Mixed Models Part 1 - A Critical Tool When You Have More Than One Source of Variation

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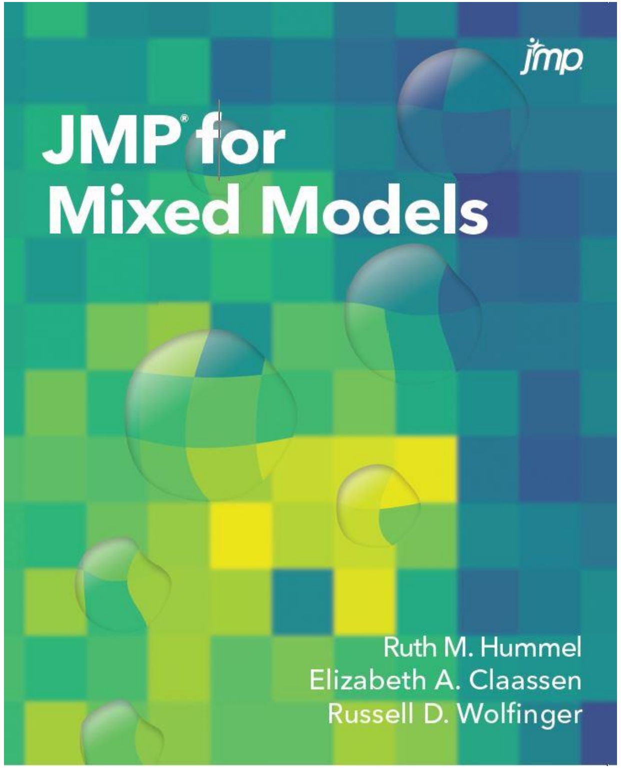
JMP for Mixed Models

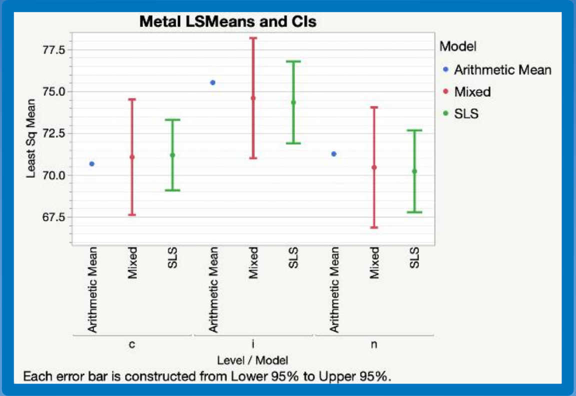
- Example driven
  - Focus on concepts rather than formulas
  - Get modelers modeling!
- Data available
  - JMP Journal organized by chapter
  - support.sas.com/hummel
  - support.sas.com/claassen
  - support.sas.com/wolfinger



Overview

- Introduction to the various concepts in mixed modeling
  - Fixed vs Random Effects
  - Correlation between experimental units
  - Different size experimental units for different effects
- Teach through example
  - Formulas only when necessary
  - Focus on conceptual understanding
- JMP and JMP Pro
  - A lot of mixed models can be fit in JMP
  - But some more complicated models require JMP Pro

Why use Mixed Models?



What we will cover:

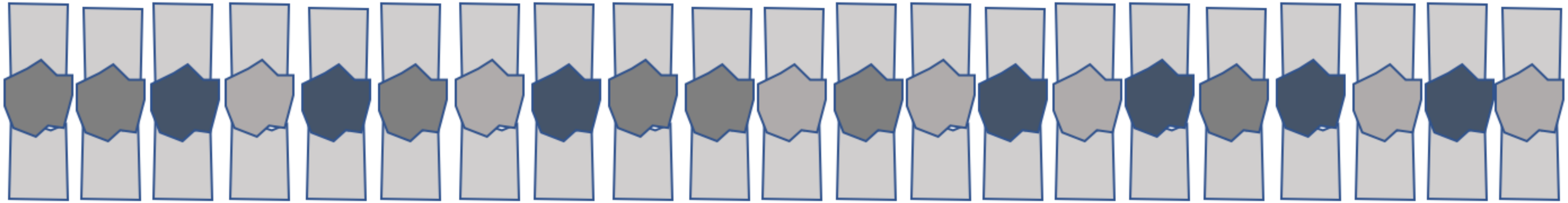
- What is a **Random Effect**?
- **Mixed Models →** possibly different results for estimates, CIs, and pairwise comparisons
- **REML** – Use it! Especially when you have missing data or unequal group sizes
- **LSMeans** – better than arithmetic means. ;)

Explore "Bond" data: No Blocks

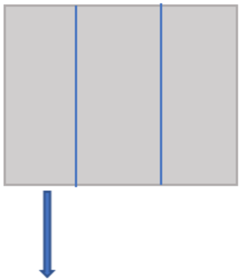
Prepare the experimental unit and assign the Treatment Level (i.e., which metal?)

Metal = Copper

Conduct the experiment: test the breaking strength of the metal bond.



Explore "Bond" data: With Blocks (Fixed? Or Random?)

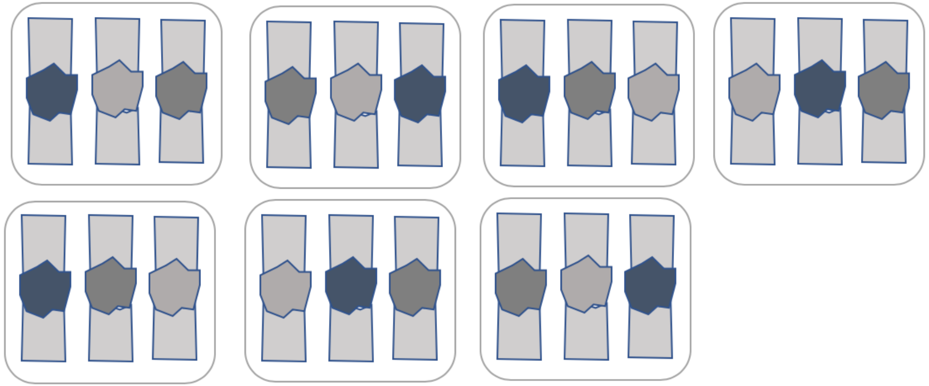


Divide each ingot into three experimental units

Prepare the experimental unit and assign the Treatment Level (i.e., which metal?)

