Statistics for the Sciences

Linear Models and Nested Designs

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Outline

- Linear Models
- Linear Mixed Models
- Nested Designs
- Example
- Lab

- A linear model or general linear model relates a dependent variable and one or more independent variables such that the relationship is modeled as a linear combination of the independent variables which are numerical or factors (categorical).
- Linear models get their name because they are linear in the model parameters. The general form of a linear model is given by

$$\mathbf{Y} = \mathbf{X}\boldsymbol{\beta} + \boldsymbol{\varepsilon},$$

where **Y** is the vector of dimension $n \times 1$ observed responses, **X** is the design matrix of $n \times (p+1)$ fixed constants, β is the vector of $(p+1) \times 1$ parameters to be estimated(unknown), and ε is the vector of $n \times 1$ random errors.

- ▶ p + 1 is used for including the intercept
- ► So MLR models are linear models

- Is the model for one-factor experimental design a liner model?
- For example, consider a fixed-effect model

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}, j = 1, 2, \dots, n_i, i = 1, 2, \dots, k$$

with $\sum_{i} \tau_{i} = 0$

• For simplicity let $n_i = 4, i = 1, 2, ..., k$ and k = 3

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}, j = 1, 2, 3, 4, i = 1, 2, 3$$

with $\tau_1 + \tau_2 + \tau_3 = 0$

$$\begin{aligned} Y_{11} &= \mu + \tau_1 + \varepsilon_{11} \\ Y_{12} &= \mu + \tau_1 + \varepsilon_{12} \\ Y_{13} &= \mu + \tau_1 + \varepsilon_{13} \\ Y_{14} &= \mu + \tau_1 + \varepsilon_{14} \\ Y_{21} &= \mu + \tau_2 + \varepsilon_{21} \\ Y_{22} &= \mu + \tau_2 + \varepsilon_{22} \\ Y_{23} &= \mu + \tau_2 + \varepsilon_{23} \\ Y_{24} &= \mu + \tau_2 + \varepsilon_{24} \\ Y_{31} &= \mu + \tau_3 + \varepsilon_{31} \\ Y_{32} &= \mu + \tau_3 + \varepsilon_{32} \\ Y_{33} &= \mu + \tau_3 + \varepsilon_{33} \\ Y_{34} &= \mu + \tau_3 + \varepsilon_{34} \end{aligned}$$

• Due to $\tau_1 + \tau_2 + \tau_3 = 0$, we only need three parameters: μ , τ_1 and τ_2 .

$$\begin{aligned} Y_{11} &= \mu + \tau_1 + \varepsilon_{11} \\ Y_{12} &= \mu + \tau_1 + \varepsilon_{12} \\ Y_{13} &= \mu + \tau_1 + \varepsilon_{13} \\ Y_{14} &= \mu + \tau_1 + \varepsilon_{14} \\ Y_{21} &= \mu + \tau_2 + \varepsilon_{21} \\ Y_{22} &= \mu + \tau_2 + \varepsilon_{22} \\ Y_{23} &= \mu + \tau_2 + \varepsilon_{23} \\ Y_{24} &= \mu + \tau_2 + \varepsilon_{24} \\ Y_{31} &= \mu - \tau_1 - \tau_2 + \varepsilon_{31} \\ Y_{32} &= \mu - \tau_1 - \tau_2 + \varepsilon_{33} \\ Y_{33} &= \mu - \tau_1 - \tau_2 + \varepsilon_{33} \\ Y_{34} &= \mu - \tau_1 - \tau_2 + \varepsilon_{34} \end{aligned}$$

In matrix notation

- Is the model for two-factor block design a liner model?
- Consider the statistical model (both effects are fixed):

$$Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}, j = 1, 2, \dots, b, i = 1, 2, \dots, a,$$

with $\sum_i \tau_i = 0$ and $\sum_i \beta_i = 0$

• For simplicity, consider a = 3 and b = 4:

$$Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}, j = 1, 2, 3, 4, i = 1, 2, 3,$$

with $\tau_1 + \tau_2 + \tau_3 = 0$ and $\beta_1 + \beta_2 + \beta_3 + \beta_4 = 0$.

