

Lecture 23

1. Mixed model example: assay plates
2. Multilevel/Hierarchical models
3. SAS versions of R models in Gelman and Hill, chapter 12

1

Mixed Model example: Rat Diets

In Lecture 10, we fit a linear model to data from a study by Sabrina Peterson (Food Science and Nutrition) which examined effects on MROD (liver enzyme) over time from diets containing:

- cruciferous (C) vegetables : broccoli, cabbage, and watercress
- apiaceous (A) vegetables: parsnips and celery

2×2 factorial in diets: Basal (control), A, C, A+C, with 30 rats assigned to each

At three times (7, 30, 60 days), they sacrificed 10 rats from each diet group to measure MROD.

$2 \times 2 \times 3$ factorial : A \times C \times day (time)

2

day diet

Frequency	Control	A	C	AC	Total
7	10	10	10	10	40
30	10	10	10	10	40
60	10	10	9	9	38
Total	30	30	29	29	118

Slightly unbalanced because 2 rats not measured.

3

Part of the data:

Obs	Plate	animal	Liver_wt	MROD	Api	Cru	day	diet
107	15	109	11.32	4.1450	0	1	60	C
108	15	110	14.22	4.2353	0	1	60	C
109	11	112	14.63	3.5352	1	1	60	AC
110	12	113	19.91	2.5222	1	1	60	AC

Two different ways to specify the 4 diets:

- by diet (0, A, C, AC) — used to make interaction plot with time
- by combinations of Cru and Api — used in model

4

Fixed-effects model: Proc GLM

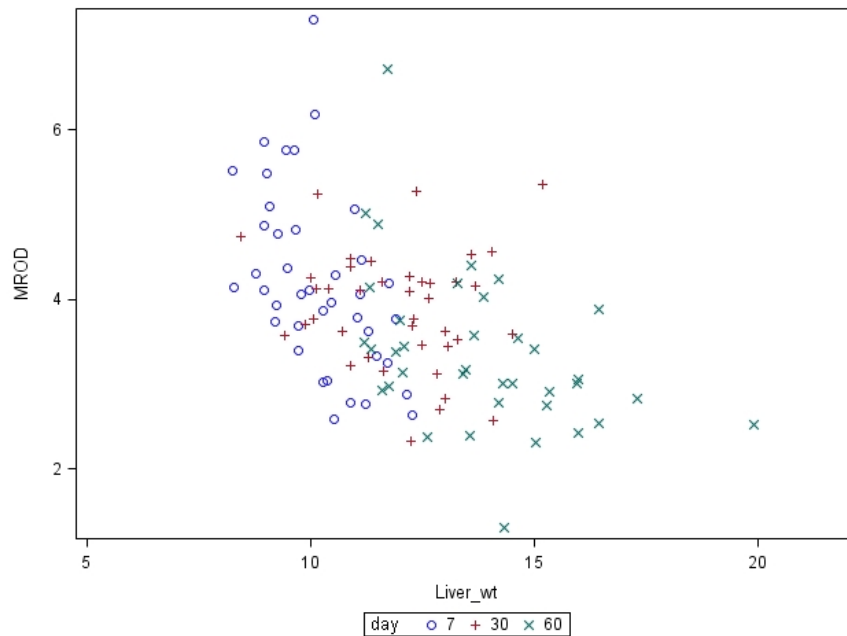
Preliminary model: $2 \times 2 \times 3$ factorial: $A \times C \times \text{day}$

```
Proc GLM  data=ph6470.rat_diets;  
  class day api cru ;  
  
  model MROD = liver_wt  api  cru  api*cru  
    day  day*api  day*cru  day*api*cru;  
  
  lsmeans day*api*cru /  slice=day;
```

All 2-factor and 3-factor interactions between experimental factors.

5

Adjust for weight of liver, which was strongly associated with MROD level, but not balanced across days.



