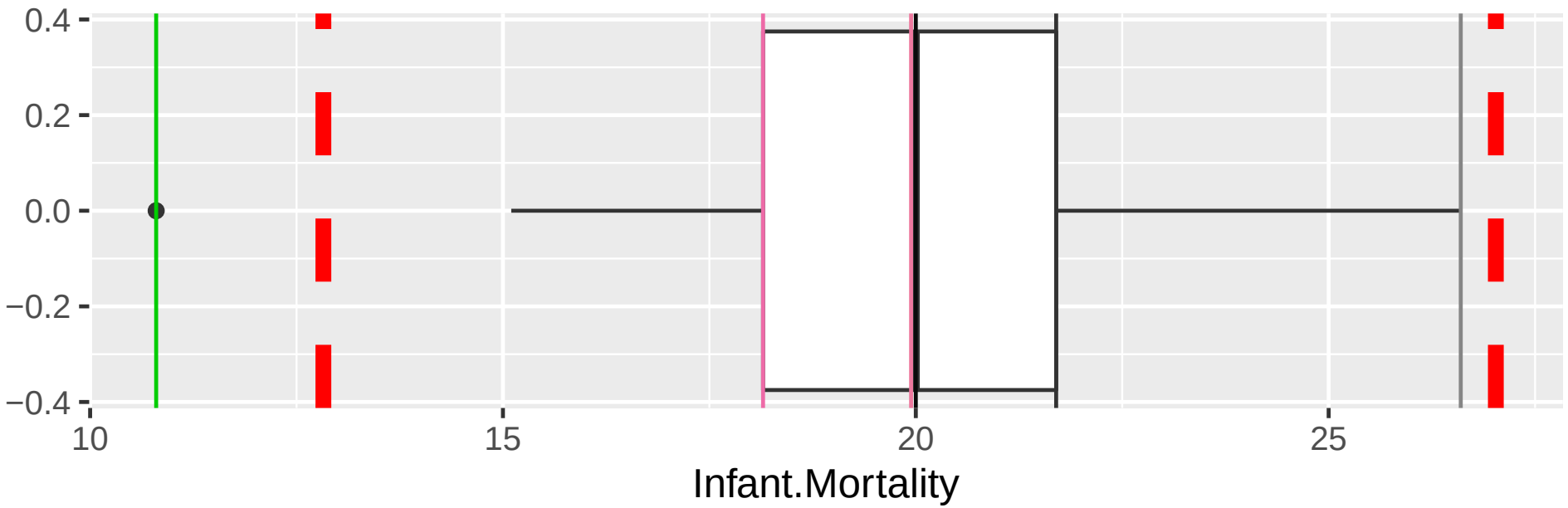


Figure 2: Tukey's method for outlier detection

an example of outlier values

```
s <- summary( swiss$Infant.Mortality );
irq <- IQR(swiss$Infant.Mortality);
ggplot( swiss, aes( y =  Infant.Mortality ) ) + geom_boxplot() + coord_flip() +
   geom_hline( yintercept = s, colour = sample( colors(), length(s) ) ) +
   geom_hline( yintercept = c( s["1st Qu."] - 1.5 * irq, s["3rd Qu."] + 1.5 * irq ),
               colour = "red", size = 2, linetype = 2);
```



other methods for detect outliers

1. https://conversionxl.com/blog/outliers/
2. https://www.r-bloggers.com/outlier-detection-and-treatment-with-r/
3. https://machinelearningmastery.com/how-to-identify-outliers-in-your-data/
4. https://www.amazon.com/dp/1461463955
5. https://www.itl.nist.gov/div898/handbook/eda/section3/eda35h.htm

one sample t-test

检测分布是否与预期一致；比如：男生每天的步数是否显著区别于 1 万

```
with( Data, t.test( Steps[ Sex == "male" ], mu = 1000 ) );
```

```
##
##  One Sample t-test
##
## data:  Steps[Sex == "male"]
## t = 18.166, df = 10, p-value = 5.484e-09
## alternative hypothesis: true mean is not equal to 1000
## 95 percent confidence interval:
##  6264.07 7735.93
## sample estimates:
## mean of x
##      7000
```