Metal = Copper

Conduct the experiment: test the breaking strength of the metal bond.



Definitions

- Fixed Effect
  - When you care about exactly those levels, and you want to make inference for a future observation from exactly those levels

- Random Effect
  - when the levels are a sample from a larger population, and you want to make inference to the larger population and not just to those specific levels, and/or
  - when you are primarily interested in explaining the variability coming from that effect rather than, say, pairwise comparisons

Do I care about only those seven ingots? Or do I want to understand which bonding metal will work best on a future ingot?

Determining a 'Sensible' Model

Repurposing the ANOVA table

- ANOVA tables originally designed to aid construction of F-tests
  - Degrees of freedom
  - Expected Mean Squares
  - F-ratios
- Modern software computes these statistics automatically
- Repurpose the ANOVA table to describe a 'sensible' model
  - Sources of Variation
    - Experiment Design
    - Treatment Design
  - One-to-one match of ANOVA effects and model parameters

Skeleton ANOVA

Experiment Design		Treatment Design		Skeleton ANOVA	
Source	df	Source	df	Source	df
Total				Total	

Skeleton ANOVA

Experiment Design		Treatment Design		Skeleton ANOVA	
Source	df	Source	df	Source	df
Ingot	7-1=6				
Total				Total	

Skeleton ANOVA

Experiment Design		Treatment Design		Skeleton ANOVA	
Source	df	Source	df	Source	df
Ingot	7-1=6				
Ingot-third	(3-1)*7=14				
Total				Total	

Skeleton ANOVA

Experiment Design		Treatment Design		Skeleton ANOVA	
Source	df	Source	df	Source	df
Ingot	7-1=6				
Ingot-third	(3-1)*7=14				
Total	21-1=20			Total	

Skeleton ANOVA

Experiment Design		Treatment Design		Skeleton ANOVA	
Source	df	Source	df	Source	df
Ingot	7-1=6				
		Metal	3-1=2		
Ingot-third	(3-1)*7=14				
Total	21-1=20			Total	

Skeleton ANOVA

Experiment Design		Treatment Design		Skeleton ANOVA	
Source	df	Source	df	Source	df
Ingot	7-1=6			Ingot	6
		Metal	3-1=2		
Ingot-third	(3-1)*7=14				
Total	21-1=20			Total	

Skeleton ANOVA

Experiment Design		Treatment Design		Skeleton ANOVA	
Source	df	Source	df	Source	df
Ingot	7-1=6			Ingot	6
		Metal	3-1=2	Metal	2
Ingot-third	(3-1)*7=14			Ingot-third|Metal -> Residual	12
Total	21-1=20			Total	20

From ANOVA to Model

One-to-one ANOVA source & Model Parameter

Skeleton ANOVA	
Source	df
Ingot	6
Metal	2
Ingot-third|Metal -> Residual	12
Total	20

$y_{ij} = \mu + r_j + \alpha_i + e_{ij}$

y_{ij} is the observation of the i^{th} Metal in the j^{th} Ingot

μ is the intercept

r_j is the j^{th} Ingot effect and $\sim N(0,\sigma_r^2)$

α_i is the i^{th} Metal effect

e_{ij} is the residual error and $\sim N(0,\sigma^2)$

Bond with random Ingots

Fit Mixed

Fit Statistics

-2 Residual Log Likelihood	109.98743
-2 Log Likelihood	115.94455
AICc	129.94455
BIC	131.16716

Random Effects Covariance Parameter Estimates

Variance Component	Estimate	Std Error	95% Lower	95% Upper	Wald p-Value
ingot	11.447778	8.7203658	-5.643825	28.539381	0.1893
Residual	10.371587	4.2341828	5.3331979	28.261813	
Total	21.819365	9.056538	11.11697	60.718883	

Fixed Effects Parameter Estimates

Random Coefficients

Fixed Effects Tests

Source	Nparm	DFNum	DFDen	F Ratio	Prob > F
metal	2	2	12.0	6.358764	0.0131*

Bond with random Ingots

Fit Mixed

Fixed Effects Tests

Multiple Comparisons for metal

Least Squares Means Estimates

metal	Estimate	Std Error	DF	Lower 95%	Upper 95%
c	70.185714	1.7655175	11.609	66.324558	74.046870
i	75.900000	1.7655175	11.609	72.038844	79.761156
n	71.100000	1.7655175	11.609	67.238844	74.961156

Tukey HSD All Pairwise Comparisons

Quantile = 2.66776, Adjusted DF = 12.0, Adjustment = Tukey-Kramer

All Pairwise Differences

metal	-metal	Difference	Std Error	t Ratio	Prob>|t|	Lower 95%	Upper 95%
c	i	-5.71429	1.721427	-3.32	0.0156*	-10.3066	-1.12194
c	n	-0.91429	1.721427	-0.53	0.8578	-5.5066	3.67806
i	n	4.80000	1.721427	2.79	0.0404*	0.2077	9.39235

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Why bother using a mixed model? Why not just include the random effect like a fixed effect?

