Model Based Statistics in Biology.

Part II. Quantifying Uncertainty.

Chapter 7.2 Hypothesis Testing with an Empirical Distribution

Material from page 808 in Sokal and Rohlf 1995 used,for Scutum widths

For each example,

draw graph of cumulative histogram.

Show one arrow up and across for one-tailed test

Show two arrows up and across for two-tailed test

ReCap Part I (Chapters 1,2,3,4)
Quantitative reasoning: Example of scallops, which combined models (what is the relation of scallop density to substrate?) with statistics (how certain can we be?)

ReCap (Ch5)
Data equations summarize pattern in data.

ReCap (Ch 6)
Frequency distributions are another key concept in statistics.
They are used to quantify uncertainty.
Empirical distributions are constructed from data
Theoretical distributions are models of data.

ReCap (Ch 7)
Inferential statistics are a logical procedure for making decisions when there is uncertainty due to variable outcomes. Frequentist decision making is based on the logic of eliminating chance as an explanation for an outcome.

Today: An example, using a generic recipe for statistical inference.

Wrap-up
We used a generic recipe for statistical decision making based on the logic of the null hypothesis.
To calculate a p-value, we used a distribution of outcomes generated by randomizing the data.

Table 7.1 Generic recipe for decision making with statistics

1. State population, conditions for taking sample
2. State the model or measure of pattern……ST
3. State null hypothesis about population…… H_o
4. State alternative hypothesis…… H_A
5. State tolerance for Type I error……α
6. State frequency distribution that gives probability of outcomes when the Null Hypothesis is true. Choices:
    a) Permutations: distributions of all possible outcomes
    b) Empirical distribution obtained by random sampling of all possible outcomes when H_o is true
    c) Cumulative distribution function (cdf) that applies when H_o is true
    State assumptions when using a cdf such as Normal, F, t or chisquare
7. Calculate the statistic. This is the observed outcome
8. Calculate p-value for observed outcome relative to distribution of outcomes when H0 is true
9. If p less than α then reject H_o in favour of H_A
    If greater than α then accept H_o
10. Report statistic, p-value, sample size
    Declare decision

Equivalent method (less informative) based on just a statistical table, no computer

8. Calculate outcome corresponding to α
9. If observed outcome > outcome @ α then reject H_o, accept H_A.
    If observed outcome $\leq$ outcome @ α then accept H_o.
10. Report statistic, p-value, and sample size. Declare decision.

This latter method is less informative, because the observed p-value does not get reported. This method was made necessary by the cumbersome tables for frequency distribution. With modern computers it is possible to calculate an exact p-value for any statistic. The method of reporting an exact p-value is preferred to the method based on tables.

The simplest way of understanding quite rigorously, yet without mathematics, what the calculations of the test of significance amount to, is to consider what would happen if our two hundred actual measurements [of stature of Englishmen and Frenchmen] were written on cards, shuffled without regard to nationality, and divided at random into two new groups of a hundred each. Actually, the statistician does not carry out this very simple and very tedious process, but his conclusions have no justification beyond the fact that they agree with those which could have been arrived at by this elementary method."

Fisher R.A. 1936. The "coefficient of racial likeness" and the future of craniometry. Journal of the Royal Anthropological Institute of Great Britain and Ireland 66: 57-63.

Example of Hypothesis Testing, Using the Generic Recipe - Jackal Bones

Example is length of bones from 10 male and 10 female jackals.
L = length of mandible (L= mm) of Golden Jackals
Canis aureus from the British Museum.
Data from Manly (1991).

Male	Female	
120	110	
107	111	
110	107	
116	108	
114	110	
111	105	
113	107	
117	106	
114	111	
112	111	
113.4	108.6	mean
13.82	5.16	var

Generic recipe. Set-up = steps 1-6. Execution = steps 7-10.

1. State population.
   It could be taken as all possible measurements
   on these particular bones.
   The values would vary because of measurement error.
   It would be very safe to infer to this population.
   It could be all jackals of this species in the world.
   The values would vary because of individual variation, on top of error.
   Inference to this population is less safe. We need to know whether this sample was representative of the population (all jackals).

2. Measure of pattern. ST = D_o = mean(L_{male}) − mean(L_{female})

3. H_o: $D_o \not> 0$ (i.e., $L_{male} \leq L_{female}$) for the population.

4. H_A: $D_o > 0$ (i.e., $L_{male} > L_{female}$) for the population.
   This is better test than H_A: $D_o \neq 0$ because it reduces Type II error.