two samples t-test

比较 sd 和 mean ， 可应用于正态分布。几种使用方法：

```
with( Data, t.test( Steps ~ Sex ) )
```

```
##
##  Welch Two Sample t-test
##
## data:  Steps by Sex
## t = 2.6424, df = 22.816, p-value = 0.01461
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##   260.1421 2139.8579
## sample estimates:
## mean in group female   mean in group male
##                 8200                 7000
```

two sample t-test 使用方法 2

```
with( Data, t.test( Steps[ Sex == "male" ], Steps[ Sex == "female" ] ) );
```

```
##
##  Welch Two Sample t-test
##
## data:  Steps[Sex == "male"] and Steps[Sex == "female"]
## t = -2.6424, df = 22.816, p-value = 0.01461
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -2139.8579  -260.1421
## sample estimates:
## mean of x mean of y
##      7000      8200
```

two sample t test 检测结果

```
res <- with( Data, t.test( Steps ~ Sex ) );
str(res);
```

```
## List of 10
##  $ statistic  : Named num 2.64
##   ..- attr(*, "names")= chr "t"
##  $ parameter  : Named num 22.8
##   ..- attr(*, "names")= chr "df"
##  $ p.value    : num 0.0146
##  $ conf.int   : num [1:2] 260 2140
##   ..- attr(*, "conf.level")= num 0.95
##  $ estimate   : Named num [1:2] 8200 7000
##   ..- attr(*, "names")= chr [1:2] "mean in group female" "mean in group male"
##  $ null.value : Named num 0
##   ..- attr(*, "names")= chr "difference in means"
##  $ stderr     : num 454
##  $ alternative: chr "two.sided"
##  $ method     : chr "Welch Two Sample t-test"
##  $ data.name  : chr "Steps by Sex"
##  - attr(*, "class")= chr "htest"
```

paired two sample t test

例如：辅导前后的学生成绩：

```
source("data/talk10/input_data2.R");

head(scores);
```

```
##     Time Student Score
## 1 Before       a    65
## 2 Before       b    75
## 3 Before       c    86
## 4 Before       d    69
## 5 Before       e    60
## 6 Before       f    81
```

paired two sample t test

```
scores.wide <- scores %>% spread( Time, Score );
head(scores.wide, n = 3);
```

```
##   Student After Before
## 1       a    77     65
## 2       b    98     75
## 3       c    92     86
```

```
with( scores.wide, t.test( After, Before,  paired = T ) );
```

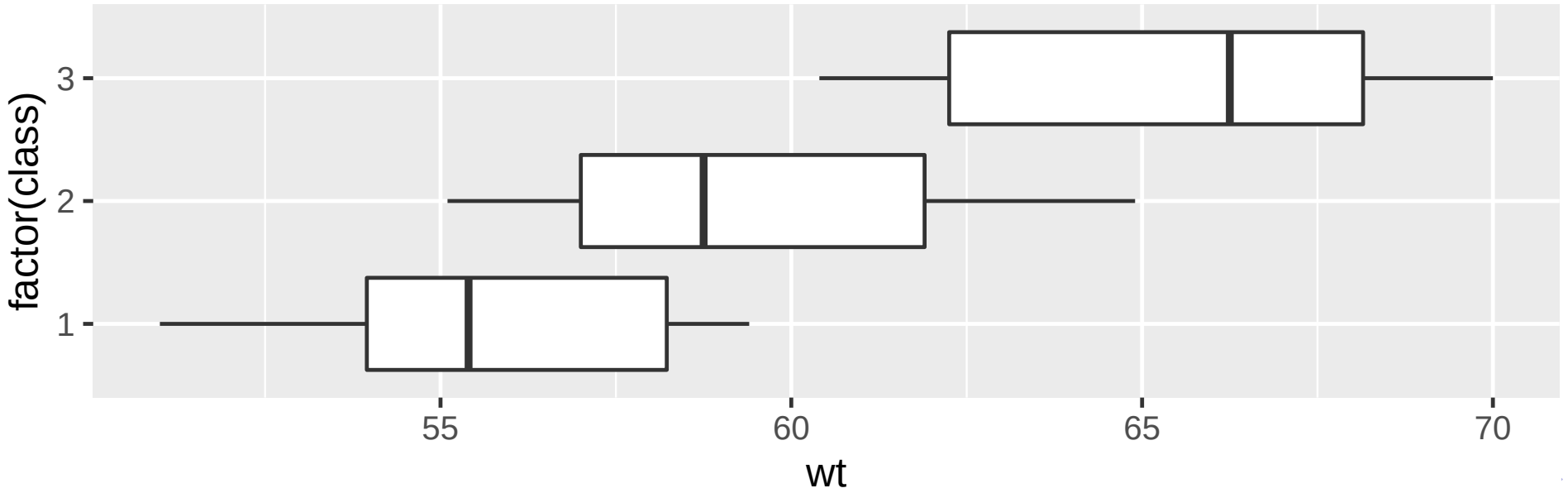
```
##
##  Paired t-test
##
## data:  After and Before
## t = 3.8084, df = 9, p-value = 0.004163
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##   4.141247 16.258753
## sample estimates:
## mean of the differences
##                    10.2
```