• Again, there are only six parameters: $\mu, \tau_1, \tau_2, \beta_1, \beta_2$ and β_3 .

$$\begin{aligned} Y_{11} &= \mu + \tau_1 + \beta_1 + \varepsilon_{11} \\ Y_{12} &= \mu + \tau_1 + \beta_2 + \varepsilon_{12} \\ Y_{13} &= \mu + \tau_1 + \beta_3 + \varepsilon_{13} \\ Y_{14} &= \mu + \tau_1 - (\beta_1 + \beta_2 + \beta_3) + \varepsilon_{14} \\ Y_{21} &= \mu + \tau_2 + \beta_1 + \varepsilon_{21} \\ Y_{22} &= \mu + \tau_2 + \beta_2 + \varepsilon_{22} \\ Y_{23} &= \mu + \tau_2 + \beta_3 + \varepsilon_{23} \\ Y_{24} &= \mu + \tau_2 - (\beta_1 + \beta_2 + \beta_3) + \varepsilon_{24} \\ Y_{31} &= \mu - (\tau_1 + \tau_2) + \beta_1 + \varepsilon_{31} \\ Y_{32} &= \mu - (\tau_1 + \tau_2) + \beta_2 + \varepsilon_{32} \\ Y_{33} &= \mu - (\tau_1 + \tau_2) + \beta_3 + \varepsilon_{33} \\ Y_{34} &= \mu - (\tau_1 + \tau_2) - (\beta_1 + \beta_2 + \beta_3) + \varepsilon_{34} \end{aligned}$$

In matrix notation,

$$\begin{bmatrix} Y_{11} \\ Y_{12} \\ Y_{13} \\ Y_{14} \\ Y_{21} \\ Y_{22} \\ Y_{23} \\ Y_{24} \\ Y_{31} \\ Y_{32} \\ Y_{33} \\ Y_{34} \end{bmatrix} = \begin{bmatrix} 1 & 1 & 0 & 1 & 0 & 0 \\ 1 & 1 & 0 & 0 & 1 & 0 \\ 1 & 1 & 0 & 0 & 0 & 1 \\ 1 & 1 & 0 & -1 & -1 & -1 \\ 1 & 0 & 1 & 1 & 0 & 0 \\ 1 & 0 & 1 & 0 & 1 & 0 \\ 1 & 0 & 1 & -1 & -1 & -1 \\ 1 & 0 & 1 & -1 & -1 & -1 \\ 1 & -1 & -1 & 1 & 0 & 0 \\ 1 & -1 & -1 & 0 & 1 & 0 \\ 1 & -1 & -1 & 0 & 0 & 1 \\ 1 & -1 & -1 & -1 & -1 & -1 \end{bmatrix} \begin{bmatrix} \mu \\ \tau_1 \\ \tau_2 \\ \beta_1 \\ \beta_2 \\ \beta_3 \end{bmatrix} + \begin{bmatrix} \varepsilon_{11} \\ \varepsilon_{12} \\ \varepsilon_{23} \\ \varepsilon_{24} \\ \varepsilon_{31} \\ \varepsilon_{32} \\ \varepsilon_{33} \\ \varepsilon_{34} \end{bmatrix}$$

Linear Mixed Models

- **Linear Mixed Models** are models which are linear in their parameters and incorporate both fixed and random effects.
 - Response is numerical
 - ► Fixed effects can be numerical or categorical factors
 - Random effects are categorical factors with factor levels drawn from a larger population of factor levels.
- The matrix notation for a mixed model is highly similar to that for a fixed effects(systematic) model.
- In matrix notation,a linear mixed model can be represented as

$$\mathbf{Y} = \mathbf{X}\beta + \mathbf{Z}b + \boldsymbol{\varepsilon}$$

- Systematic part $X\beta$: β are the parameters for the fixed effects
- Random part Zb: b is the random effects and Z is the design matrix for the random effects
- ightharpoonup arepsilon is the experimental random error vector
- ▶ In general, **b** and ε are assumed to be independent normal vectors.

Linear Mixed Models

• Consider the mixed model for the randomized block design (treatment effects fixed, block effects random). For simplicity, consider a=3 and b=4 again.

$$Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}, j = 1, 2, 3, 4, i = 1, 2, 3,$$

with $\tau_1 + \tau_2 + \tau_3 = 0$ and $\beta_i \sim N(0, \sigma_b^2), j = 1, 2, 3, 4$.

