GLMM workshop March 2021 Instructors: David Schneider, Victor Valdez, with assistance of Taurai Matengu

First session Writing the model Break Second session F-ratios from Expected Mean Squares Break Third session Executing the analyses in R

Goal of the first session – Writing Statistical ModelsGLMThe General Linear ModelGzLMThe Generalized Linear modelFixed Effects + Normal ErrorsGLMMThe General Linear Mixed ModelFixed + Random + NormalGzLMMThe Generalized Linear Mixed ModelFixed + Random + Normal

Goal of the second session - Writing out the expected mean squares Forming unambiguous likelihood ratio tests (*F*, *t*, χ^2)

Goal of the third session - Executing a GLMM in a statistical package Interpreting the output Second session F-ratios from Expected Mean Squares

Review of fixed effect, random effect, and mixed models.

Write the model and construct the ANOVA table.

Step 1. Assign symbols to the response variable and explanatory variables and write the model (Session 1)

Step 2. Identify each explanatory variable as Factor or Covarate (Session 1)

Step 3. For explanatory variable and each interaction term, identify as Random or Fixed. (Session 1)

Step 4. For explanatory variable and each interaction term, identify as Crossed or Nested. (Session 1)

Step 5. Rewrite the model as an ANOVA table, showing results of Steps 2, 3, and 4.

Here is an example. We have data on response of plants to CO_2 at three levels (none, medium, high). The response variable is plant size at 5 successive times to produce an estimate of growth rate. The model term of interest is $Trt \times Time$ - does growth rate depend on Trt? The experiment is repeated at 6 locations taken to be representative of a larger area. The full model has three main effects, three pairwise interaction terms, and a three way interaction.

Note that $Fixed \times Fixed = Fixed$

Fixed \times Random = Mixed Random \times Random = Random

Time is a covariate – we expect monotonic response.

*In this example TIME was not measured at the same intervals in each Block, so TIME×Block fails the cross test.

TIME×Block cannot be estimated. However, this mixed term is still there, lurking. We cannot assume it is small, with little effect. To cancel out its effect we need to keep track of it.

To do this we use the "random within" rule.

| Source | df | Factor or | Random | Crossed |
|-------------|-----------------------|-----------|----------|---------|
| | | Covariate | or Fixed | or |
| | | | | Nested |
| Trt | 3-1 | Factor | Fixed | NA |
| TIME (n=5) | 1 | Cov | Fixed | NA |
| Block | 6-1 | Factor | Random | NA |
| Trt×TIME | 2×1 | | Fixed | Crossed |
| Trt×Block | 2×5 | | Mixed | Crossed |
| TIME×Block | 1×5 | | Mixed | Nested* |
| Trt×Time×Bl | $2 \times 1 \times 5$ | | Mixed | Nested |
| Model | 35 | | | |
| Residual | 89-35 | | | |
| Total | (3×5×6)-1 | | | |

TIME×Block: Block(Time) is random within. Time(Block) is not random within. We assign TIME×Block to Block(Time)

Second session F-ratios from Expected Mean Squares

Write the model and construct the ANOVA table.

Step 1. Assign symbols to the response variable and explanatory variables and write the model (Session 1)

Step 2. Identify each explanatory variable as Factor or Covarate (Session 1)

Step 3. For explanatory variable and each interactive effect term, identify as Random or Fixed. (Session 1)

Step 4. For explanatory variable and each interactive effect term, identify as Crossed or Nested. (Session 1)

Step 5. Rewrite the model as an ANOVA table, showing results of Steps 2, 3, and 4.

<u>Step 6</u>. Revise ANOVA table for nested terms.

| Source | df |
|-------------|-----------------------|
| Trt | 3-1 |
| TIME (n=5) | 1 |
| Block | 6-1 |
| Trt×TIME | 2×1 |
| Trt×Block | 2×5 |
| TIME×Block | 1×5 |
| Trt×Time×Bl | $2 \times 1 \times 5$ |
| Model | 35 |
| Residual | 89-35 |
| Total | (3×5×6)-1 |

| Source | df |
|-------------|-----------------------|
| Trt | 3-1 |
| TIME (n=5) | 1 |
| TIME×Block | 1×5 |
| Block | 6-1 |
| Trt×TIME | 2×1 |
| Trt×Block | 2×5 |
| Trt×Time×Bl | $2 \times 1 \times 5$ |
| Model | 35 |
| Residual | 89-35 |
| Total | (3×5×6)-1 |
| | |

| Source | df |
|-------------|-----------------------|
| Trt | 3-1 |
| TIME (n=5) | 1 |
| Block(Time) | 1×5+6-1 |
| Trt×TIME | 2×1 |
| Trt×Block | 2×5 |
| Trt×Time×Bl | $2 \times 1 \times 5$ |
| Model | 35 |
| Residual | 89-35 |
| Total | (3×5×6)-1 |
| | |
| | |

12. Fisher (1925 *Statistical Methods for Research Workers*) introduced the latin square design, which controls for two sources of random variation, rows and columns in a square array in field experiment.

"The following root weights for mangolds were found by Mercer and Hall in 25 plots... Then out of the 24 degrees of freedom.... 12 will remain for the estimation of error. The 12 will provide an unbiased estimate of the errors in the comparison of treatments, provided that every pair of plots, not in the same row or column, belong equally frequently to the same treatment.." Here is the ANOVA table as reported by Fisher (p267 in 13th ed, 1958, Table 61)

Note that all three factors can be crossed but there are insufficient df to estimate any of the interaction terms.