one way ANOVA

ANOVA: similar to indepedent t-test, but can be applied to multiple groups

比如：3 个班学生的体重

```
wts <- bind_rows( tibble( class = 1, wt = sample( seq(50, 60, by = 0.1), 20 ) ),
                  tibble( class = 2, wt = sample( seq(55, 65, by = 0.1), 20 ) ),
                  tibble( class = 3, wt = sample( seq(60, 70, by = 0.1), 20 ) )
                  );

ggplot(wts, aes( factor( class ), wt ) ) + geom_boxplot() + coord_flip();
```



one way ANOVA, cont.

```
library(FSA); ## 如果没有这个包，请先安装 ...
```

```
## ## FSA v0.8.25. See citation('FSA') if used in publication.
## ## Run fishR() for related website and fishR('IFAR') for related book.

##
## Attaching package: 'FSA'

## The following object is masked from 'package:psych':
##
##     headtail
```

```
with( wts, Summarize( wt ~ class, digits = 3 ) );
```

```
##   class  n   mean    sd  min    Q1 median     Q3  max
## 1     1 20 55.490 2.812 51.0 53.95  55.40 58.225 59.4
## 2     2 20 59.250 3.059 55.1 57.00  58.75 61.900 64.9
## 3     3 20 65.395 3.193 60.4 62.25  66.25 68.150 70.0
```

linear model

两个问题：

1. 组间有显著区别吗？

```
model <- lm( wt ~ class, data = wts );

anova( model );
```

```
## Analysis of Variance Table
##
## Response: wt
##           Df Sum Sq Mean Sq F value    Pr(>F)
## class      1 981.09  981.09  105.24 1.195e-14 ***
## Residuals 58 540.72    9.32
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

ANOVA

2. 分组对变量的贡献（r-square, aka. variance explained ）

```
summary( model );
```

```
##
## Call:
## lm(formula = wt ~ class, data = wts)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -4.9450 -2.7700 -0.2187  2.5419  5.0025
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  50.1400     1.0429   48.08  < 2e-16 ***
## class         4.9525     0.4828   10.26  1.2e-14 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 3.053 on 58 degrees of freedom
## Multiple R-squared:  0.6447, Adjusted R-squared:  0.6386
## F-statistic: 105.2 on 1 and 58 DF,  p-value: 1.195e-14
```

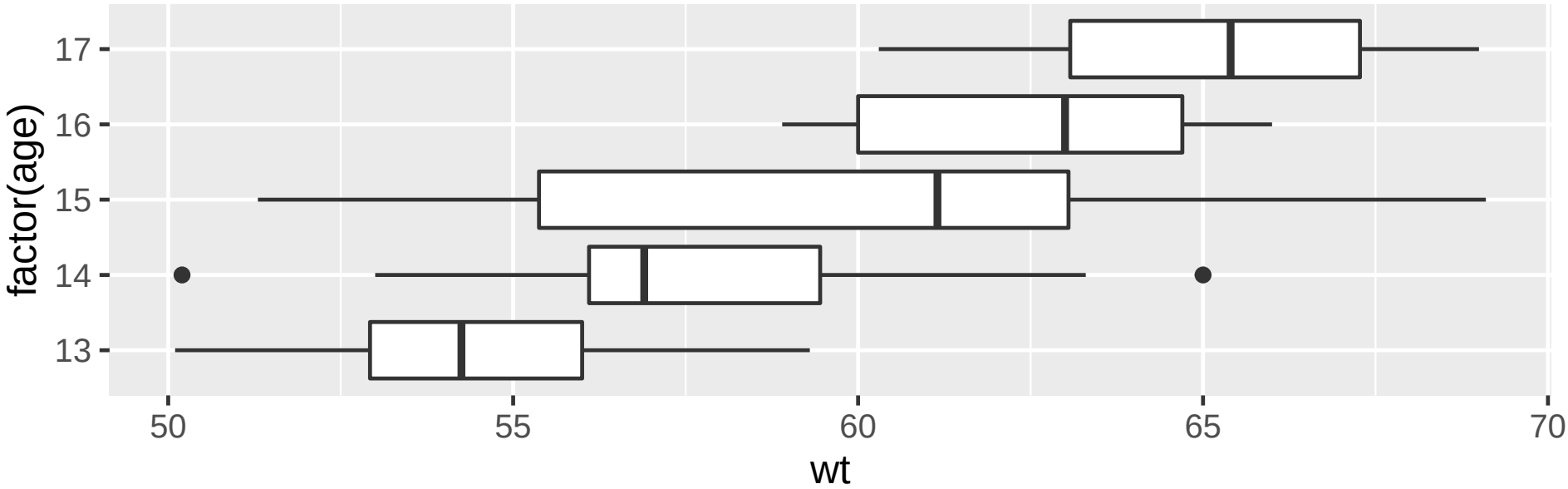
其中的值都是什么意思 ???

更多内容见：http://rcompanion.org/handbook/I_05.html

one way ANOVA with blocks

同时有多个因素影响体重时，哪些才是主要的？