Metal and Ingot both fixed effects

Response pressure

Summary of Fit

AICc	BIC
138.964	127.4092

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	8	400.19048	50.0238	4.8232
Error	12	124.45905	10.3716	Prob > F
C. Total	20	524.64952		0.0076*

Parameter Estimates

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
metal	2	2	131.90095	6.3588	0.0131*
ingot	6	6	268.28952	4.3113	0.0151*

Bond with random Ingots

Fit Mixed

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-2 Residual Log Likelihood	109.98743
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Random Coefficients

Fixed Effects Tests

Source	Nparm	DFNum	DFDen	F Ratio	Prob > F
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Answer #1: Ingot is used in the model to explain variance.

Bond with random Ingots: adjustments to one-group CIs

Metal and Ingot both fixed effects

Response pressure

Multiple Comparisons for metal

Least Squares Means Estimates

metal	Estimate	Std Error	DF	Lower 95%	Upper 95%	Arithmetic Mean Estimate	N
c	70.185714	1.2172327	12	67.533592	72.837836	70.185714	7
i	75.900000	1.2172327	12	73.247878	78.552122	75.900000	7
n	71.100000	1.2172327	12	68.447878	73.752122	71.100000	7

Tukey HSD All Pairwise Comparisons

Quantile = 2.66776, Adjusted DF = 12.0, Adjustment = Tukey

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Answer #2: And that effects CIs for the fixed effects.

Bond with random Ingots: adjustments to one-group CIs

Metal and Ingot both fixed effects

Response pressure

Multiple Comparisons for metal

Least Squares Means Estimates

metal	Estimate	Std Error	DF	Lower 95%	Upper 95%	Arithmetic Mean Estimate	N
c	70.185714	1.2172327	12	67.533592	72.837836	70.185714	7
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n	71.100000	1.2172327	12	68.447878	73.752122	71.100000	7

Tukey HSD All Pairwise Comparisons

Quantile = 2.66776, Adjusted DF = 12.0, Adjustment = Tukey

All Pairwise Differences

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i	n	4.80000	1.721427	2.79	0.0404*	0.2077	9.39235

Fit Mixed

Fixed Effects Tests

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i	n	4.80000	1.721427	2.79	0.0404*	0.2077	9.39235

Answer #2: (But not the CIs for differences if the design is balanced).

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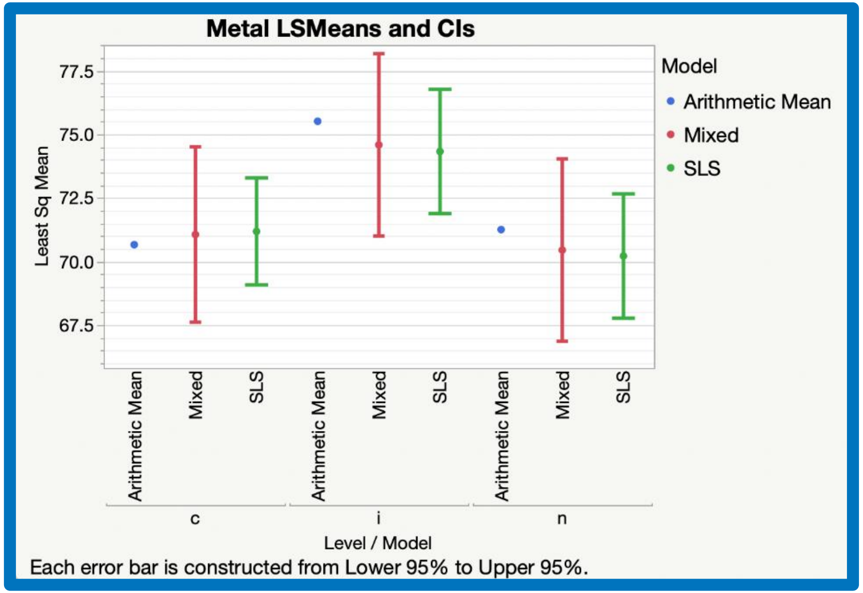
Summary/Tips

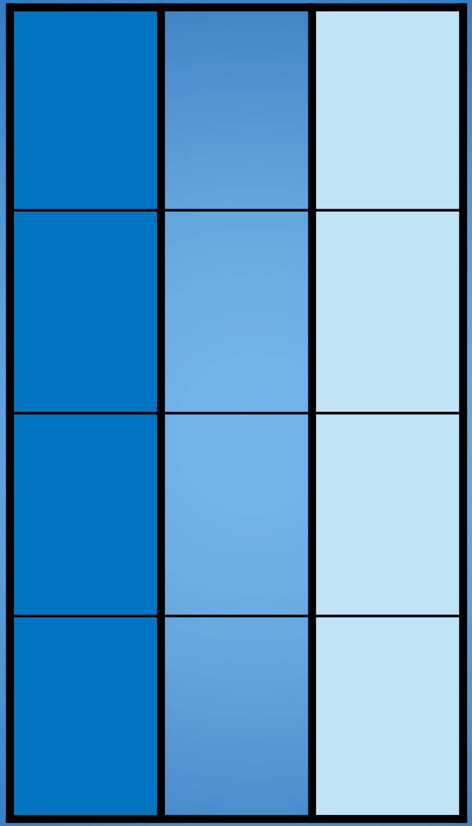
- You are dealing with Random Effects:
  - when the levels are a sample from a larger population, and/or
  - when you are primarily interested in explaining the variability rather than, say, pairwise comparisons.



- If you treat a random effect as fixed, your standard errors (and therefore your CIs on the other fixed effects) will be less appropriate. **"All models are wrong, but some are useful." "When you know better, do better."**

- LSMeans are not necessarily the arithmetic means. Missing data / imbalance needs REML to better estimate the LSMeans.
- With missing data / imbalance, the pairwise comparisons for the other fixed effects are also affected by the choice to model an effect as random.
- Don't rely only on the p-value – remember that p=0.049 and p=0.050 are basically the same thing.