In matrix notation

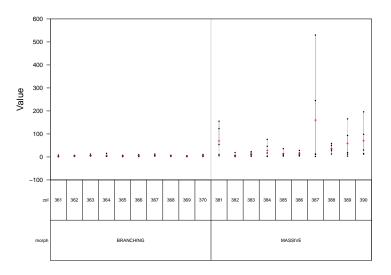
- Estimation Methods
 - Method of Moments
 - Maximum likelihood(ML)
 - Restricted maximum likelihood(REML)
- Maximum likelihood (ML) is obtained from the likelihood functions for the distribution of $\mathbf{Y}|\mathbf{b}$ and \mathbf{b}
- Restricted maximum likelihood(REML): REML is based on the maximum likelihood method but, instead of maximizing the likelihood function of the original data, it maximizes the likelihood function of the residuals obtained by removing the fixed effects from the original response variables, which are assumed to be known.

- In certain multifactor experiments, the levels of one factor (e.g., factor B) are similar but not identical for different levels of another factor (e.g., A). Such an arrangement is called a **nested**, **or hierarchical**, design, with the levels of factor B nested under the levels of factor A.
- Example (lesions.csv): Lozada-Misa et al. (2015) compared two different morphology types of the coral genus Porites for lesions caused by white syndrome disease. They collected ten random colonies of the branching P. cylindrica and ten colonies of the massive Porites spp., and five random lesions per colony were photographed and measured.
 - Morphology type was a fixed factor,
 - with colony a random factor nested within morphology type, and
 - size of individual lesions as the observations (responses).

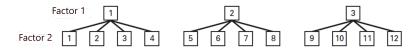
```
##
          morph col size
## 1
      BRANCHING 361 1.8912
      BRANCHING 361 8.2259
##
## 3
      BRANCHING 361 3.0047
## 4
      BRANCHING 361 2.0495
## 5
      BRANCHING 361 0.5167
      BRANCHING 362 6.8058
## 6
## 7
      BRANCHING 362 5.6315
      BRANCHING 362 2 7840
```

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Colony nested within morphology type



 This is a two-stage nested design, with Colony nested under morphology types.



- In the example, the ten random colonies under BRANCHING have no connection with the ten random colonies under MASSIVE.
- The number of stages can be more than two.

- There could be three possible models
 - Random effects model
 - Fixed effects model
 - Mixed Effects Models
 - * Treatment effects fixed and nested factor random
- Random effects model:

$$Y_{ijk} = \mu + \tau_i + \beta_{j(i)} + \varepsilon_{(ij)k}, k = 1, 2, \dots, n, j = 1, 2, \dots, b, i = 1, 2, \dots, a,$$

- where the j(i) indicates that the factor corresponding to j (factor B) is nested in the factor corresponding to i (factor A),
- ▶ The random errors $\varepsilon_{(ij)k}$ iid $\sim N(0, \sigma^2)$ are nested in factor A-B combinations.
- $ightharpoonup au_i \ iid \sim N(0, \sigma_{\tau}^2)$
- \triangleright $\beta_{j(i)}$ iid $\sim N(0, \sigma_{\beta}^2)$

ANOVA Table for the Random effects model

Analysis of Variance Table for the Two-Stage Nested Design

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square
A	$bn\sum_{i}(\overline{y}_{i}-\overline{y}_{})^{2}$	a - 1	MS_A
B within A	$n\sum\sum(\overline{y}_{ij.}-\overline{y}_{i})^2$	a(b-1)	$MS_{B(A)}$
Error	$\sum \sum \sum (y_{ijk} - \bar{y}_{ij.})^2$	ab(n-1)	MS_E
Total	$\sum \sum \sum (y_{ijk} - \overline{y}_{})^2$	abn-1	

•
$$E(MS_A) = \sigma^2 + bn\sigma_{\tau}^2 + n\sigma_{\beta}^2$$

•
$$E(MS_{B(A)}) = \sigma^2 + n\sigma_\beta^2$$

•
$$E(MS_E) = \sigma^2$$

Fixed effects model:

$$Y_{ijk} = \mu + \tau_i + \beta_{j(i)} + \varepsilon_{(ij)k}, k = 1, 2, \dots, n, j = 1, 2, \dots, b, i = 1, 2, \dots, a,$$

- where the j(i) indicates that the factor corresponding to j (factor B) is nested in the factor corresponding to i (factor A),
- ▶ The random errors $\varepsilon_{(ij)k}$ iid $\sim N(0, \sigma^2)$ are nested in factor A-B combinations.
- $\sum_{i} \tau_{i} = 0$
- $\sum_{i}^{n} \beta_{j(i)} = 0$ for all $i = 1, 2, \ldots, a$

