

Analysis of repeated measurements (KL MED8008)

Eirik Skogvoll, MD PhD

Professor and Consultant

Institute of Circulation and Medical Imaging
Dept. of Anaesthesiology and Intensive Care

Day 3

- Practical issues ...?
 - Lectures
 - Textbook
 - Software
 - Exercise 1: a brief introduction
- Robust variance estimation in linear regression («sandwich»)
- A quick look at statistical models
- Variance components
 - So what is «nesting»?

ROBUST («SANDWICH») VARIANCE ESTIMATION

Linear model - requirements

- Independent observations
- Residuals are Normally distributed
- Homoskedasticity

Linear model with robust standard error

- If we use the Huber-White «sandwich» estimator of the variance/covariance matrix, these assumptions may be relaxed
- Textbook:
«By fitting an ordinary regression model with robust standard errors for clustered data instead of fitting variance-components models, we are treating the within-cluster dependence as a «nuisance», not as a phenomenon we are interested in.
We learn nothing about the between and within-cluster variances or intraclass correlation».

. use wi ner, repl ace
(T4. 3 -- Wi ner, Brown, Mi chel s)

. regress score i .drug

Source	SS	df	MS	Number of obs =	20
Model	698.2	3	232.733333	F(3, 16) =	4.69
Residual	793.6	16	49.6	Prob > F =	0.0155
Total	1491.8	19	78.5157895	R-squared =	0.4680
				Adj R-squared =	0.3683
				Root MSE =	7.0427

score	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
drug						
2	-.8	4.454211	-0.18	0.860	-10.24251	8.642507
3	-10.8	4.454211	-2.42	0.028	-20.24251	-1.357493
4	5.6	4.454211	1.26	0.227	-3.842507	15.04251
_cons	26.4	3.149603	8.38	0.000	19.72314	33.07686

. regress score i .drug, **robust** cluster(person)

Linear regression

Number of obs = 20
F(3, 4) = 47.85
Prob > F = 0.0014
R-squared = 0.4680
Root MSE = 7.0427

(Std. Err. adjusted for 5 clusters in person)

score	Coef.	Robust Std. Err.	t	P> t	[95% Conf. Interval]	
drug						
2	-.8	1.770593	-0.45	0.675	-5.715955	4.115955
3	-10.8	2.808024	-3.85	0.018	-18.59633	-3.003675
4	5.6	.8154753	6.87	0.002	3.335878	7.864122
_cons	26.4	4.270831	6.18	0.003	14.54227	38.25773

. pwcompare drug, bonferroni effects

Pairwise comparisons of marginal linear predictions

Margins : asbalanced

	Number of Comparisons
drug	6

	Contrast	Std. Err.	Bonferroni t	P> t	Bonferroni [95% Conf. Interval]	
drug						
2 vs 1	-.8	4.454211	-0.18	1.000	-14.19976	12.59976
3 vs 1	-10.8	4.454211	-2.42	0.165	-24.19976	2.599755
4 vs 1	5.6	4.454211	1.26	1.000	-7.799755	18.99976
3 vs 2	-10	4.454211	-2.25	0.235	-23.39976	3.399755
4 vs 2	6.4	4.454211	1.44	1.000	-6.999755	19.79976
4 vs 3	16.4	4.454211	3.68	0.012	3.000245	29.79976

After "robust" estimation:

	Contrast	Std. Err.	Bonferroni t	P> t	Bonferroni [95% Conf. Interval]	
drug						
2 vs 1	-.8	1.770593	-0.45	1.000	-9.389162	7.789162
3 vs 1	-10.8	2.808024	-3.85	0.110	-24.42175	2.821749
4 vs 1	5.6	.8154753	6.87	0.014	1.644122	9.555878
3 vs 2	-10	2.484955	-4.02	0.095	-22.05454	2.054536
4 vs 2	6.4	1.74356	3.67	0.128	-2.058022	14.85802
4 vs 3	16.4	2.426932	6.76	0.015	4.626931	28.17307

A QUICK LOOK AT STATISTICAL MODELS

One group

$$y_i = \mu + \xi_i \quad \xi_i \sim N(0, \sigma^2) \quad i = 1, 2, \dots, n \text{ (subject)}$$