Models with Factorial Designs



What we will cover:

- What is a Factorial Design and why would we use one?
- How is discussion of Factorials a Mixed Model topic?
- Beyond the split plot

Factorial Treatment Designs

- A factorial treatment design occurs when the experiment has two or more treatment factors of interest
- These designs are more efficient than one factor at a time experiments
  - Reduce the number of experiments and therefore experimental units required
  - Enables the measurement of *interactions* between the factors
  - Improved statistical properties for *main effects* and *simple effects*
- Factorial treatment designs are often used with split-plot experiment designs
  - Often one factor is harder to change treatment levels than the other
  - The hard-to-change factor is the *whole plot* factor
  - The easier-to-change factor is the *split plot* factor

Main Effects

When there's negligible interaction

- A main effect is the effect of a single factor averaging over any other factors in the model
- Because we have determined there is no interaction, it does not matter what level the other factor(s) are at. Thus, we can use the "average" level/effect.
- Use LSMeans comparisons of the factor to make decisions about optimal factor settings.

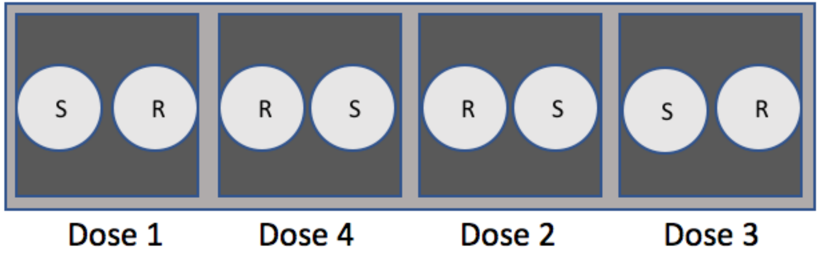
Simple effects

When an interaction is non-negligible

- A simple effect is the effect of one factor given a particular level of the other factor(s).
- An interaction by definition is when the simple effect *changes* depending on the level of the other factor.
- Look at *slices* of the interaction LSMeans to determine optimal factor settings.

Greenhouse Example

- A plant researcher has two plant varieties and a pesticide meant to protect the plants against disease
- The amount of pesticide can be applied to sections of greenhouse benches.
- Bench sections can hold multiple plants.
- From past experiments the researcher knows there is variability between benches within the greenhouse, so benches should be a blocking factor.



Skeleton ANOVA

Experiment Design		Treatment Design		Skeleton ANOVA	
Source	df	Source	df	Source	df
Block	5-1=4				
Total				Total	

Skeleton ANOVA

Experiment Design		Treatment Design		Skeleton ANOVA	
Source	df	Source	df	Source	df
Block	5-1=4				
WP(Block)	(4-1)*5=15				
Total				Total	

Skeleton ANOVA

Experiment Design		Treatment Design		Skeleton ANOVA	
Source	df	Source	df	Source	df
Block	5-1=4				
WP(Block)	(4-1)*5=15				
SP(WP)	(2-1)*20=20				
Total				Total	

Skeleton ANOVA

Experiment Design		Treatment Design		Skeleton ANOVA	
Source	df	Source	df	Source	df
Block	5-1=4				
WP(Block)	(4-1)*5=15				
SP(WP)	(2-1)*20=20				
Total	40-1=39			Total	

Skeleton ANOVA

Experiment Design		Treatment Design		Skeleton ANOVA	
Source	df	Source	df	Source	df
Block	5-1=4				
		Dose	4-1=3		
WP(Block)	(4-1)*5=15				
SP(WP)	(2-1)*20=20				
Total	40-1=39			Total	

Skeleton ANOVA

Experiment Design		Treatment Design		Skeleton ANOVA	
Source	df	Source	df	Source	df
Block	5-1=4				
		Dose	4-1=3		
WP(Block)	(4-1)*5=15				
		Type	2-1=1		
		Type*Dose	3		
SP(WP)	(2-1)*20=20				
Total	40-1=39			Total	

Skeleton ANOVA

Experiment Design		Treatment Design		Skeleton ANOVA	
Source	df	Source	df	Source	df
Block	5-1=4			Block	4
		Dose	4-1=3		
WP(Block)	(4-1)*5=15				
		Type	2-1=1		
		Type*Dose	3		
SP(WP)	(2-1)*20=20				
Total	40-1=39			Total	

Skeleton ANOVA

Experiment Design		Treatment Design		Skeleton ANOVA	
Source	df	Source	df	Source	df
Block	5-1=4			Block	4
		Dose	4-1=3	Dose	3
WP(Block)	(4-1)*5=15			WP(Block)|Dose -> Block*Dose	12
		Type	2-1=1		
		Type*Dose	3		
SP(WP)	(2-1)*20=20				
Total	40-1=39			Total	39

Skeleton ANOVA

Experiment Design		Treatment Design		Skeleton ANOVA	
Source	df	Source	df	Source	df
Block	5-1=4			Block	4
		Dose	4-1=3	Dose	3
WP(Block)	(4-1)*5=15			WP(Block)|Dose -> Block*Dose	12
		Type	2-1=1	Type	1
		Type*Dose	3	Type*Dose	3
SP(WP)	(2-1)*20=20			SP(WP)|Dose,Type -> Residual	16
Total	40-1=39			Total	39