5. α = 5% We will tolerate 5% rate of Type I error,
   i.e. error in accepting a difference that does not exist
   In order to make a decision, we need a criterion for what is 'improbable'
   This is usually fixed at 5% by tradition.
   Criterion must be stated before test, in rigorous decision-theoretical approach.
   In practice, the best thing is to fix the criterion then state the exact p-value,

Hypothesis Testing, Using the Generic Recipe - Jackal Bones

5. What is a good criterion for significance?

A criterion of 5% is traditional in biology

1% sometimes seen in physical sciences

Symbol for criterion is greek letter α

The criterion level, or tolerance for Type I error, depends on whether Type I or Type II error is more important because there is a trade-off. Lowering the tolerance of Type I error (lower α) usually increases Type II error.

In an exploratory analysis the tolerance is often raised (higher α), so as to reduce the chance that something will be missed.

6. The frequency distribution of the statistic D_o when the null hypothesis H_o is true will be calculated by randomization. This method does not require assumptions about the frequency distribution of the statistic.

To obtain the distribution we assign the 20 bones randomly to two groups, regardless of whether the bones were from a male or female.
Next, we compute the mean for each group.
Then we calculate D_o the <u>random</u> difference in means. This is the difference when the null hypothesis is true. We made the null hypothesis true by assigning bones randomly to two groups.
Next, we repeat this many times to obtain many random differences
(the more the better)
We assemble these random differences into a frequency distribution.

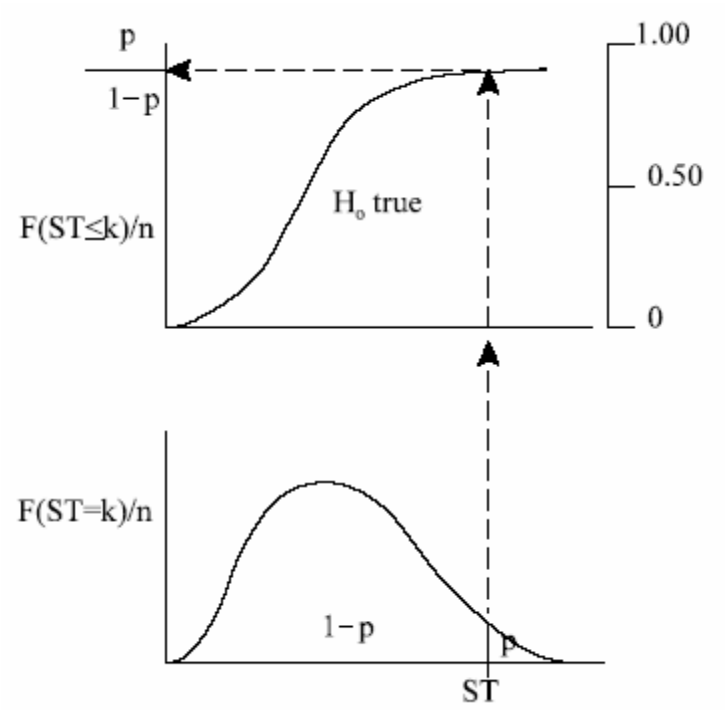
7. The observed difference is $D_o = 113.4 - 108.6 = 4.8$ mm

8. In this example we compute
5000 values of D_o
9 values exceed 4.8
$p = 9/5000$
$= 0.18\%$
$p < 5\%$

9. $p < \alpha$ so reject H_o ($D_o = 0$)
in favour of H_A ($D_o > 0$)

10. $D_o = 4.8$ mm,
$n = 20$,
$p = 0.0018$
male jackal bones
significantly longer
than female bones

Figure L10a



Compare general procedure (A,B,C,D) with recipe.

A. Define the population (step 1) and the signal (step 2, step 4, step 7)
B. Describe the noise (step 3, step 6)
C. Evaluate signal relative to noise (step 8)
D. Declare a decision (step 9, step 10)

Not used in 1997 onward

Hypothesis testing–Direct versus indirect method.