```
wts2 <- bind_rows(
   tibble( class = 1, age = sample( 13:15, 20, replace = T ), wt = sample( seq(50, 60, by = 0.1
   tibble( class = 2, age = sample( 14:16, 20, replace = T ), wt = sample( seq(55, 65, by = 0.1
   tibble( class = 3, age = sample( 15:17, 20, replace = T ), wt = sample( seq(60, 70, by = 0.1
);

ggplot(wts2, aes( factor( age ), wt ) ) + geom_boxplot() + coord_flip();
```



one way ANOVA with blocks, cont.

```
model2 <- lm( wt ~ class + age, data = wts2);
anova( model2 );
```

```
## Analysis of Variance Table
##
## Response: wt
##           Df  Sum Sq Mean Sq  F value Pr(>F)
## class      1 1084.72 1084.72 136.0612 <2e-16 ***
## age        1    0.35    0.35   0.0442 0.8342
## Residuals 57  454.42    7.97
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

如何获得 r.squre value ???

one way ANOVA with blocks, 各个 factor 的重要性 ??

```
library(relaimpo);
```

```
res3 <- calc.relimp( wt ~ factor(class) + age, data = wts2 );
res3$R2; ## 总 R2
```

```
## [1] 0.7048711
```

```
res3$lmg; ## 每个因素的贡献;
```

```
## factor(class)           age
##     0.4920945     0.2127766
```

```
## 测试 rela 参数:
res4 <- calc.relimp( wt ~ factor(class) + age, data = wts2, rela = T);
res4$R2; ## 总 R2
```

```
## [1] 0.7048711
```

```
res4$lmg; ## 每个因素的贡献;
```

```
## factor(class)           age
##     0.6981341     0.3018659
```

更多请见: http://rcompanion.org/handbook/I_06.html

two way ANOVA

一个变量受另外两个因素影响的分；比如上例中 **体重受 年级和 年龄的影响**。

年级和 年龄至少有 4 个 unique combinations .

实际上，上面的 block test 可以认为是 two-way ANOVA 分析

```
Summarize(wt ~ age + class, data = wts2, digits=3);
```

```
##    age class  n   mean    sd  min     Q1 median     Q3  max
## 1   13     1 12 54.575 2.784 50.1 52.925  54.25 56.000 59.3
## 2   14     1  4 54.650 3.760 50.2 52.300  54.95 57.300 58.5
## 3   15     1  4 52.875 1.307 51.3 52.425  52.85 53.300 54.5
## 4   14     2  7 59.386 3.621 55.7 56.600  58.10 61.850 65.0
## 5   15     2  8 59.200 3.365 55.3 56.525  59.00 62.425 62.9
## 6   16     2  5 60.300 1.594 58.9 59.400  60.00 60.200 63.0
## 7   15     3  8 64.062 3.034 60.1 61.375  64.45 65.725 69.1
## 8   16     3  4 65.025 0.655 64.6 64.675  64.75 65.100 66.0
## 9   17     3  8 65.088 3.158 60.3 63.075  65.40 67.275 69.0
```