Complete Steps 1-6 for Fisher's three factor table. For step 5 show zero df for all interactive effect terms.

| | df | SS | MS | sd |
|-----------|----|---------|--------|-------|
| Columns | 4 | 701.84 | | |
| Rows | 4 | 4240.24 | _ | |
| Trt | 4 | 330.24 | 130.29 | 11.41 |
| Remainder | 12 | 1754.32 | | |
| Total | 24 | 7026.64 | 292.78 | 17.11 |

Writing out the expected mean squares.

Once the model is written and its components identified we can write out the expected means squares. From there we can write the correct (unambiguous) F-ratios.

With fixed effect models we do not need to do this because we always use the residual MS to form F-ratios.

With mixed models we need to write out expected mean squares to form the correct F-ratio, because the residual is **NOT** always the error term that isolates the each term from all others.

To see why the residual MS is not always the correct F-ratio denominator, we will write out the expected mean squares for Example 11, Mosquito Winglengths.

In this example there are 3 factors, all are random, and all are nested within another term. That is, all three pairs of terms fail the cross test.

Writing out the expected mean squares to form F-ratios - Random Effects

List the terms in the model, as in the ANOVA table List the same term horizontally. In each row, show the row term.

Cage Fly%in% Cage Error Each EMS includes itself EMS Cage Cage EMS Fly%in% Cage Fly%in% Cage EMS Error Error Cage Fly%in% Cage Error Each EMS includes the EMS Cage Error Cage fixed error term EMS Fly%in% Cage Fly%in% Cage Error EMS Error Error Fly%in% Cage Error Cage Each EMS includes crossed EMS Cage Fly%in% Cage Error (or nested) random terms Cage EMS Fly%in% Cage Fly%in% Cage Error EMS Error Error Correct Fly%in% Cage Error Denominator MS Identify the denominator MS Cage EMS Cage Fly%in% Cage Error Fly%in% Cage for the F-ratio Cade EMS Fly%in% Cage Fly%in% Cage Error Error The denominator MS cancels all but EMS Error Error the term of interest Incorrect Cage Fly%in% Cage **Denominator MS** Error EMS Cage Cage Fly%in% Cage Cage MS / Error results in Error Fly%in% Cage *2* uncancelled terms. EMS Error The F-test is ambiguous Error Error

Writing out the expected mean squares to form F-ratios - Mixed Effects

List the terms in the model, as in the ANOVA table (Example 14 Sleep data) List the same term horizontally

| | | Subj | Drug | Subj×Drug | Error |
|-----|-----------|------|------|-----------|-------|
| EMS | Subject | Subj | | | |
| EMS | Drug | | Drug | | |
| EMS | Subj×Drug | | | Subj×Drug | |
| EMS | Error | | | | Error |

| | | Subj | Drug | Subj×Drug | Error |
|-----|-----------|------|------|-----------|-------|
| EMS | Subject | Subj | | | Error |
| EMS | Drug | | Drug | | Error |
| EMS | Subj×Drug | | | Subj×Drug | Error |
| EMS | Error | | | | Error |

| | | Subj | Drug | Subj×Drug | Error |
|-----|-----------|------|------|------------|-------|
| EMS | Subject | Subj | | Drug(Subj) | Error |
| EMS | Drug | | Drug | Subj(Drug) | Error |
| EMS | Subj×Drug | | | Subj×Drug | Error |
| EMS | Error | | | | Error |

| | | Subj | Drug | Subj×Drug | Error |
|-----|-----------|------|------|------------|-------|
| EMS | Subject | Subj | | | Error |
| EMS | Drug | | Drug | Subj(Drug) | Error |
| EMS | Subj×Drug | | | Subj×Drug | Error |
| EMS | Error | | | | Error |

| | | Subj | Drug | Subj×Drug | Error |
|-----|-----------|------|------|------------|-------|
| EMS | Subject | Subj | | | Error |
| EMS | Drug | | Drug | Subj(Drug) | Error |
| EMS | Subj×Drug | | | Subj×Drug | Error |
| EMS | Error | | | | Error |

Each EMS includes itself

Each EMS includes the constant error term

For each mixed term, display as if nested Display Drug within Subj for EMS Subj Display Subj within Drug for EMS Drug

Drop fixed terms, Retain random terms Drug is fixed, Drug(Subj) is dropped, Subj is random, Subj(Drug) is retained,

| Identify denominator MS that isolates a single term |
|---|
|---|

| Error | Isolates Subj : F = 1 if Subj = 0 |
|--------------------|-----------------------------------|
| Subj(Drug) + Error | Isolates Drug : F = 1 if Drug = 0 |
| Error | |

Writing out the expected mean squares to form F-ratios - Mixed Effects

Gossett data has only one measurement per subject with each drug There are too few df to estimate both the error term and the Drug×Subject term. The residual term includes the error and the mixed term . Residual = Subj×Drug + Error We test over the residual term.

| | | Subj | Drug | Subj×Drug | Residual | Denominator MS |
|-----|----------|------|------|------------|----------|-------------------------------|
| EMS | Subject | Subj | | | Residual | (no test) |
| EMS | Drug | | Drug | Subj(Drug) | Residual | Residual = Subj(Drug) + Error |
| EMS | Residual | | | | Residual | |