Then $y \sim N(\mu, \sigma^2)$

Hypotheses (usually)

$$H_0 : \mu = \mu_0 \quad \text{vs.} \quad H_1 : \mu \neq \mu_0$$

Estimators

$$\hat{\mu} = \bar{y} \quad \hat{\sigma}^2 = \text{Var}(y) \quad \hat{\sigma} = \sqrt{\text{Var}(y)} \quad \text{Var}(y) : \text{sample variance}$$

Example

[Day2: Vickers.dta]

$$y_i = \mu + \xi_i \quad \xi_i \sim N(0, \sigma^2) \quad i = 1, 2, \dots, n \text{ (subject)}$$

Estimators

$$\hat{\mu} = \bar{y} \quad \hat{\sigma}^2 = \text{Var}(y) \quad \hat{\sigma} = \sqrt{\text{Var}(y)} \quad \text{Var}(y) : \text{sample variance}$$

. ttest post = 60 // bare outcome, modell-eksempel

One-sample t test

Variable	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf. Interval]	
post	52	70.61538	2.697291	19.45044	65.20035	76.03042

mean = mean(post)
Ho: mean = 60

$\hat{\mu}$

$\hat{\sigma}$

t = 3.9356
degrees of freedom = 51

Ha: mean < 60
Pr(T < t) = 0.9999

Ha: mean != 60
Pr(|T| > |t|) = 0.0003

Ha: mean > 60
Pr(T > t) = 0.0001

Two independent groups

$$y_{ij} = \mu_j + \xi_{ij} \quad \xi_{ij} \sim N(0, \sigma^2) \quad j = 1, 2 \text{ (groups)} \quad i = 1, 2, \dots, n_j \text{ (subject within group)}$$

$$\text{Then } y_j \sim N(\mu_j, \sigma^2)$$

Hypotheses (usually)

$$H_0 : \mu_1 = \mu_2 \leftrightarrow \mu_1 - \mu_2 = 0 \quad \text{vs.} \quad H_1 : \mu_1 \neq \mu_2 \leftrightarrow \mu_1 - \mu_2 \neq 0$$

Estimators

$$\hat{\mu}_j = \bar{y}_j \quad \hat{\sigma}^2 = \text{Var}(y_j) \quad \hat{\sigma}_j = \sqrt{\text{Var}(y_j)}$$

Example

[Day2: Vickers.dta]

$$y_{ij} = \mu_j + \xi_{ij} \quad \xi_{ij} \sim N(0, \sigma^2) \quad j = 1, 2 \text{ (groups)} \quad i = 1, 2, \dots, n_j \text{ (subject within group)}$$

Estimators

$$\hat{\mu}_j = \bar{y}_j \quad \hat{\sigma}^2 = \text{Var}(y_j) \quad \hat{\sigma}_j = \sqrt{\text{Var}(y_j)}$$

. ttest post, by (group) // bare outcome

Two-sample t test with equal variances

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf. Interval]
pl acebo	27	62.2963	3.449602	17.92466	55.20554 69.38706
acupunct	25	79.6	3.4288	17.144	72.52331 86.67669
combi ned	52	70.61538	2.697291	19.45044	65.20035 76.03042
di ff		-17.3037	4.872285		-27.08998 -7.517431

di ff = mean(pl acebo) - mean(acupunct) t = -3.5515
 Ho: di ff = 0 degrees of freedom = 50

Ha: di ff < 0
 Pr(T < t) = 0.0004

Ha: di ff != 0
 Pr(|T| > |t|) = 0.0008

Ha: di ff > 0
 Pr(T > t) = 0.9996

ANOVA (more than two groups)

$$y_{ij} = \mu_j + \xi_{ij} \quad \xi_{ij} \sim N(0, \sigma^2) \quad j = 1, 2, 3, \dots, m \text{ (groups)}$$

$i = 1, 2, \dots, n_j$ subject within group

Then $y_j \sim N(\mu_j, \sigma^2)$

Hypotheses (usually)

$H_0 : \mu_1 = \mu_2 = \dots = \mu_j$ vs. $H_1 : \text{At least one } \mu_j \text{ differs from the other}$

Estimators

$$\hat{\mu}_j = \bar{y}_j \quad \hat{\sigma}^2 = MSE \quad \hat{\sigma} = \sqrt{MSE}$$

Example

[Day2: winer.dta]