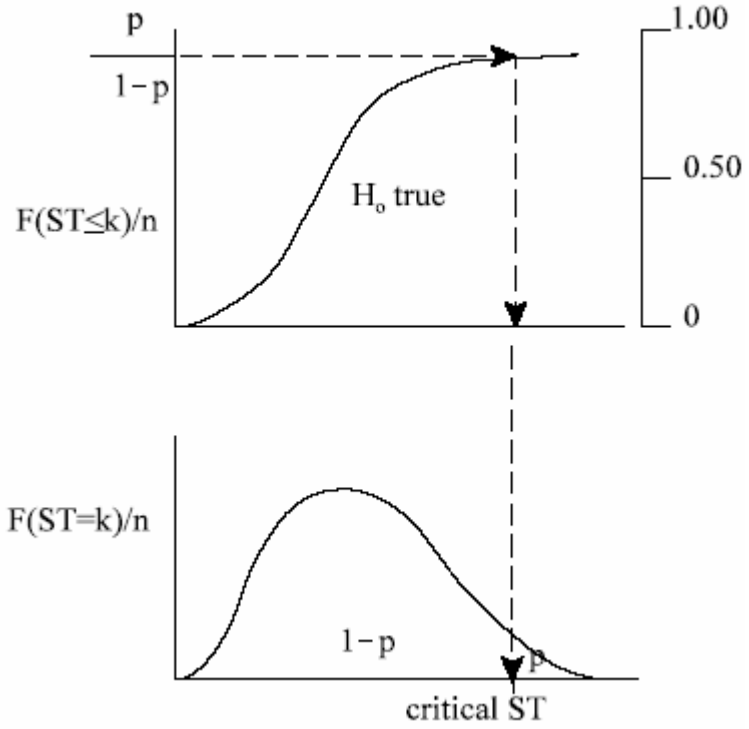
Direct method–compare p-value to criterion.

compute the p-value and compare it with the 5% criterion.
show arrow from statistic, upward to cdf, across to p-value
(Figure L10a on previous page).

Indirect method–compare observed statistic to critical value of statistic.

use criterion (e.g. 5%) to compute critical value corresponding to 5%
show arrow from 1−p across to cdf, downward to critical value.
compare observed value of statistic to this critical value.

Figure L10b



The indirect method is less informative.
It is used when there is no computer available to compute exact p-value.

This course will emphasize the direct method–comparing p-values.
The direct method provides more information to the reader of a report.
It is consistent with modern practice.
It demonstrates the machinery of hypothesis testing,
which is based on making a decision from a p-value relative to a fixed criterion.
Critical values do this indirectly, rather than directly.

Recall material on data equations. Showing improvement (reduction in sum of square deviations)

Hypothesis Testing. 2nd example. Heterozygosity data.

H_o: $H = \hat{\beta}_o + \epsilon$ $\quad SS_{tot}$ $\Sigma res^2 = \underline{0.1171}$

H_A: $H = \hat{\beta}_E \cdot E + Offset + \epsilon$ $\quad \Sigma res^2 = \underline{0.0204}$

The reduction in squared deviation is: $\quad SS_{model}$ $\Sigma res^2 = \underline{0.0966}$

H = heterozygosity, E = elevation

$H = -0.1273 \cdot E + 0.58 + res$

Data =	Model	+ residual
0.1171 =	0.0966	+ 0.0204
SS_{tot} =	SS_{model}	+ SS_{error}

Is this improvement better than random ?

Set up the analysis

1. Sample = 7 measurements.
   Population = all possible measurements taken with a stated procedure.
2. ST = SS_{model} = 0.0966, in the improvement in SS
   going from $H = \beta_o$ to $H = \beta_o + \beta_E E$ (E = elevation)
3. H_o: $SS_{model} = 0$ (in population) Improvement is 'just chance'
4. H_A: $SS_{model} > 0$ (in population) Improvement is more then 'just chance'
5. α = 5% (Type I error held to 5%)
6. go to key. SS_{model} not listed, hence use empirical.
7. Execute the analysis. SS_{model} = 0.096644 This is the observed improvement.

Calculate frequency distribution of improvements when H_o is true.
Do this by randomizing the heterozygosity data relative to explanatory variable Elev
The estimate parameters and improvement in fit.

Here is R code for to calculate random improvement in fit.