ANOVA Table for the Fixed effects model

Analysis of Variance Table for the Two-Stage Nested Design

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square
A	$bn\sum (\overline{y}_{i}-\overline{y}_{})^2$	a - 1	MS_A
B within A	$n\sum\sum(\overline{y}_{ij.}-\overline{y}_{i})^2$	a(b-1)	$MS_{B(A)}$
Error	$\sum \sum \sum (y_{ijk} - \overline{y}_{ij.})^2$	ab(n-1)	MS_E
Total	$\sum \sum \sum (y_{ijk} - \overline{y}_{})^2$	abn-1	

•
$$E(MS_A) = \sigma^2 + bn \frac{\sum_i \tau_i^2}{a-1}$$

•
$$E(MS_A) = \sigma^2 + bn \frac{\sum_i \tau_i^2}{a - 1}$$

• $E(MS_{B(A)}) = \sigma^2 + n \frac{\sum_i \sum_j \beta_{j(i)}}{a(b - 1)}$

•
$$E(MS_E) = \sigma^2$$

- In practice we often encounter models which contain both random and fixed effects. We call them mixed effects models.
- Mixed Effects Model:

$$Y_{ijk} = \mu + \tau_i + \beta_{j(i)} + \varepsilon_{(ij)k}, k = 1, 2, \dots, n, j = 1, 2, \dots, b, i = 1, 2, \dots, a,$$

- where the j(i) indicates that the factor corresponding to j (factor B) is nested in the factor corresponding to i (factor A),
- ▶ The random errors $\varepsilon_{(ij)k}$ iid $\sim N(0, \sigma^2)$ are nested in factor A-B combinations.
- $ightharpoonup au_i$ is fixed: $\sum_{i=1}^a au_i = 0$.
- $\beta_{j(i)}$ iid $\sim N(0, \sigma_{\beta}^2)$

ANOVA Table for the Mixed effects model

Analysis of Variance Table for the Two-Stage Nested Design

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square
A	$bn\sum (\overline{y}_{i}-\overline{y}_{})^2$	a - 1	MS_A
B within A	$n\sum\sum(\overline{y}_{ij.}-\overline{y}_{i})^2$	a(b-1)	$MS_{B(A)}$
Error	$\sum \sum \sum (y_{ijk} - \overline{y}_{ij.})^2$	ab(n-1)	MS_E
Total	$\sum \sum \sum (y_{ijk} - \overline{y}_{})^2$	abn-1	

•
$$E(MS_A) = \sigma^2 + bn \frac{\sum_i \tau_i^2}{a-1} + n\sigma_\beta^2$$

• $E(MS_{B(A)}) = \sigma^2 + n\sigma_\beta^2$

- $E(MS_F) = \sigma^2$

• The first import hypothesis testing problem in nested designs is to test

 H_0 : The levels of B have the same effect on the response within every given level. Under this null hypothesis, the model is reduced to

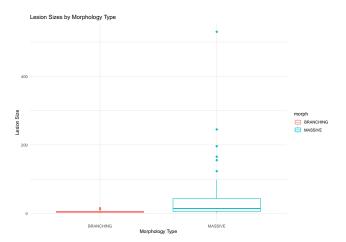
$$Y_{ijk} = \mu + \tau_i + \varepsilon_{ijk}, k = 1, 2, \dots, n, j = 1, 2, \dots, b, i = 1, 2, \dots, a,$$

• Another important hypothesis testing problem is to test if the *a* levels of A (main factor; fixed effects) averaged over the levels of B have a same effect. That is, we check if the main effects are equal.