. anova score drug

Number of obs = 20
 Root MSE = 7.04273
 R-squared = 0.4680
 Adj R-squared = 0.3683

Source	Partial SS	df	MS	F	Prob > F
Model	698.2	3	232.733333	4.69	0.0155
drug	698.2	3	232.733333	4.69	0.0155
Residual	793.6	16	49.6		
Total	1491.8	19	78.5157895		

. margins drug

Adjusted predictions Number of obs = 20

Expression : Linear prediction, predict()

	$\hat{\mu}_1$ Margin	Delta-method Std. Err.	z	P> z	[95% Conf. Interval]
drug					
1	$\hat{\mu}_2$ 26.4	3.149603	8.38	0.000	20.22689 32.57311
2	25.6	3.149603	8.13	0.000	19.42689 31.77311
3	15.6	3.149603	4.95	0.000	9.426891 21.77311
4	$\hat{\mu}_3$ 32	3.149603	10.16	0.000	25.82689 38.17311

Simple linear regression

$$y_i = \beta_0 + \beta_1 x + \xi_i$$

$$\xi_i \sim N(0, \sigma^2)$$

$i = 1, 2, \dots, n$ (subjects)

Then $Y | x \sim N(\beta_0 + \beta_1 x, \sigma^2)$

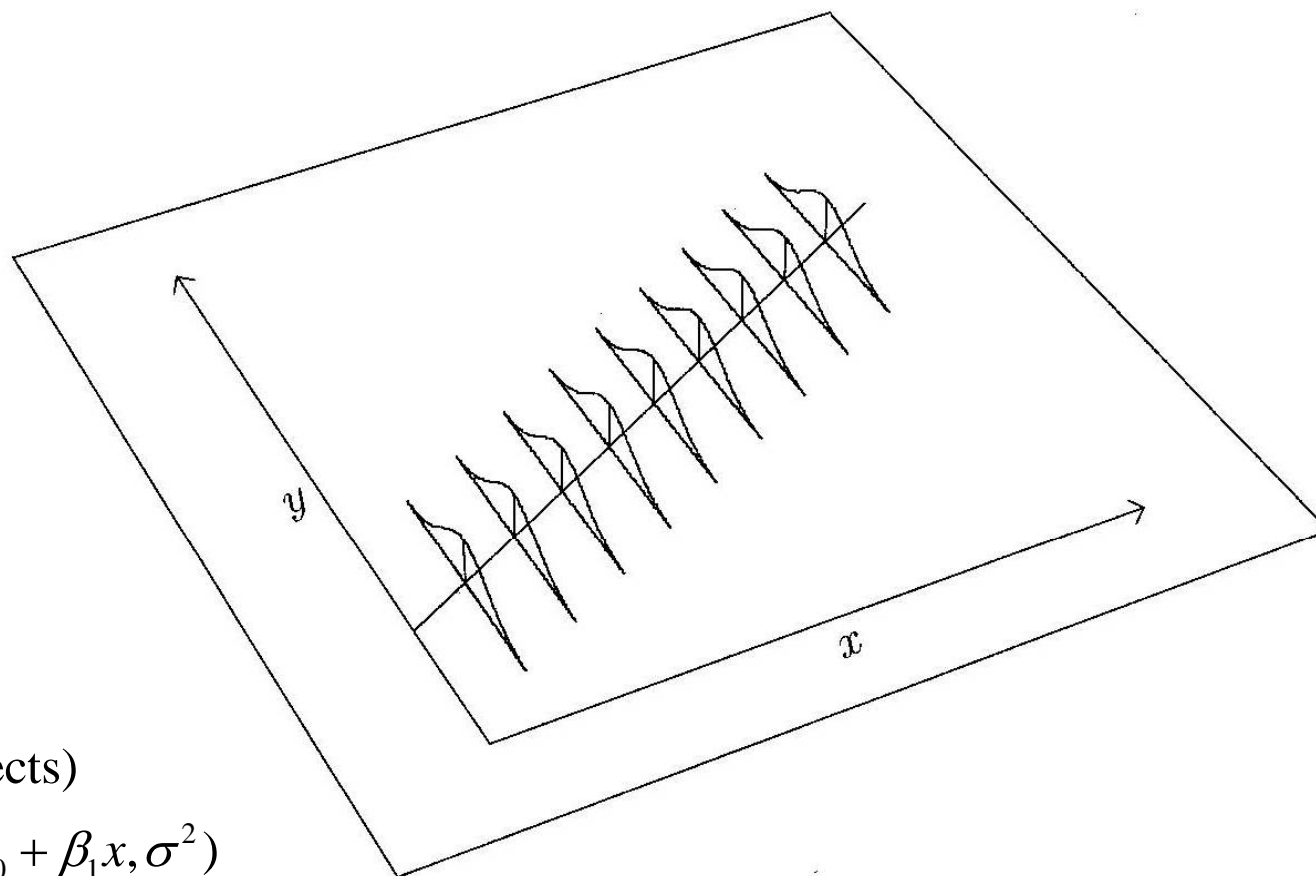


Figure : Illustration of simple linear regression model

Example

[Day2: Vickers.dta]

$$y_i = \beta_0 + \beta_1 x_1 + \xi_i$$

$i = 1, 2, \dots, n$ (subjects)

X_1 : continuous predictor variable

```
. regress post pre
```

Source	SS	df	MS
Model	6323.33822	1	6323.33822
Residual	12970.9695	50	259.419389
Total	19294.3077	51	378.319759

$\hat{\sigma}^2$

Number of obs = 52
 F(1, 50) = 24.37
 Prob > F = 0.0000
 R-squared = 0.3277
 Adj R-squared = 0.3143
 Root MSE = 16.107