6

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Liver_wt	1	10.06110559	10.06110559	15.53	0.0001
Api	1	0.85420092	0.85420092	1.32	0.2536
Cru	1	12.90007396	12.90007396	19.91	<.0001
Api*Cru	1	0.75793715	0.75793715	1.17	0.2820
day	2	0.34908168	0.17454084	0.27	0.7644
day*Api	2	0.51629446	0.25814723	0.40	0.6724
day*Cru	2	0.43713415	0.21856708	0.34	0.7145
day*Api*Cru	2	1.07263533	0.53631766	0.83	0.4400

Which factors affect response MROD?

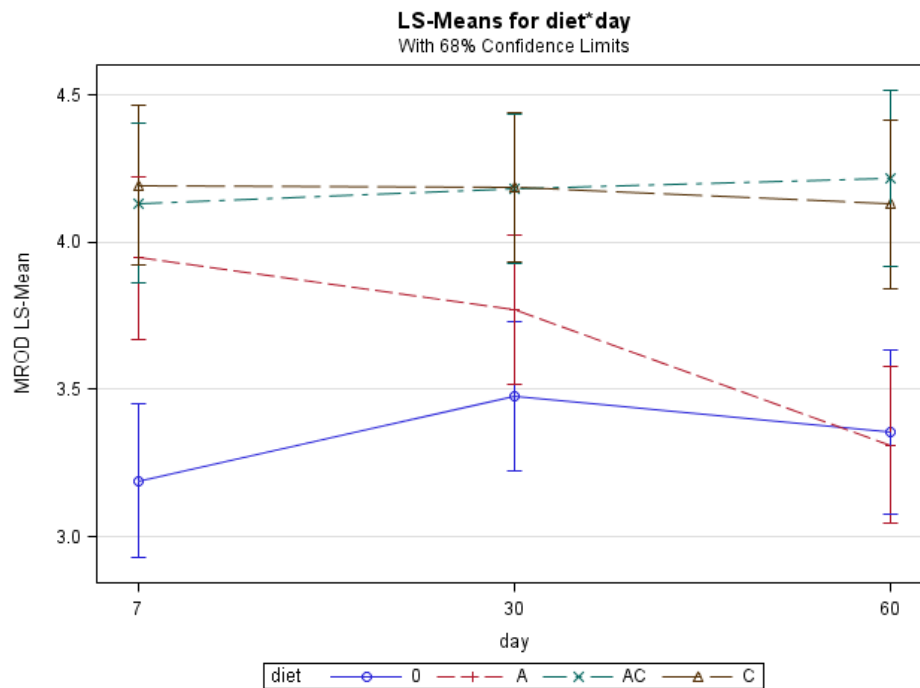
7

LSmeans adjusted for liver weight:

Least Squares Means						
day	Api	Cru	MROD LSMEAN	Standard Error	Pr > t	
7	0	0	3.19037736	0.26254278	<.0001	
7	0	1	4.19380460	0.27126807	<.0001	
7	1	0	3.94692888	0.27513287	<.0001	
7	1	1	4.13322903	0.27041769	<.0001	
30	0	0	3.47668375	0.25462926	<.0001	
30	0	1	4.18853602	0.25462283	<.0001	
30	1	0	3.77017422	0.25465618	<.0001	
30	1	1	4.18165666	0.25457044	<.0001	
60	0	0	3.35529699	0.27806812	<.0001	
60	0	1	4.12976077	0.28882738	<.0001	
60	1	0	3.31199757	0.26548906	<.0001	
60	1	1	4.21805146	0.30036140	<.0001	

8

Interaction plot:



9

Two main reasons for adjusting comparison of groups:

- For **balance**, when groups have different distributions of the predictor Z

Adjust for liver weight, which is associated with response and not balanced between time groups

