Statistics for Biologists

Outline

- estimation and hypothesis testing
- two sample comparisons
- linear models
- non-linear models
- application to genome scale data

Warning

- while the quantities often seem simple
- **NEVER**

  IMPLEMENT THEM

  YOURSELF
- use good software that already exists (R, SAS, MatLab)
  - numerical/scientific computing has many pitfalls for the unwary

Warning

- what went wrong:

```
> x = sqrt(2)
> x
[1] 1.414214
> x * x == 2
[1] FALSE
```

- R FAQ 7.31, Why doesn’t R think these numbers are equal?

Estimation

- given some set of data one might want to estimate some parameters of that data
  - mean, variance, 95th percentile
- point estimates
  - mean=122.2
- interval estimates
  - the mean is between 101 and 133
- in general we make assumptions about the underlying probability model (randomness) and choose estimates with specific properties
  - unbiased, minimum variance
  - we can be frequentist or Bayesian
  - confidence intervals (have a frequentist interpretation)

Hypothesis Testing

- a hypothesis is a statement about the real world
  - I think the mean is 100 ($H_0 : \mu = 100$)
- the null hypothesis should typically represent the *status quo*, or a presumption of no effect
- we use the data, plus our chosen inference paradigm to compute quantities that help us determine whether the null hypothesis is reasonable, or not

Two-types of mistake

- there are two kinds of mistakes that can be made
  - reject the null hypothesis when it is true
  - accept the null hypothesis when it is false
  - the size of a test is the probability that we reject the null when true
- the power of a test is the probability of rejecting the null hypothesis when it is false
  - this generally requires us to specify how it is false
- in general we use the size of the test to control the first type of mistake at some fixed level
- for a given size there are many tests, we attempt to choose ones that are more

p-values

- are quantities that relate to the null hypothesis
  - you cannot have a p-value without a null hypothesis
  - the p-value measures how likely it is to see evidence as extreme or more extreme as that observed assuming the null hypothesis is true
  - small p-values are evidence against the null hypothesis; they are **not** the probability it is true!
  - Bayesian’s use a different approach and

Size of the effect

- our point estimate gives us our best guess of the true value of the parameter we are interested in
- suppose we want to know if the FC > 1
  - H_0: FC = 1 in this case our one sided alternative is H_A: FC > 1
  - suppose our data provide an estimate of the FC to be 1.5, with a 95% CI (0.1, 2.2)
  - do we accept H_0 or reject it?

Size of the Effect

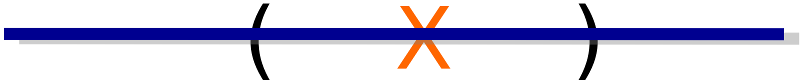
- what went wrong? (if anything)
- we might have been under-powered
- that is we did not have enough data to detect the difference
- the size of the CI is determined by the amount of variation in the data
- and that is largely controlled by the sample size

Equivalence

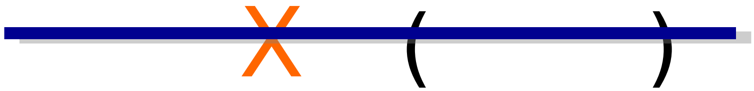
- there is a very direct relationship between confidence intervals and hypothesis tests

$$H_0 : \theta = X$$

  - if the value, X, lies inside of a 95% CI then the null hypothesis would not be rejected at the 5% level
  - if X, lies outside the 95% CI, then the null hypothesis would be rejected at the 5% level



- do not reject H_0



- reject H_0

Significance

- statistical significance should never be confused with scientific significance
- statistical significance tells us the surprise factor:
  - if all my assumptions are correct, and the null hypothesis is true, how surprised should I be by my data
  - at some level of surprise we choose to decide that our null hypothesis is unlikely to be true (usually we check to be sure our assumptions are reasonable)
- scientific significance is concerned with whether what we found is likely to have any relevance to our understanding of

Significance

- statistical significance is affected by sample size
- scientific significance is not
- getting more data often ensures statistical significance
  - new data technologies give us too much data
  - eg flow cytometry, sequencing
  - many things are scientifically uninteresting, but statistically significant

Two Concepts

- variance: when we estimate a quantity using data, we generally get both a point estimate and some estimate of the variability of that estimate
  - as sample sizes increase this variance tends to decrease
- bias: this is the difference between what we intended to measure and what we did measure
  - we estimate RPKMs incorrectly due to mapping issues
  - bias is never improved by sampling more, it usually requires changes in technology to