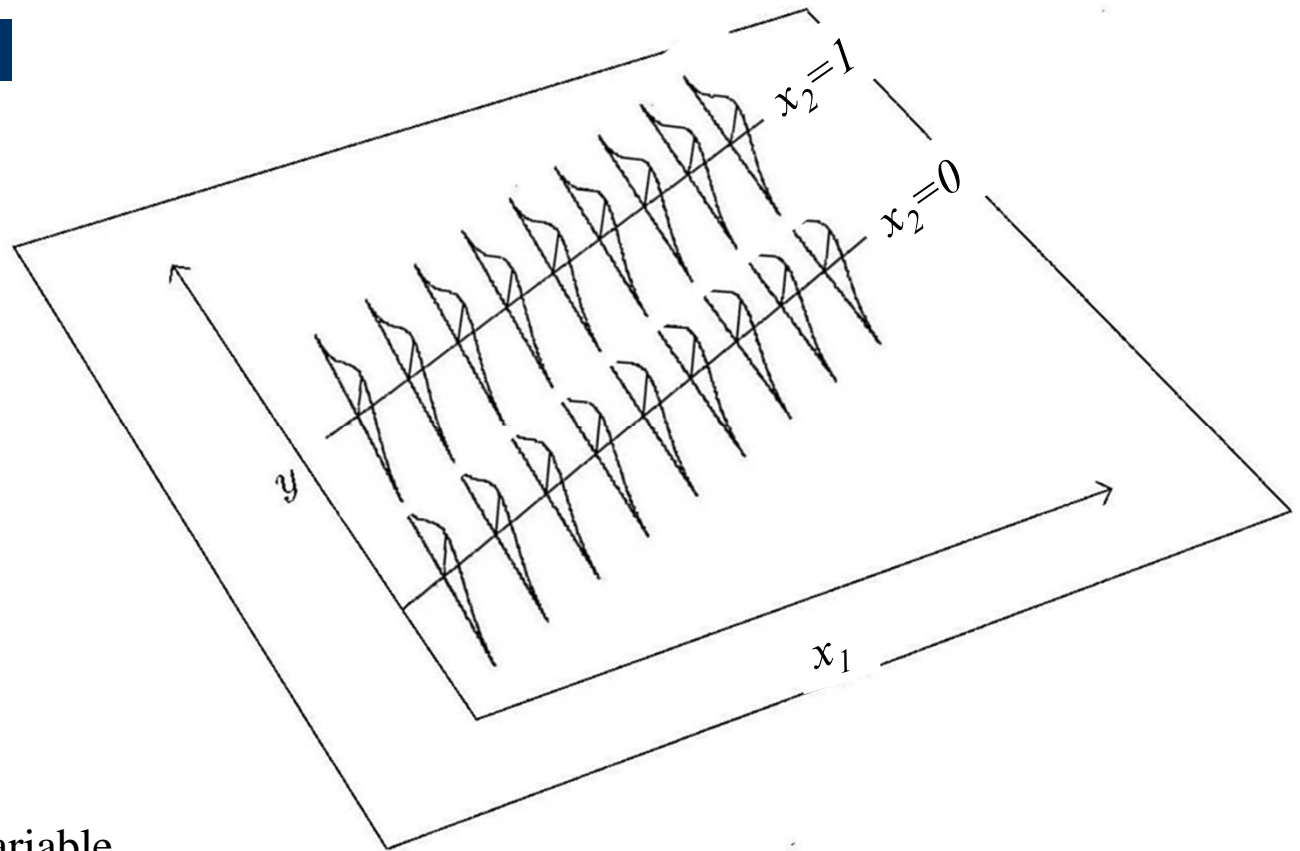
$\hat{\sigma}$

	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
post	$\hat{\beta}_1$				
pre	.8252027	.1671432	4.94	0.000	.4894858 1.16092
_cons	23.54709	9.79174	2.40	0.020	3.8798 43.21438

$\hat{\beta}_1$

$\hat{\beta}_0$

ANCOVA (= linear regression + categorical variables)



$$y_i = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \xi_i$$

$$\xi_i \sim N(0, \sigma^2)$$

$i = 1, 2, \dots, n$ (subjects)

X_1 : continuous predictor variable

X_2 : categorical predictor variable ($X_2 = 0, 1$)