two way ANOVA, cont.

```
model3 <- lm( wt ~ class + age + class:age, data = wts2);
anova( model3 );
```

```
## Analysis of Variance Table
##
## Response: wt
##           Df  Sum Sq Mean Sq F value Pr(>F)
## class      1 1084.72 1084.72 135.290 <2e-16 ***
## age        1    0.35    0.35   0.044 0.8346
## class:age  1    5.43    5.43   0.677 0.4141
## Residuals 56  448.99    8.02
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

其中：class 和 age 称为 **main effects**，class:age 称为 **interaction effects**

relative importance of interactions

```
res5 <- calc.relimp( wt ~ factor(class) + age + factor(class):age, data = wts2);
res5$R2; ## 总 R2
```

```
## [1] 0.7131791
```

```
res5$lmg; ## 每个因素的贡献;
```

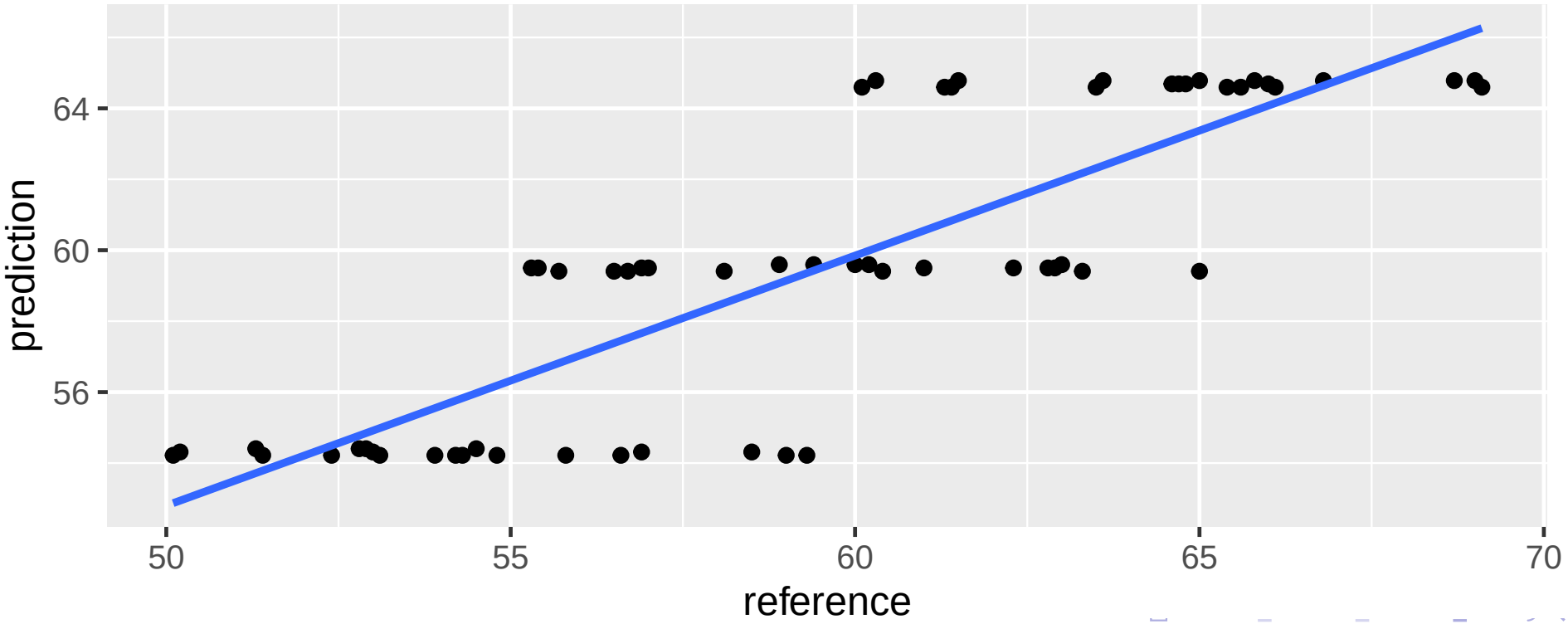
```
##     factor(class) factor(class):age                 age
##       0.492094503       0.008308066       0.212776574
```

more to read about the interaction effects:
http://oak.ucc.nau.edu/rh232/courses/EPS625/Handouts/Two-way%20ANOVA/Understanding%20the%20Two-way%20ANOVA.pdf

用模型进行预测 predict