Two Important Theorems

- a central limit theorem basically says that the average (mean) of a set of numbers (assumed to come from some distribution) will behave approximately like a Normal random variable as the set grows
- the law of large numbers says that the mean of a set of numbers (assumed to come from some distribution) will get arbitrarily close to the mean (expected value) of the distribution

Two Sample Comparisons

- paired vs non-paired comparisons
  - eg. before/after, or two related measurements
  - a paired comparison usually increases power
- non-parametric tests vs parametric tests
  - parametric tests tend to be more powerful, for a given sample size, but they often achieve that at the expense of making assumptions

t-test

- test is for equality of the means

$$H_0 : \mu_1 = \mu_2$$

- various versions can address different underlying assumptions
  - paired vs independent
- assumptions:
  - no strong ones, the CLT provides rationale for reasonable samples
  - this is a parametric test (μ is the parameter)

Non-parametric two-sample tests

- Mann-Whitney (two independent samples)
- Wilcoxon (paired samples)
- they have a different null hypothesis

$$H_0 : F_1 = F_2$$

- equality of the two underlying distributions
- while this includes equality of the means, it is more restrictive
- in particular we do not expect correspondence between these tests

When to use tests

- non-parametric tests are often used when one does not want to make specific assumptions about the data
  - but they are less powerful, so if you don’t have much data they won’t work very well
- when you have lots of data and the assumptions are reasonable both parametric and non-parametric methods have similar behavior
- so I would use the non-parametric tests when I want to test $H_0 : F_1 = F_2$
- and the parametric tests when I want to test $H_0 : \mu_1 = \mu_2$

Limitations

- the two sample tests can be extended in a number of ways
  - inclusion of covariates; linear and non-linear regression
  - multiple groups; ANOVA (and friends)