Then $Y | x_1, x_2 \sim N(\beta_0 + \beta_1 x_1 + \beta_2 x_2, \sigma^2)$

Example

[Day2: Vickers.dta]

$$y_i = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \xi_i$$

$i = 1, 2, \dots, n$ (subjects)

X_1 : continuous predictor variable

X_2 : categorical predictor variable ($X_2 = 0,1$)

```
. regress post pre i.group // rett metode
```

Source	SS	df	MS
Model	8296.11243	2	4148.05621
Residual	10998.1953	49	224.452965
Total	19294.3077	51	378.319759

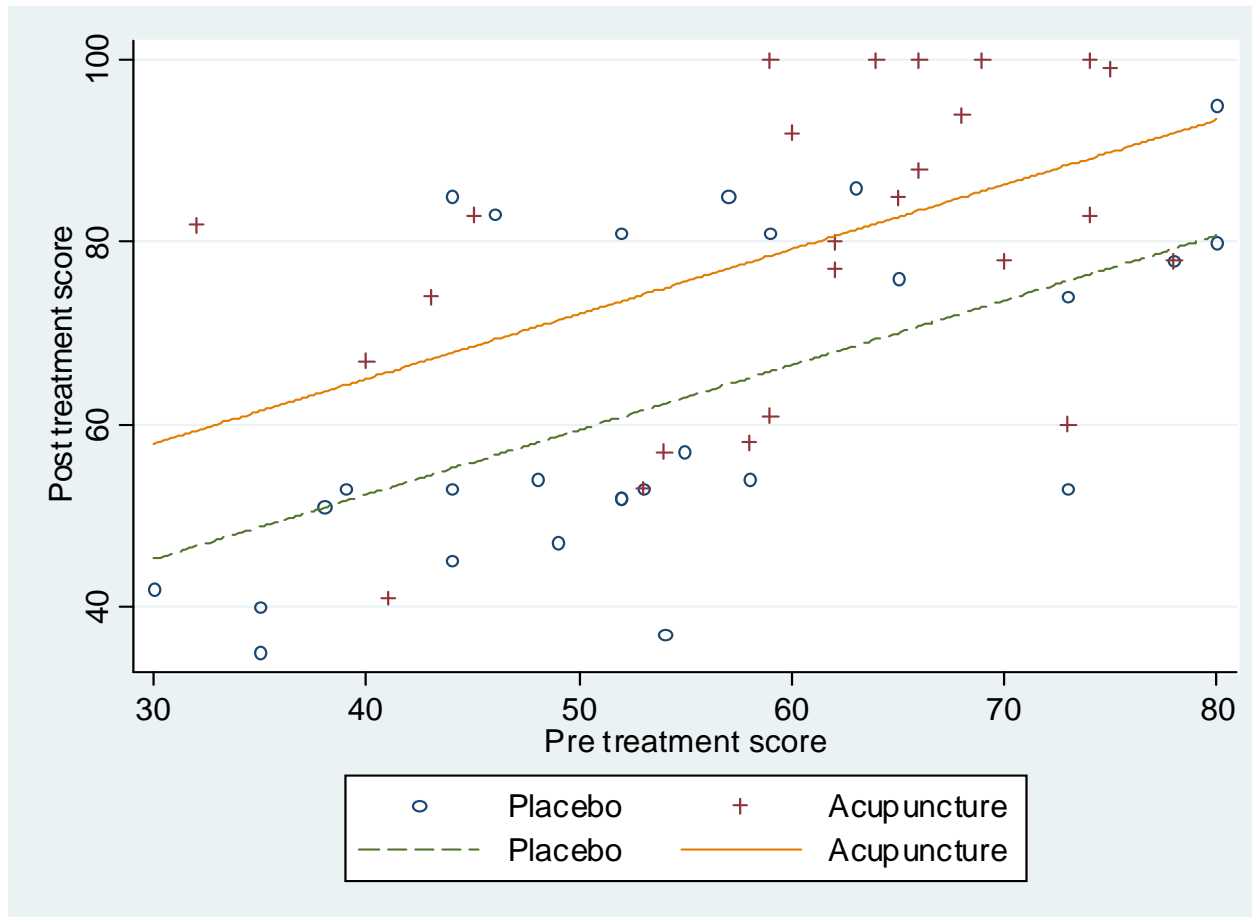
Number of obs = 52
 F(2, 49) = 18.48
 Prob > F = 0.0000
 R-squared = 0.4300
 Adj R-squared = 0.4067
 Root MSE = 14.982