```
model2 = lm(formula = wt ~ class + age, data = wts2)
```

```
newdata <- wts2 %>% dplyr::select( class, age );
wt.predicted <- predict( model2, newdata );

dat <- data.frame( reference = wts2$wt, prediction = wt.predicted );
ggplot( dat , aes( x = reference, y = prediction ) ) + geom_point() +
   geom_smooth( method = "lm", se = F );
```



prediction 与 original data 的 correlation 是多少 ??

```
with( dat, cor.test( prediction, reference ) )$estimate;
```

```
##       cor
## 0.8395383
```

```
## R ^ 2
with( dat, cor.test( prediction, reference ) )$estimate ^2;
```

```
##       cor
## 0.7048245
```

```
## 正好是 model2 的 r.squred ...
summary( model2 )$r.squared;
```

```
## [1] 0.7048245
```

手动计算 prediction

在一个 linear model 中，`wt = intercept + a * class + b * age`

而 `intercept`，a, b 的值分别为：

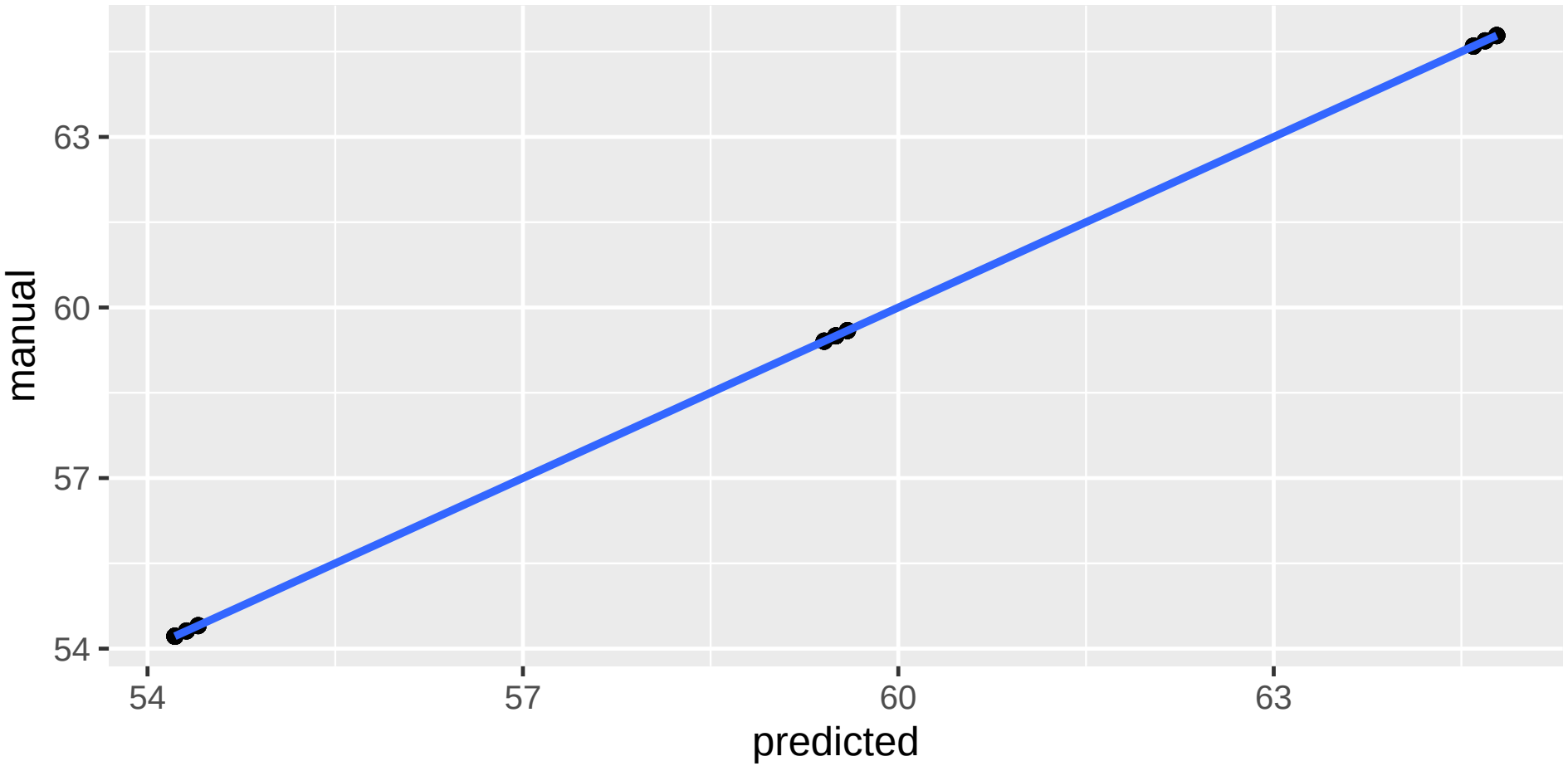
```
( paras <- coef( model2 ) );
```