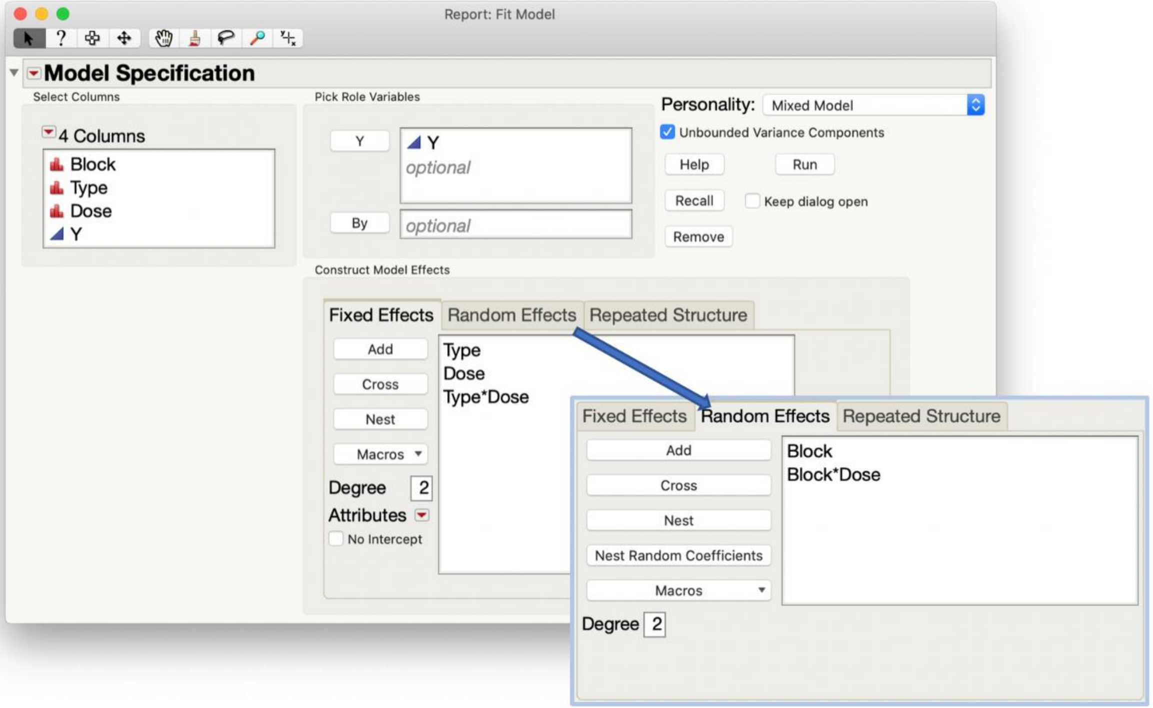
ANOVA to Model

Skeleton ANOVA	
Source	df
Block	4
Dose	3
WP(Block)|Dose -> Block*Dose	12
Type	1
Type*Dose	3
SP(WP)|Dose,Type -> Residual	16
Total	39

- $y_{ijk} = \mu + r_k + \delta_i + w_{ik} + \tau_j + \delta\tau_{ij} + e_{ijk}$
- y_{ijk} is the observation of the i^{th} Dose, j^{th} Type, and k^{th} block.
- μ is the intercept
- r_k is the k^{th} block effect and $\sim N(0,\sigma_r^2)$
- δ_i is the i^{th} Dose effect
- w_{ik} is the ik^{th} whole-plot (Block*Dose) effect and $\sim N(0,\sigma_w^2)$
- τ_j is the j^{th} Type effect
- $\delta\tau_{ij}$ is the ij^{th} Dose*Type interaction effect
- e_{ijk} is the split-plot, residual error and $\sim N(0,\sigma^2)$

Dialog boxes

$$y_{ijk} = \mu + r_k + \delta_i + w_{ik} + \tau_j + \delta\tau_{ij} + e_{ijk}$$

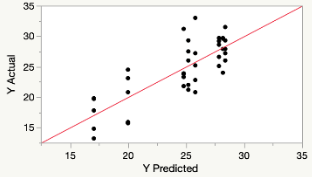


Results

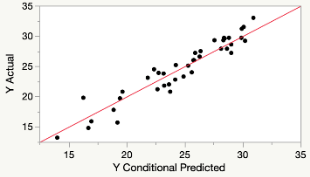
Variety-Pesticide Evaluation - Fit Mixed

Fit Mixed

Actual by Predicted Plot



Actual by Conditional Predicted Plot



Fit Statistics

-2 Residual Log Likelihood	182.87576
-2 Log Likelihood	190.63737
AICc	222.06595
BIC	231.21505

Random Effects Covariance Parameter Estimates

Variance Component	Estimate	Std Error	95% Lower	95% Upper	Wald p-Value
Block	2.0735208	2.7320156	-3.281131	7.4281731	0.4479
Block*Dose	4.5132292	2.8290711	-1.031648	10.058107	0.1106
Residual	4.318875	1.5269529	2.3956027	10.003671	
Total	10.905625	3.4288743	6.4002154	22.628698	

Fixed Effects Parameter Estimates

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|	95% Lower	95% Upper
Intercept	24.6575	0.865065	4.0	28.50	<.0001*	22.255694	27.059306
Type[r]	0.5475	0.3285907	16.0	1.67	0.1151	-0.149081	1.2440813
Dose[1]	-6.1275	1.0004499	12.0	-6.12	<.0001*	-8.307293	-3.947707
Dose[2]	1.8625	1.0004499	12.0	1.86	0.0873	-0.317293	4.0422931
Dose[4]	3.6225	1.0004499	12.0	3.62	0.0035*	1.4427069	5.8022931
Type[r]*Dose[1]	0.9225	0.5691359	16.0	1.62	0.1246	-0.284014	2.1290141
Type[r]*Dose[2]	0.7725	0.5691359	16.0	1.36	0.1935	-0.434014	1.9790141
Type[r]*Dose[4]	-0.6475	0.5691359	16.0	-1.14	0.2720	-1.854014	0.5590141