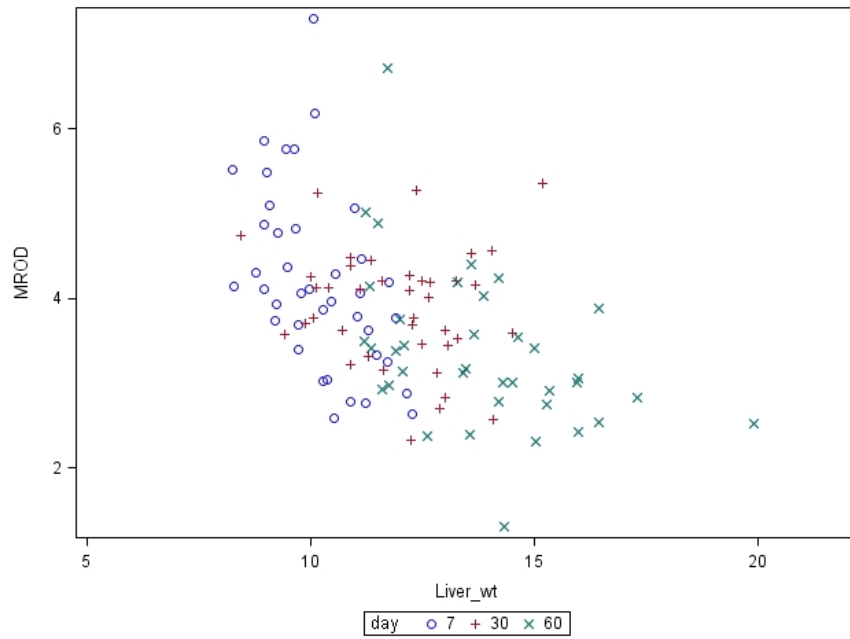
- To **reduce error variance**, when predictor Z is associated with response and known to vary across subjects

Liver enzyme MROD assayed in plates, 8 samples at a time.

Adjusting for plate will remove variability between plates from the error term.

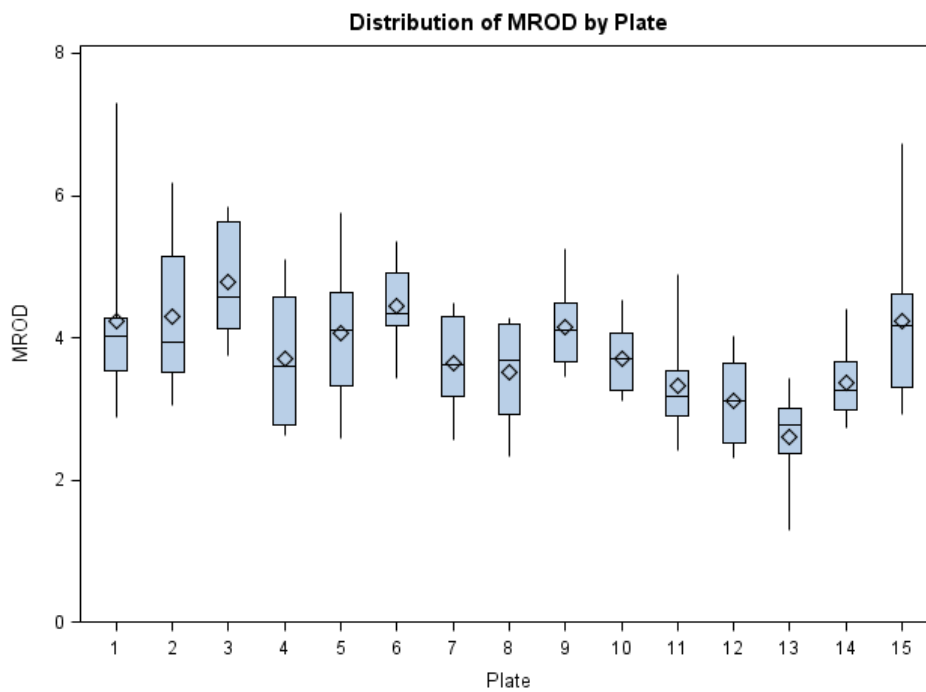
Why is it good to reduce error variance?

Adjust for weight of liver, which was strongly associated with MROD level, but not balanced across days.