	post	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
	pre	.7102148	.1602363	4.43	0.000	.3882079	1.032222
	1.group	12.70572	4.285715	2.96	0.005	4.093254	21.31819
	_cons	23.99731	9.109231	2.63	0.011	5.691621	42.30299

$\hat{\sigma}^2$

$\hat{\sigma}$

$\hat{\beta}_0$



$$y_i = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \xi_i$$

$i = 1, 2, \dots, n$ (subjects)

X_1 : continuous predictor variable

X_2 : categorical predictor variable ($X_2 = 0, 1$)

VARIANCE COMPONENTS

Example: PEFR

```
. use http://www.stata-press.com/data/mlmus3/pefr, clear
```

```
. list
```

	id	wp1	wp2	wm1	wm2
1.	1	494	490	512	525
2.	2	395	397	430	415
3.	3	516	512	520	508
4.	4	434	401	428	444
5.	5	476	470	500	500
6.	6	557	611	600	625
7.	7	413	415	364	460
8.	8	442	431	380	390
9.	9	650	638	658	642
10.	10	433	429	445	432
11.	11	417	420	432	420
12.	12	656	633	626	605
13.	13	267	275	260	227
14.	14	478	492	477	467
15.	15	178	165	259	268
16.	16	423	372	350	370
17.	17	427	421	451	443

Lung function (peak expiratory flow)

- Two different instruments:
 - Mini Wright peak flow meter ("wm")
 - Wright peak flow meter ("wp")
- Measured in 17 subjects ("id"), on 2 occasions using both instruments (wp1 and wp2, and wm1 and wm2, respectively)
- We will model wm1 and wm2