Variety-Pesticide Evaluation - Fit Mixed

Fit Mixed

Random Coefficients

Fixed Effects Tests

Source	Nparm	DFNum	DFDen	F Ratio	Prob > F
Type	1	1	16.0	2.7762438	0.1151
Dose	3	3	12.0	13.625356	0.0004*
Type*Dose	3	3	16.0	2.287845	0.1176

Multiple Comparisons for Dose

Least Squares Means Estimates

Tukey HSD All Pairwise Comparisons

Quantile = 2.9688, Adjusted DF = 12.0, Adjustment = Tukey-Kramer

All Pairwise Differences

Dose	-Dose	Difference	Std Error	t Ratio	Prob>|t|	Lower 95%	Upper 95%
1	2	-7.99000	1.633728	-4.89	0.0018*	-12.8402	-3.13978
1	4	-9.75000	1.633728	-5.97	0.0003*	-14.6002	-4.89978
1	8	-6.77000	1.633728	-4.14	0.0064*	-11.6202	-1.91978
2	4	-1.76000	1.633728	-1.08	0.7092	-6.6102	3.09022
2	8	1.22000	1.633728	0.75	0.8763	-3.6302	6.07022
4	8	2.98000	1.633728	1.82	0.3095	-1.8702	7.83022

All Pairwise Comparisons Scatterplot

Results

Variety-Pesticide Evaluation - Fit Least Squares

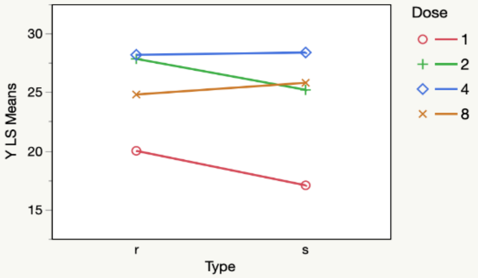
Response Y

Multiple Comparisons for Type*Dose

Least Squares Means Estimates

Type	Dose	Estimate	Std Error	DF	Lower 95%	Upper 95%
r	1	20.000000	1.4768632	20.231	16.921576	23.078424
r	2	27.840000	1.4768632	20.231	24.761576	30.918424
r	4	28.180000	1.4768632	20.231	25.101576	31.258424
r	8	24.800000	1.4768632	20.231	21.721576	27.878424
s	1	17.060000	1.4768632	20.231	13.981576	20.138424
s	2	25.200000	1.4768632	20.231	22.121576	28.278424
s	4	28.380000	1.4768632	20.231	25.301576	31.458424
s	8	25.800000	1.4768632	20.231	22.721576	28.878424

Least Squares Means Plot



Variety-Pesticide Evaluation - Fit Least Squares

Response Y

Multiple Comparisons for Type*Dose

Tukey HSD All Pairwise Comparisons

Quantile = 3.46215, Adjusted DF = 16.0, Adjustment = Tukey-Kramer

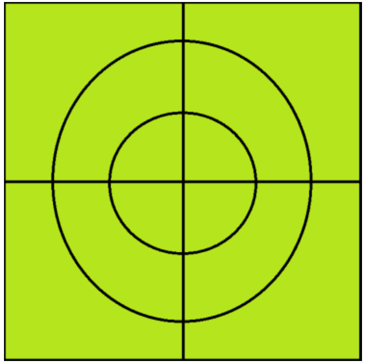
All Pairwise Differences

Type	Dose	-Type	-Dose	Difference	Std Error	t Ratio	Prob>|t|	Lower 95%	Upper 95%
r	1	r	2	-7.8400	1.879586	-4.17	0.0128*	-14.3474	-1.3326
r	1	r	4	-8.1800	1.879586	-4.35	0.0090*	-14.6874	-1.6726
r	1	r	8	-4.8000	1.879586	-2.55	0.2417	-11.3074	1.7074
r	1	s	1	2.9400	1.314363	2.24	0.3814	-1.6105	7.4905
r	1	s	2	-5.2000	1.879586	-2.77	0.1721	-11.7074	1.3074
r	1	s	4	-8.3800	1.879586	-4.46	0.0073*	-14.8874	-1.8726
r	1	s	8	-5.8000	1.879586	-3.09	0.0995	-12.3074	0.7074
r	2	r	4	-0.3400	1.879586	-0.18	1.0000	-6.8474	6.1674
r	2	r	8	3.0400	1.879586	1.62	0.7345	-3.4674	9.5474
r	2	s	1	10.7800	1.879586	5.74	0.0006*	4.2726	17.2874
r	2	s	2	2.6400	1.314363	2.01	0.5057	-1.9105	7.1905
r	2	s	4	-0.5400	1.879586	-0.29	1.0000	-7.0474	5.9674
r	2	s	8	2.0400	1.879586	1.09	0.9510	-4.4674	8.5474
r	4	r	8	3.3800	1.879586	1.80	0.6298	-3.1274	9.8874
r	4	s	1	11.1200	1.879586	5.92	0.0005*	4.6126	17.6274
r	4	s	2	2.9800	1.879586	1.59	0.7521	-3.5274	9.4874
r	4	s	4	-0.2000	1.314363	-0.15	1.0000	-4.7505	4.3505
r	4	s	8	2.3800	1.879586	1.27	0.8986	-4.1274	8.8874
r	8	s	1	7.7400	1.879586	4.12	0.0142*	1.2326	14.2474
r	8	s	2	-0.4000	1.879586	-0.21	1.0000	-6.9074	6.1074
r	8	s	4	-3.5800	1.879586	-1.90	0.5666	-10.0874	2.9274
r	8	s	8	-1.0000	1.314363	-0.76	0.9930	-5.5505	3.5505
s	1	s	2	-8.1400	1.879586	-4.33	0.0094*	-14.6474	-1.6326
s	1	s	4	-11.3200	1.879586	-6.02	0.0004*	-17.8274	-4.8126
s	1	s	8	-8.7400	1.879586	-4.65	0.0050*	-15.2474	-2.2326
s	2	s	4	-3.1800	1.879586	-1.69	0.6922	-9.6874	3.3274
s	2	s	8	-0.6000	1.879586	-0.32	1.0000	-7.1074	5.9074
s	4	s	8	2.5800	1.879586	1.37	0.8567	-3.9274	9.0874