Here is Minitab code to calculate random improvement in fit.

```
MTB > sample 7 c2 c6
MTB > regress c6 1 c1;
SUBC> residuals c7.
```

Hypothesis Testing. 2nd Example. Improvement in fit, heterozygosity data

Here are the parameter estimates and ANOVA table for a single randomization.

```
The regression equation is
C6 = 0.263 +0.000015 Elev

Predictor       Coef       Stdev    t-ratio        p
Constant      0.2627      0.1184       2.22    0.077
Elev      0.00001506  0.00001787       0.84    0.438

s = 0.1432      R-sq = 12.4%     R-sq(adj) = 0.0%

Analysis of Variance

SOURCE       DF          SS          MS         F        p
Regression    1     0.01455     0.01455      0.71    0.438
Error         5     0.10253     0.02051
Total         6     0.11709
```

Random improvement is 0.01455

Run this repeatedly with a computer (2000 runs in BrusRN2.out)
[Handout unique randomization to each student]
Then tabulate on chalkboard, to construct frequency distribution.
k = Outcomes(SS_{model}) $F(SS_{model}=k)$

```
MTB > hist c10
Midpoint   Count
    0.00     692  **************************************************
    0.01     422  ******************************
    0.02     283  ********************
    0.03     219  ***************
    0.04     118  ********
    0.05      88  ******
    0.06      69  *****
    0.07      39  ***
    0.08      36  ***
    0.09      16  **
    0.10      16  **
    0.11       2  *
```

Hypothesis Testing. 2nd Example. Improvement in fit, heterozygosity data.

Now compute the number of random improvements that were larger than the observed improvement of 0.096644

```
MTB > hist c10;
SUBC> start 0.096644.

Histogram of C10   N = 2000
1983 Obs. below the first class

Midpoint   Count
   0.097       2  **
   0.098       6  ******
   0.099       0
   0.100       4  ****
   0.101       0
   0.102       3  ***
   0.103       0
   0.104       0
   0.105       0
   0.106       1  *
   0.107       0
   0.108       1  *

MTB > let k1 = 2000-1783
MTB > print k1
K1        17

MTB > let k2 = 17/2000
MTB > print k2
K2        0.00850000
```

8. p = ___/_____ (class result, show of hands to obtain distribution)

Figure 10a, one line coming across cdf frequency distribution, one tailed test.

9. $p = 0.0085 < 0.05 = \alpha$ so reject H_o
The improvement is better than random.

10. $SS_{model} = 0.096644$ $n = 7$ $p = 0.0085$ so reject H_o we can reject chance, that is, we can reject the JUST LUCK hypothesis.

Hypothesis Testing. 3rd Example. Improvement in fit, Oat Yield data.

Recall material on data equations. Showing improvement (reduction in sum of square deviations)

H_o: $Q = \beta_o$ $\Sigma res^2 = 493.14$ = SStotal

H_A: $Q = \beta_o + \beta_x X$ $\Sigma res^2 = 301.06$

The reduction in squared deviation: $\Sigma res^2 = 192.08$ = SSmodel

Is this improvement better than random ?

Set up the analysis

1. Sample = 8 measurements.
   Population = all possible measurements taken with a stated procedure.
2. ST = SS_{model} the improvement in SS going from $H = \beta_o$
   to $H = \beta_o + \beta_E X$ (X = group)
3. H_o: $E(SS_{model}) = 0$ The expected value in the population is zero.
4. H_A: $(SS_{model}) > 0$ The expected value in the population is not zero.
   Note that sums of squares (SS) cannot be less than zero.
5. $\alpha = 5\%$
6. go to key. SS_{model} not listed, hence use empirical (randomization test)

Execute the analysis.

7. $SS_{model} = 192.08$ This is the observed improvement.

Data Equations for null and alternative models

	data	null model	res	res^2	alt. model	res	res^2	observed improvement
0	42.90	40.95	1.95	3.80	36.05	6.85	46.92	
0	41.60	40.95	0.65	0.42	36.05	5.55	30.80	
0	28.90	40.95	-12.05	145.20	36.05	-7.15	51.12	
0	30.80	40.95	-10.15	103.02	36.05	-5.25	27.56	
1	49.50	40.95	8.55	73.10	45.85	3.65	13.32	
1	53.80	40.95	12.85	165.12	45.85	7.95	63.20	
1	40.70	40.95	-0.25	0.06	45.85	-5.15	26.52	
1	39.40	40.95	-1.55	2.40	45.85	-6.45	41.60	
	40.95		0.00	493.14			301.06	192.08

Hypothesis Testing. 3rd Example. Improvement in fit, Oat Yield data.