• Calculate summary stats by groups

			,			,		
##		col	morph		size	sd	se	ci
##	1	361	BRANCHING	5	3.13760	2.979725	1.3325737	3.699818
##	2	362	BRANCHING	5	4.41892	1.768847	0.7910522	2.196313
##	3	363	${\tt BRANCHING}$	5	6.39866	3.451261	1.5434508	4.285306
##	4	364	BRANCHING	5	5.77804	5.594773	2.5020583	6.946828
##	5	365	BRANCHING	5	4.06686	2.398199	1.0725071	2.977757
##	6	366	${\tt BRANCHING}$	5	5.01400	3.148717	1.4081489	3.909648
##	7	367	BRANCHING	5	7.37804	4.299565	1.9228237	5.338614
##	8	368	${\tt BRANCHING}$	5	4.82584	2.134307	0.9544910	2.650092
##	9	369	BRANCHING	5	3.59112	1.931891	0.8639679	2.398759
##	10	370	BRANCHING	5	5.40918	3.791108	1.6954351	4.707282
##	11	381	MASSIVE	5	69.53184	67.443147	30.1614921	83.741727
##	12	382	MASSIVE	5	7.01802	7.054432	3.1548380	8.759234
##	13	383	MASSIVE	5	11.10566	8.543465	3.8207537	10.608113
##	14	384	MASSIVE	5	29.10582	31.698167	14.1758511	39.358472
##	15	385	MASSIVE	5	13.52838	13.171769	5.8905943	16.354912
##	16	386	MASSIVE	5	13.28264	9.758733	4.3642380	12.117067
##	17	387	MASSIVE	5	160.17892	230.708834	103.1761270	286.462853
##	18	388	MASSIVE	5	36.23336	18.281438	8.1757075	22.699403
##	19	389	MASSIVE	5	58.51856	69.802447	31.2166031	86.671185
##	20	390	MASSIVE	5	70.19178	78.626406	35.1627978	97.627578

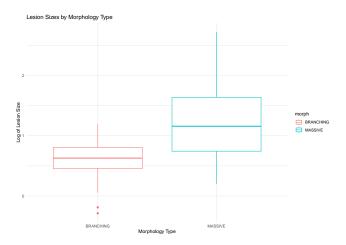
• Compare the main effect means



Consider log10 transformation of the response variable size

```
##
          morph col size
                              logsize
## 1
     BRANCHING 361 1.8912
                            0.2767375
     BRANCHING 361 8.2259 0.9151834
##
     BRANCHING 361 3.0047
                            0.4778011
##
                            0.3116479
## 4
     BRANCHING 361 2.0495
##
  5
     BRANCHING 361 0.5167 -0.2867615
## 6
     BRANCHING 362 6.8058
                            0.8328792
     BRANCHING 362 5.6315
                            0.7506241
##
  7
     BRANCHING 362 2.7840
                            0.4446692
##
  8
     BRANCHING 362 4.0470
                            0.6071332
##
  10 BRANCHING 362 2.8263
                            0.4512183
```

• Check the box plots again



ANOVA table

```
## # A tibble: 3 x 7
##
    stratum term
                     df sumsq meansq statistic p.value
   <chr> <chr> <dbl> <dbl> <dbl>
                                     <dbl>
                                              <dbl>
##
## 1 col
          morph
                      1 9.82 9.82
                                      32.3 0.0000217
## 2 col Residuals
                     18 5.47 0.304
                                      NA
                                          NΑ
## 3 Within Residuals
                     80 17.1 0.214
                                      NΑ
                                          NΑ
```

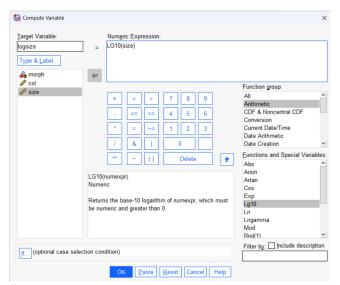
REML-estimation with hypothesis testing

```
## Linear mixed model fit by REML. t-tests use Kenward-Roger's method [
## lmerModLmerTest]
## Formula: logsize ~ morph + (1 | col)
     Data: lesions
##
##
## REML criterion at convergence: 141
##
## Scaled residuals:
       Min 10 Median
##
                                30
                                       Max
## -2.23033 -0.54021 -0.00755 0.49227 3.04868
##
## Random effects:
## Groups Name
                 Variance Std.Dev.
## col (Intercept) 0.01809 0.1345
## Residual
                      0.21368 0.4623
## Number of obs: 100, groups: col, 20
##
## Fixed effects:
              Estimate Std. Error df t value Pr(>|t|)
##
## (Intercept) 0.60557 0.07799 18.00000 7.765 3.74e-07 ***
## morphMASSIVE 0.62674 0.11029 18.00000 5.683 2.17e-05 ***
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

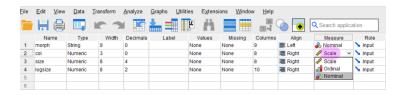
• Variance component for random-effects