11

Adjust for assay plate:



12

Fixed-effects model 2

Problem: 10 rats per treatment group, 8 rats per plate

```
Proc GLM  data=ph6470.rat_diets;
  class day api cru plate ;

  model mrod = plate liver_wt  api cru api*cru
    day day*api day*cru day*api*cru / solution;

  lsmeans day*api*cru / stderr slice=day;
```

13

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Plate	12	14.20216234	1.18351353	2.05	0.0284
Liver_wt	1	4.69052329	4.69052329	8.11	0.0054
Api	1	1.01404063	1.01404063	1.75	0.1887
Cru	1	12.58240943	12.58240943	21.76	<.0001
Api*Cru	1	0.76569779	0.76569779	1.32	0.2528
day	0	0.00000000	.	.	.
day*Api	2	0.46902814	0.23451407	0.41	0.6678
day*Cru	2	0.36275345	0.18137673	0.31	0.7315
day*Api*Cru	2	1.15928428	0.57964214	1.00	0.3709

Plate is significant, and requires estimating 12 *fixed-effect* parameters.

14

Regression coefficients:

Parameter		Estimate		Standard Error	t Value	Pr > t
Plate	5	-0.698295930	B	0.51489447	-1.36	0.1784
Plate	6	0.074369757	B	0.48722087	0.15	0.8790
Plate	7	-0.730576335	B	0.48734556	-1.50	0.1373
Plate	8	-0.878905030	B	0.48797407	-1.80	0.0750
Plate	9	-0.527842029	B	0.50663906	-1.04	0.3002
Plate	10	-0.783407547	B	0.49204226	-1.59	0.1148
Plate	11	-0.393801367	B	0.43302798	-0.91	0.3655
Plate	12	-0.535796083	B	0.43126369	-1.24	0.2173
Plate	13	-1.333303779	B	0.40193771	-3.32	0.0013
Plate	14	-0.698241765	B	0.38424775	-1.82	0.0724
Plate	15	0.000000000	B	.	.	.
Liver_wt		-0.165951032		0.05826511	-2.85	0.0054
Api	0	-0.125917178	B	0.38459969	-0.33	0.7441
Api	1	0.000000000	B	.	.	.
Cru	0	-0.891814454	B	0.35839193	-2.49	0.0146
Cru	1	0.000000000	B	.	.	.
Api*Cru	0 0	0.146979284	B	0.52354680	0.28	0.7795
Api*Cru	0 1	0.000000000	B	.	.	.
Api*Cru	1 0	0.000000000	B	.	.	.
Api*Cru	1 1	0.000000000	B	.	.	.
day	7	0.000000000	B	.	.	.
day	30	0.000000000	B	.	.	.

15

day	60	0.000000000	B	.	.	.
day*Api	7 0	0.188009626	B	0.51280670	0.37	0.7147
day*Api	7 1	0.000000000	B	.	.	.
day*Api	30 0	0.130339212	B	0.51433739	0.25	0.8005
day*Api	30 1	0.000000000	B	.	.	.
day*Api	60 0	0.000000000	B	.	.	.
day*Api	60 1	0.000000000	B	.	.	.
day*Cru	7 0	0.713523382	B	0.49198415	1.45	0.1504
day*Cru	7 1	0.000000000	B	.	.	.
day*Cru	30 0	0.485216338	B	0.49273386	0.98	0.3273
day*Cru	30 1	0.000000000	B	.	.	.
day*Cru	60 0	0.000000000	B	.	.	.
day*Cru	60 1	0.000000000	B	.	.	.
day*Api*Cru	7 0 0	-0.990075201	B	0.70244246	-1.41	0.1621
day*Api*Cru	7 0 1	0.000000000	B	.	.	.
day*Api*Cru	7 1 0	0.000000000	B	.	.	.
day*Api*Cru	7 1 1	0.000000000	B	.	.	.
day*Api*Cru	30 0 0	-0.452415462	B	0.70898402	-0.64	0.5250
day*Api*Cru	30 0 1	0.000000000	B	.	.	.
day*Api*Cru	30 1 0	0.000000000	B	.	.	.
day*Api*Cru	30 1 1	0.000000000	B	.	.	.
day*Api*Cru	60 0 0	0.000000000	B	.	.	.
day*Api*Cru	60 0 1	0.000000000	B	.	.	.
day*Api*Cru	60 1 0	0.000000000	B	.	.	.