```
## (Intercept)       class         age
## 47.91315057  5.09584697  0.09304419
```

```
predicted2 <-
   paras[1] + paras["age"] * wts2$age + paras["class"] * wts2$class;

plot <-
   ggplot( data.frame( predicted = wt.predicted, manual = predicted2 ),
           aes( predicted, manual ) ) +
      geom_point() + geom_smooth( method = "lm", se = F );
```

show the plot

```
plot;
```



更多更方便的函数

以下函数也可以用于 multivariable analysis / multiple regression

```
fit <- lm(y ~ x1 + x2 + x3, data=mydata)
summary(fit) # show results

# Other useful functions
coefficients(fit) # model coefficients
confint(fit, level=0.95) # CIs for model parameters
fitted(fit) # predicted values
residuals(fit) # residuals
anova(fit) # anova table
vcov(fit) # covariance matrix for model parameters
influence(fit) # regression diagnostics
```

linear regression 注意事项

1. 是 parametric test
2. 假设变量之间独立（比如：**年龄和班级之间没有关联**）
3. homogeneity of variance

但实际上 ...

multivariable analysis

more to read:

1. https://www.statmethods.net/stats/regression.html
2. https://data.library.virginia.edu/getting-started-with-multivariate-multiple-regression/

```
# Multiple Linear Regression Example
fit <- lm(y ~ x1 + x2 + x3, data=mydata)
summary(fit) # show results

# compare models
fit1 <- lm(y ~ x1 + x2 + x3 + x4, data=mydata)
fit2 <- lm(y ~ x1 + x2)
anova(fit1, fit2)

# K-fold cross-validation
library(DAAG)
cv.lm(df=mydata, fit, m=3) # 3 fold cross-validation

# Stepwise Regression; feature selection
library(MASS)
fit <- lm(y~x1+x2+x3,data=mydata)
step <- stepAIC(fit, direction="both")
step$anova # display results
```

extended reading

1. repeated measures ANOVA : http://rcompanion.org/handbook/I_09.html, 同一变量、不同时间段的重复测量（对上例中学生的体重进行多次测量）
2. correlation and linear regression: http://rcompanion.org/handbook/I_10.html
3. non-linear regression: https://www.amazon.com/Statistical-Tools-Nonlinear-Regression-Statistics/dp/0387400818

更多详见: http://rcompanion.org/handbook/I_08.html

section 6: non-parametric test

wilcox.test and kruskal.test

```
# independent 2-group Mann-Whitney U Test
with( Data, wilcox.test( Steps ~ Sex ) );
```

```
## Warning in wilcox.test.default(x = c(8000L, 9000L, 10000L, 7000L, 6000L, :
## cannot compute exact p-value with ties
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  Steps by Sex
## W = 127.5, p-value = 0.01773
## alternative hypothesis: true location shift is not equal to 0
```

```
# Kruskal Wallis Test One Way Anova by Ranks
with( Data, kruskal.test( Steps ~ Sex ) );
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  Steps by Sex
## Kruskal-Wallis chi-squared = 5.7494, df = 1, p-value = 0.01649
```

作业与练习

作业与练习

练习本课堂所讲的内容 ...