```
##
##
## Result Variance Component Analysis:
##
    Name DF
                                              %Total SD
                                                               CV [%]
##
                     SS
                              MS
                                      VC
## 1 total 4.555803
                                      0.422086 100
                                                       0.649681 70.699125
## 2 morph 1
                9.82009 9.82009
                                      0.19032 45.090258 0.436256 47.473953
## 3 morph:col 18 5.473944 0.304108 0.018085 4.284774 0.134482 14.634504
                   17.094469 0.213681 0.213681 50.624968 0.462256 50.303293
## 4 error
             80
##
## Mean: 0.918938 (N = 100)
##
## Experimental Design: balanced | Method: ANOVA
```

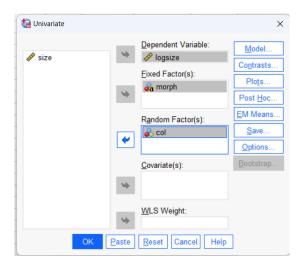
• After importing the data lesions.csv, add a new variable logsize which is log10 transformation of size.

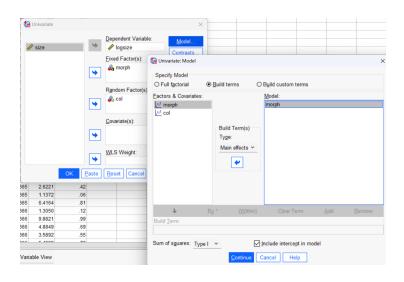


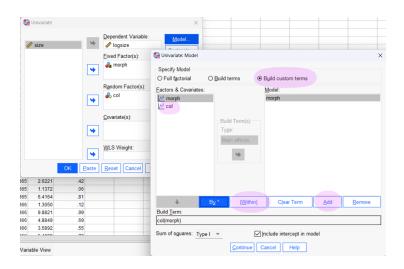
• Convert measure of col to Nominal

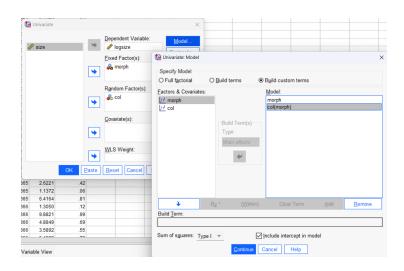


ullet Click on Analyze o General Linear Model o Univariate...

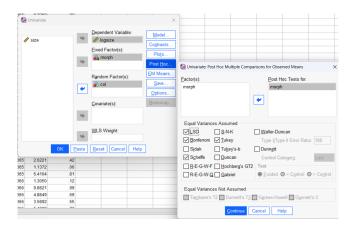




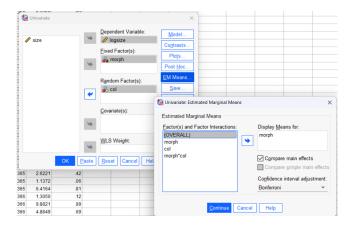




- Set up for multiple comparisons. It is not useful here.
 - It will be useful if there are more than two levels.



• Compare the means of the fixed effects



ANOVA table

Tests of Between-Subjects Effects

Dependent Variable: logsize

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	84.445	1	84.445	277.680	<.001
	Error	5.474	18	.304ª		
morph	Hypothesis	9.820	1	9.820	32.291	<.001
	Error	5.474	18	.304*		
col(morph)	Hypothesis Error	5.474 17.094	13 80	.304 .214 ^b	1.423	.144

a. MS(col(morph))

b. MS(Error)

Estimated Marginal Means

morph

Estimates

Dependent Variable: logsize

			95% Confidence Interval		
morph	Mean	Std. Error	Lower Bound	Upper Bound	
BRANCHING	.606*	.065	.475	.736	
MASSIVE	1.232ª	.065	1.102	1.362	

a. Based on modified population marginal mean.

Pairwise Comparisons

Dependent Variable: logsize

		Mean Difference			95 % Confidence Interval for Difference ^d		
(I) morph	(J) morph	(I-J)	Std. Error	Sig.d	Lower Bound	Upper Bound	
BRANCHING	MASSIVE	627*,b,c	.092	<.001	811	443	
MASSIVE	BRANCHING	.627 ^{*,b,c}	.092	<.001	.443	.811	

Based on estimated marginal means

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