16

When we adjust for plate, we cannot get estimates of the means:

Least Squares Means			
day	Api	Cru	MROD LSMEAN
7	0	0	Non-est
7	0	1	Non-est
7	1	0	Non-est
7	1	1	Non-est
30	0	0	Non-est
30	0	1	Non-est
30	1	0	Non-est
30	1	1	Non-est
60	0	0	Non-est
60	0	1	Non-est
60	1	0	Non-est
60	1	1	Non-est

Non-est means non-estimable.

17

Mixed model approach

Treat plates as a random effect:

```
Proc Mixed data=ph6470.rat_diets;
  class day api cru plate ;

  model mrod = liver_wt  api cru api*cru
    day day*api day*cru day*api*cru; only fixed effects in model

  random plate / subject=plate v vcorr;

  lsmeans day*api*cru ;
```

18

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Liver_wt	1	92	12.37	0.0007
Api	1	92	1.61	0.2074
Cru	1	92	22.03	<.0001
Api*Cru	1	92	1.30	0.2566
day	2	92	0.24	0.7868
day*Api	2	92	0.42	0.6572
day*Cru	2	92	0.34	0.7102
day*Api*Cru	2	92	0.97	0.3827

F-statistic for Cru is a little larger than in the model without plate:

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Cru	1	12.90007396	12.90007396	19.91	<.0001

19

Now we have LSmeans, adjusted for liver weight and correlations within plates:

Least Squares Means

Effect	day	Api	Cru	Estimate	Standard Error
day*Api*Cru	7	0	0	3.2075	0.2800
day*Api*Cru	7	0	1	4.2188	0.2889
day*Api*Cru	7	1	0	3.9748	0.2929
day*Api*Cru	7	1	1	4.1575	0.2881
day*Api*Cru	30	0	0	3.4752	0.2719
day*Api*Cru	30	0	1	4.1871	0.2719
day*Api*Cru	30	1	0	3.7720	0.2720
day*Api*Cru	30	1	1	4.1813	0.2719
day*Api*Cru	60	0	0	3.3255	0.2959
day*Api*Cru	60	0	1	4.0823	0.3031
day*Api*Cru	60	1	0	3.2919	0.2830
day*Api*Cru	60	1	1	4.1925	0.3180

20

How does this work?

Proc GLM tries to estimate a regression coefficient (fixed effect) for each plate: $\beta_1, \dots, \beta_{15}$ and spends 12 parameters on this.

Proc Mixed assumes plates p_j are $\text{Normal}(0, \sigma_{\text{Plates}}^2)$ and estimates the single parameter that specifies this distribution: σ_{Plates}^2