Beyond the Split-Plot

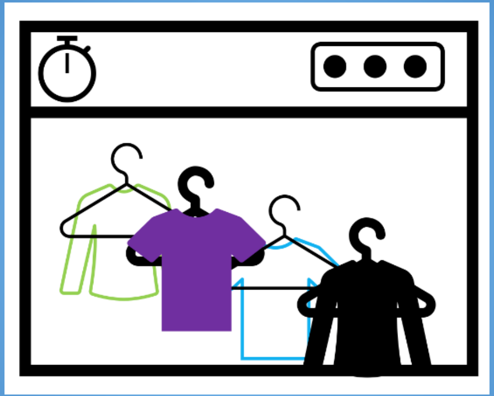
Factorial Treatments with other Experiment Designs

- Many variations and extensions of the basic split-plot exist
  - Split-split-plot
  - Strip-split-plot
  - Combinations of any of these!
- Nested designs (often look like split-plots)
- With the skeleton ANOVA process, can translate virtually any design to model



Multiple Random Effects (and negative variance components)

Fabric Shrinkage



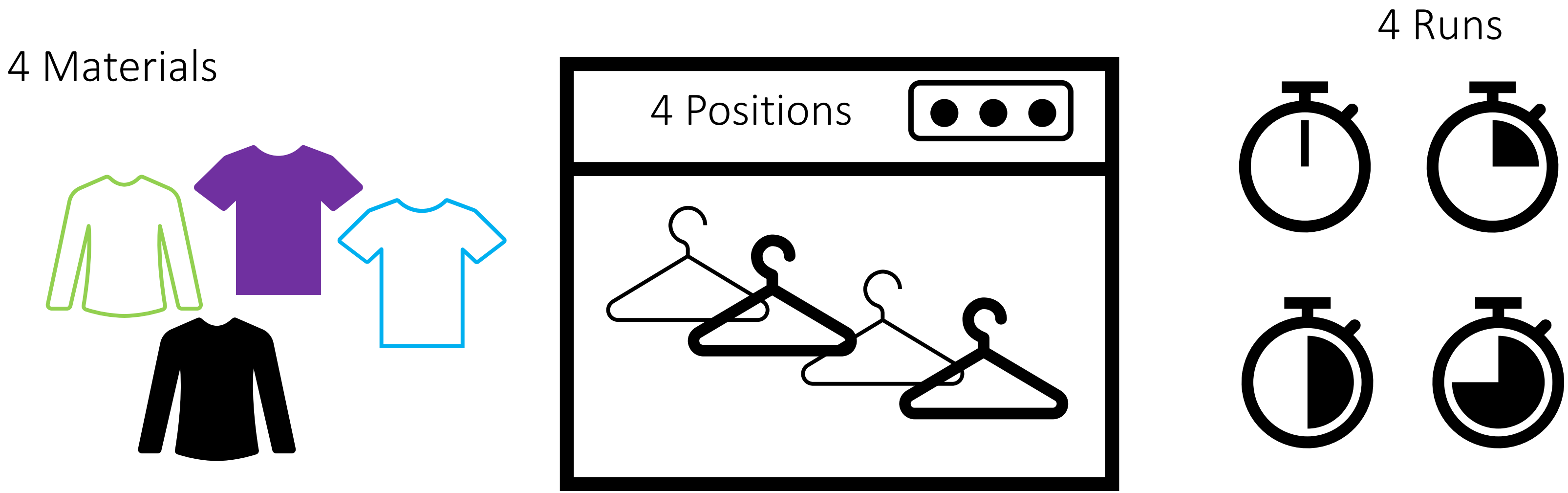
3	9	1	2	8	6	5	7	4
4	8	7	3	5	9	1	2	6
6	5	2	7	1	4	8	3	9
8	7	5	4	3	1	6	9	2
2	1	3	9	6	7	4	8	5
9	6	4	5	2	8	7	1	3
1	4	9	6	7	3	2	5	8
5	3	8	1	4	2	9	6	7
7	2	6	8	9	5	3	4	1

What we will cover:

- Just like we can have several treatment effects, and cross them (or nest them) – we can also do this with random effects
- **Latin Square** is a special kind of crossed random effects
- A note about **Negative Variance Components**

Describe the data

- **Fabric Shrinkage —** A fabric manufacturer needs to test four new materials to be used in permanent press garments. The heat chamber used to test fabrics has four positions. Each fabric should be tested under each position, and due to time constraints, the manufacturer is limited to four runs. Fabric shrinkage is the response of interest.