Linear Models

- a linear model

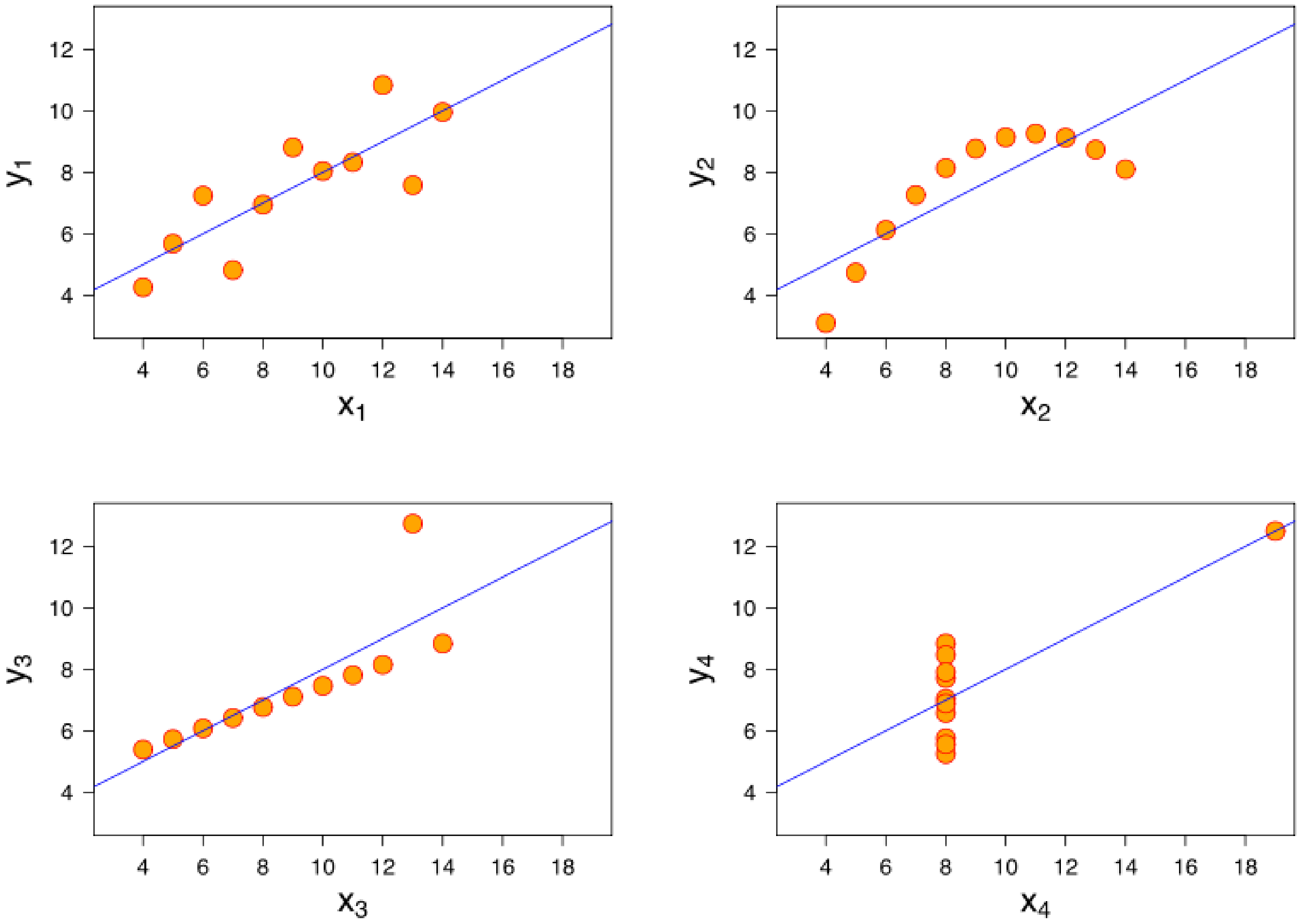
$$y = a + bx + e$$

- where y represents the independent variable
- a is the intercept (value for y when b=0)
- b is the slope of the relationship
- x are the known covariates
- e are the errors

Ancscombe’s Quartet

- four data sets for which most summary statistics and indeed, a, b and sigma^2, are identical
- but regression is appropriate for only one

Anscombe’s Quartet



Linear Models

- often the model is fit and parameters estimated using least squares
  - this gives estimates of a, b and from them the residuals can be obtained

$$\hat{e} = y - \hat{a} - \hat{b}x$$

- the residuals can be used to determine whether the model is reasonable
- hypothesis tests generally focus on questions about b

The t-test as a linear model

- if we let x be 0 or 1, depending on whether the observation is Treated or Not Treated,
- then for every observation in the treated group our model is $y = a + e$
- and for every observation in the untreated group the model is $y = a + b + e$
- so we can interpret a as the mean in the treated group, and a+b as the mean in the untreated group
- the test of b=0, is identical to the t-test, for unpaired samples

Linear model

- but the advantage of this formulation is that we can add other variables
  - eg sex, tissue, complex treatments
  - these are then adjusted for in our comparisons
- the residuals should always be examined, since they tell you about whether or not your model is appropriate
- testing $b=0$ makes the strong assumption that the model is correct
  - it is important that you learn to assess

Non-linear models

- while there is only one kind of linear model, there are lots of different non-linear models
- we will discuss generalized linear models
- this class of models includes logistic regression Poisson regression and Negative Binomial regression models
- logistic regression is used to model 0/1 data
- Poisson and Neg Binomial are suitable for modeling count data
  - the latter is more general and is being used

Non-linear models

- good software exists for fitting these
  - Modern Applied Statistics in S (MASS), Venables and Ripley
  - Julian Faraway’s books, Linear Models in R, and one on non-linear models

Application to Genome scale data

- several problems/issues became apparent
  - the test statistics seemed to often associate with other variables
    - for microarrays DE genes were those with high intensity
    - for RNA-seq, GC content seems to matter in some cases
  - these indicate the need for normalization

Genome Scale

- the test statistics could be large due to variability in the estimate of the variance
  - led to moderated t-tests, and other approaches
- how do we assess significance when doing many tests
  - p-value correction methods

Moderated t-tests

$$\frac{\hat{\mu}_1 - \hat{\mu}_2}{\sqrt{\hat{\sigma}^2 / n}}$$

- the t-test can be large if
  1. the means are different
  2. n is large
  3. our estimate of SE is small
- 1. is mostly what we are interested in
  – so we sometimes include a fold-change requirement
- 2. is a problem with flow cytometry and for some RNA-seq problems
- 3. is common in microarray experiments and limma and others use some form of moderated estimate of

Moderated tests

- they are effective for small sample sizes, the advantages of moderation drop off as the sample size increases
- there is nothing special about t-tests and limma fits more general models
  - most other methods can be similarly adapted

p-value Adjustments

- p-values are really interpreted for a single test
- when you do many some more careful thinking is required to ensure that error rates are controlled
- the false discovery rate is the expected value of the proportion of all tests for which H_0 is rejected where it is actually true
- this turns out to be a relatively easy quantity to estimate and it is of

p-value Adjustments

- we can often live with quite high FDR values
  - in some discovery projects FDR=0.5 is considered pretty good
- as with all cut-offs/approaches the FDR does not tell the whole story
  - it is attempting to control false discoveries
  - it says nothing about missing true discoveries
  - indeed, if one takes those tests just below the cut-off, they are enriched for