Cov Parm	Subject	Estimate
Plate	Plate	0.08051
Residual		0.5781

The random intercepts for plates \hat{p}_j are residuals

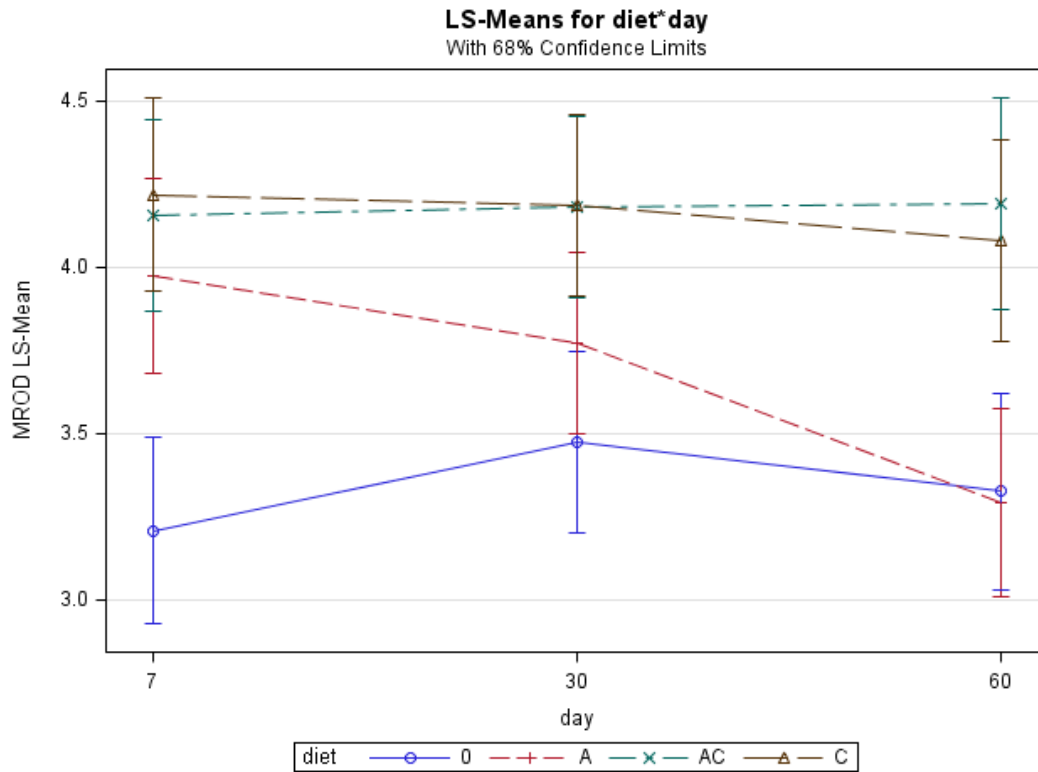
21

Code for interaction plot must include the random effect:

```
ods graphics on;  
ods select lsmeans meanplot;
```

```
Proc Glimmix data=ph6470.rat_diets;  
  class diet day plate;  
  model mrod = liver_wt diet day day*diet ;  
  random plate / subject=plate;  
  lsmeans day*diet/ alpha=.32  
  plots=(meanplot(cl join sliceby=diet));  
  
run;  
ods graphics off;
```

22



23

Multi-level or hierarchical models

Example 1. Study of standardized test scores from 4th grade students.

Sample: 8000 students at 46 schools in Wisconsin and Texas.

Student-level predictors: gender, race, pre-test scores

School-level predictors: state, school district, public/private, socio-economic status of school's neighborhood.

Effects of student-level factors probably change according to school-level factors

Regression coefficients for student-level factors vary; model these on school-level predictors

24

Example 2. Retrospective study to assess effect of surgical volume on early hospital mortality for pediatric cardiac surgery (L Kochilas, Plan B project).

Patient-level predictors: age, gender, risk-score for surgery

Hospital-level predictors: time period, surgical volume

How does effect of surgical volume on probability of survival vary between different types of patients?

Interaction terms *vs* model varying patient-level coefficients on hospital-level predictors.

25

Example 3. Measurements of radon (carcinogen gas) in samples of homes in 85 counties in Minnesota.

Aim: estimate county mean radon levels.

House-level predictor: floor where radon measurement was taken.

basement (floor=0), first floor (floor=1)

County-level predictors: uranium measurement for county

Gelman and Hill (2007) *Data Analysis Using Regression and Multilevel/Hierarchical Models*. Cambridge U Press.

In Chapter 12, they fit several multi-level models in *R*, which we will fit in Proc Mixed.

26

Model 1. Varying intercept (by county) with individual-level predictor (floor)

Following Gelman and Hill, §12.4, model radon measurements y_{ij} from house i in the county j

$$y_{ij} = \alpha_{j[i]} + \beta * \text{floor}_i + \varepsilon_{ij}$$

Assume $\alpha_{j[i]}$ are $\text{Normal}(0, \sigma_\alpha^2)$ and independent of the errors $\{\varepsilon_{ij}\} \sim \text{Normal}(0, \sigma_y^2)$.