8. Calculate frequency distribution of improvements when H_o is true.
Do this by randomizing the data with respect to explanatory variable X

Data	Equations for null and alternative models							random
	data	null model	res	res^2	alt.model	res	res^2	improvement
0	30.80	42.23	-11.43	130.53	43.70	-12.90	166.41	
0	53.80	42.23	11.58	133.98	43.70	10.10	102.01	
0	49.50	42.23	7.28	52.93	43.70	5.80	33.64	
0	40.70	42.23	-1.53	2.33	43.70	-3.00	9.00	
1	30.80	42.23	-11.43	130.53	40.75	-9.95	99.00	
1	28.90	42.23	-13.33	177.56	40.75	-11.85	140.42	
1	49.50	42.23	7.28	52.93	40.75	8.75	76.56	
1	53.80	42.23	11.58	133.98	40.75	13.05	170.30	
sum			0.00	814.76		0.00	797.35	17.41

Taken from file labeled ST237.xls
Random improvement is 814.76 – 797.35 = 17.41

8. Calculate *p*-value
Assemble 500 random improvements, compute % that exceed observed improvement of 192.08

count	n	p-value
58	500	0.116

Figure 10a, one line coming across cdf frequency distribution, one tailed test.

9. $p = 0.116 > 0.05 = \alpha$ so accept H_o
The improvement is no better than random.

10. $SS_{model} = 192.08$ $n = 8$ $p = 0.116$ so reject H_o we cannot reject chance,
that is, we cannot reject the JUST LUCK hypothesis.

Hypothesis testing. Comparing Variances

The variance of a quantity can itself be considered a quantity. This quantity is of central interest in some parts of biology, notably population biology. Population biologists (including those who do molecular genetics) think in terms of variances, as much or more than they think in terms of central tendencies measured by means.
For example, balancing selection tends to reduce the variance in a trait, while mutation tends to increase genetic variance and hence increase variance in traits in a population.

Picture here (Fig L13b) of frequency distribution of body size in lizards at time=1 (normal with wide variance), axis labelled $L_{t=1}$ and y-axis labelled $F(L_{t=1})$. Then draw another distribution on another axis labelled $L_{t=2}$ (normal with narrower variance). y-axis labelled $F(L_{t=2})$

Example: Does selection by hawks on young lizards result in balancing selection on body size of lizards ? Size as measured by length L.

Another example: what is the spatial variance in number of species?
What factors tend to reduce species diversity ?
Another example: what is the current level of genetic variability in a population?
What factors tend to increase or reduce genetic variability?

Hypothesis testing. 4th Example. Comparing Variances. Scutum widths
Here is another analysis, using the generic recipe for hypothesis testing.
The example is the analysis of scutum widths, from Sokal and Rohlf 1995, p808.

1. Population. Set of 25 measurements. Population: All possible scutum widths? Like most text examples, population is unknown. So we assume population is all possible measurements, given the procedural statement.

2. ST = F = $\mathrm{Var}(W_{dead})/\mathrm{Var}(W_{live})$

The biology here is that we expect greater variance in dead than live
under most conditions, expect lower survival by individuals with extreme traits than by individuals closer to the mean. That is, expect stabilizing selection under most conditions. Exception is episode of directional selection. Expect that only ticks from a restricted portion of the genetic spectrum will survive cold shock. Hence expect less morphological variation in live (survivors) than in dead.

3. H_o: $F \leq 1$ i.e. $\mathrm{Var}(W_{dead}) \leq \mathrm{Var}(W_{live})$
i.e. no stabilizing selection
4. H_A: $F > 1$ i.e., $\mathrm{Var}(W_{dead}) > \mathrm{Var}(W_{live})$
i.e., stabilizing selection
5. $\alpha = 5\%$

Hypothesis testing. 4th Example. Comparing Variances. Scutum widths

6. If we wish to avoid making assumptions about the distribution of the statistic when the null is true, we can generate an empirical distribution to calculate the Type I error (p-value).
   This distribution is generated by randomizing the observations, then repeatedly calculating of the statistic F, to generate a distribution based on 500 such calculations

7. $F = 3.1095$ (if $F < 1$ then accept H_o at this point)
   observed $F > 1$,
   but could this have happened by chance ?
   in other words, what is Type I error ? (if we say F really is > 1)

8. $p = 40/500 = 8\%$ (by randomization).

Figure 10a, showing one line coming across, for one tailed test, based on H_A

9. $p = 8\% > \alpha = 5\%$ so accept H_o

10. $F_{15,8} = 3.1095$ $p = 8\%$
    note the use of subscripts to show sample size
    convention is $F_{\text{numerator df, denominator df}}$
    so first number in subscript is df for W_{dead}

This was a lot of work.
There is an easier way, if we are prepared to make some assumptions.
(Next lecture)