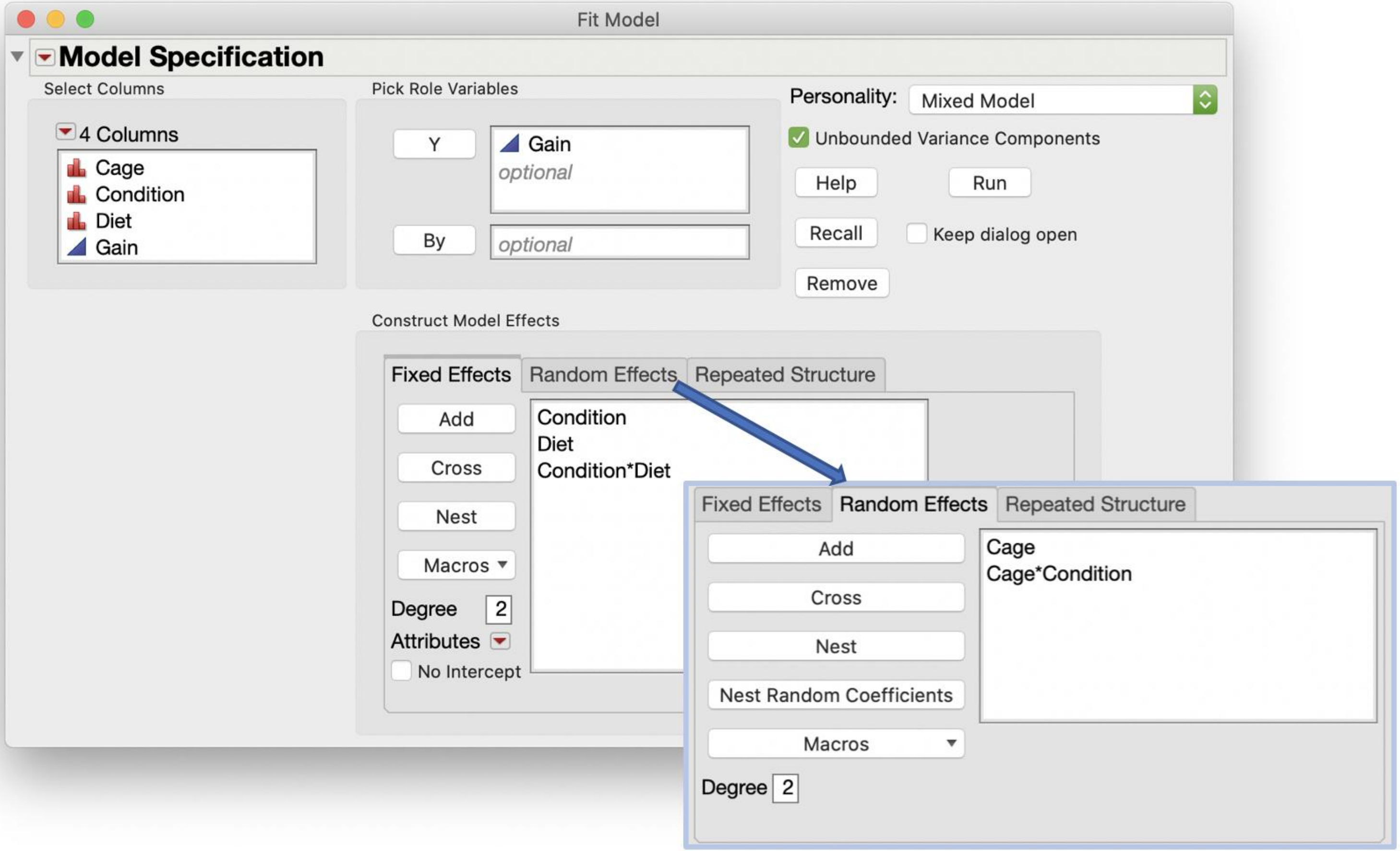


Skeleton ANOVA

Experiment Design		Treatment Design		Skeleton ANOVA	
Source	df	Source	df	Source	df
Run	4-1=3			Run	3
Position	4-1=3			Position	3
		Material	4-1=3	Material	3
Run*Position (the e.u.)	(4-1)*(4-1)=9			(Run*Position)|Material → Residual	9-3=6
Total	16-1=15			Total	15

jmp

Negative Variance Components?



Mouse Condition - Fit Least Squares

Window Tools Graph Tools Show Data Table Local Data Filter

Response Gain

Summary of Fit

RSquare	0.351969
RSquare Adj	0.054955
Root Mean Square Error	5.617433
Mean of Response	57.19444
Observations (or Sum Wgts)	36

Parameter Estimates

Random Effect Predictions

REML Variance Component Estimates

Random Effect	Var Ratio	Var Component	Std Error	95% Lower	95% Upper	Wald p-Value	Pct of Total
Cage	0.1593643	5.0288294	4.7149355	-4.212274	14.269933	0.2862	13.746
Condition*Cage	-0.197757	-6.240346	4.8693074	-15.78401	3.3033207	0.2000	0.000
Residual		31.555556	11.156574	17.503302	73.091115		86.254
Total		36.584385	12.111967	20.956243	79.50077		100.000

-2 LogLikelihood = 179.29117789

Note: Total is the sum of the positive variance components.

Total including negative estimates = 30.344039

Covariance Matrix of Variance Component Estimates

Iterations

Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Condition	3	3	4.718	4.3123	0.0798
Diet	2	2	16	0.8248	0.4561
Condition*Diet	6	6	16	1.5232	0.2333

Effect Details

BUT WHY????

- Negative variance component estimates might happen when, for example, a variance is very small or when there is negative correlation among experimental units. This latter situation often happens when there is competition for resources among plots or units, as here with mice in a cage. In such cases, the REML optimal value for the variance estimate can cross into the negative region.

- Although it might seem strange to report negative variance component estimates, this unbounded fit is the best model for estimating the fixed effects comparisons.

- Allowing the negative variance component(s), you get better control over Type 1 error for your fixed effects comparisons, and in some cases better power.

Summary

- You can have multiple random effects to account for multiple sources of variance (multiple restrictions on randomization for the treatment).
- A Latin Square is a special restriction when you have the (number of treatments) = (number of runs from restriction 1) = (number of runs from restriction 2).
- Allow negative variance estimates. It's better for your model fit and better inference.