SAS version of this model fits random intercept for each county:

$$y_{ij} = (\beta_0 + b_j) + \beta * \text{floor}_i + \varepsilon_{ij}$$

Estimate only σ_α^2 instead of 85 regression coefficients for 85 counties

27

```
Proc Mixed data= arhm.radon; * p 259;

class county_number;

model radon = floor / solution ddfm=bw; solution option gives output p 260

random intercept / subject=county_number v vcorr solution;

ODS output SolutionR = A; saves random effects to A
```

28

Class Level Information

Class	Levels	Values
county_number	85	1 2 3 4 5 6 7 8 9 10 11 12 13 . . .

Dimensions

Covariance Parameters	2
Columns in X	2
Columns in Z Per Subject	1
Subjects	85
Max Obs Per Subject	116

Number of Observations

Number of Observations Read	919
Number of Observations Used	919
Number of Observations Not Used	0

29

Fixed effects model is averaged across counties:

Solution for Fixed Effects

Effect	Estimate	Standard Error	DF	t Value	Pr > t
Intercept	1.4616	0.05157	84	28.34	<.0001
floor	-0.6930	0.07043	833	-9.84	<.0001

These are the error variance estimates of σ_α^2 and σ_y^2 (R gives the square roots)

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
Intercept	county_number	0.1077
Residual		0.5709

30

Random effects for each county (Gelman and Hill: “county-level errors” p 260):

Solution for Random Effects						
Effect	county_ number	Estimate	Std Err Pred	DF	t Value	Pr > t
Intercept	1	-0.2701	0.2487	917	-1.09	0.2778
Intercept	2	-0.5339	0.1099	917	-4.86	<.0001
Intercept	3	0.01761	0.2631	917	0.07	0.9466
. . .						
Intercept	85	-0.07536	0.2800	917	-0.27	0.7879

Model 2. Group-level predictor + subject-level predictor (Gelman & Hill, §12.6)

Two regression models: lower level for houses, upper level for counties

Model radon measurements y_{ij} from house i in the county j

$$y_{ij} = \alpha_{j[i]} + \beta * \text{floor}_i + \varepsilon_{ij}$$

$$\alpha_j = \gamma_0 + \gamma_1 u_j + e_j$$

where errors $\{\varepsilon_{ij}\} \sim \text{Normal}(0, \sigma_y^2)$ and errors $e_j \sim \text{Normal}(0, \sigma_\alpha^2)$

```
Proc Mixed data= arhm.radon; * p 266;
  class county_number;
  model radon = floor uranium / solution ddfm=bw;
  random intercept / subject=county_number v vcorr solution;
```

Fixed effects gives summary output for the regression coefficients:

Solution for Fixed Effects					
		Standard			
Effect	Estimate	Error	DF	t Value	Pr > t
Intercept	1.4658	0.03794	83	38.63	<.0001
floor	-0.6682	0.06880	833	-9.71	<.0001
uranium	0.7203	0.09176	83	7.85	<.0001

$$y_{ij} = \bar{\alpha}_j + \beta * \text{floor}_i + \gamma_1 u_j$$

33

Error variance estimates of σ_α^2 and σ_y^2

(R gives the square roots = standard deviations)

Cov Parm	Subject	Estimate
Intercept	county_number	0.02446
Residual		0.5752

Gelman and Hill give estimates $\hat{\alpha}_j = (\hat{\beta}_0 + \hat{b}_j)$ from SAS

34

References:

Gelman and Hill (2007) *Data Analysis Using Regression and Multilevel/Hierarchical Models*. Cambridge U Press.

Chapter 1 gives overview and examples

Chapter 11 on multilevel structures

Chapter 12 fits simplest multilevel models compared to regular regression

J Singer: Using SAS Proc Mixed to fit multilevel models, hierarchical models, and individual growth models. *Journal of Educational and Behavioral Statistics*, 1998; 24: 323-355.