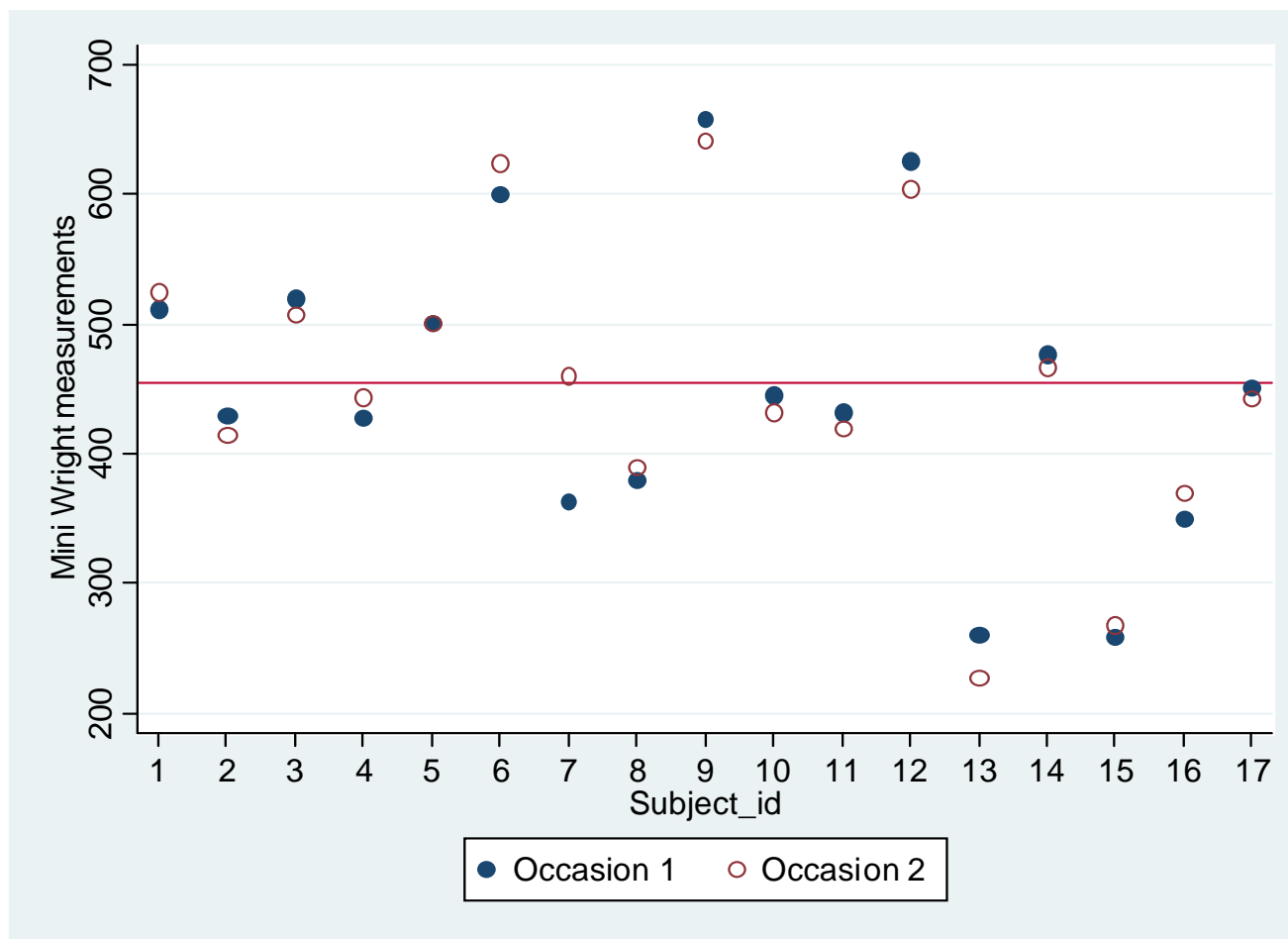
```

. gen mean_wm = (wm1 + wm2)/2 // slide 21, very simple
. egen mean_wm2 = rowmean (wm1 wm2) // using "egen"
. summarize // slide 21

```

Variabl e	Obs	Mean	Std. Dev.	Mi n	Max
id	17	9	5.049752	1	17
wp1	17	450.3529	116.3126	178	656
wp2	17	445.4118	119.6129	165	638
wm1	17	452.4706	113.1151	259	658
wm2	17	455.3529	111.3249	227	642
mean_wm	17	453.9118	111.2912	243.5	650
mean_wm2	17	453.9118	111.2912	243.5	650

```
twoway (scatter wm1 id, msymbol(circle)) ///  
(scatter wm2 id, msymbol(circle_hollow)), ///  
xtitle (Subject_id) xlabel(1/17) ///  
ytitle (Mini Wright measurements) ///  
legend(order(1 "Occasion 1" 2 "Occasion 2")) ///  
yline (453.9118)
```



Variance components

- Total variance is considered to be the sum of (two) independent components:
 - Between subjects
 - Within subjects (i.e. the residual)
- One aspect:
Is variation between subjects larger than the variation of repeated measurements within the same subjects (i.e. on different occasions)?
- No particular interest in the measurement on the subject itself (other than for the subject, of course)
 - ordinary fixed-effects ANOVA is uninteresting

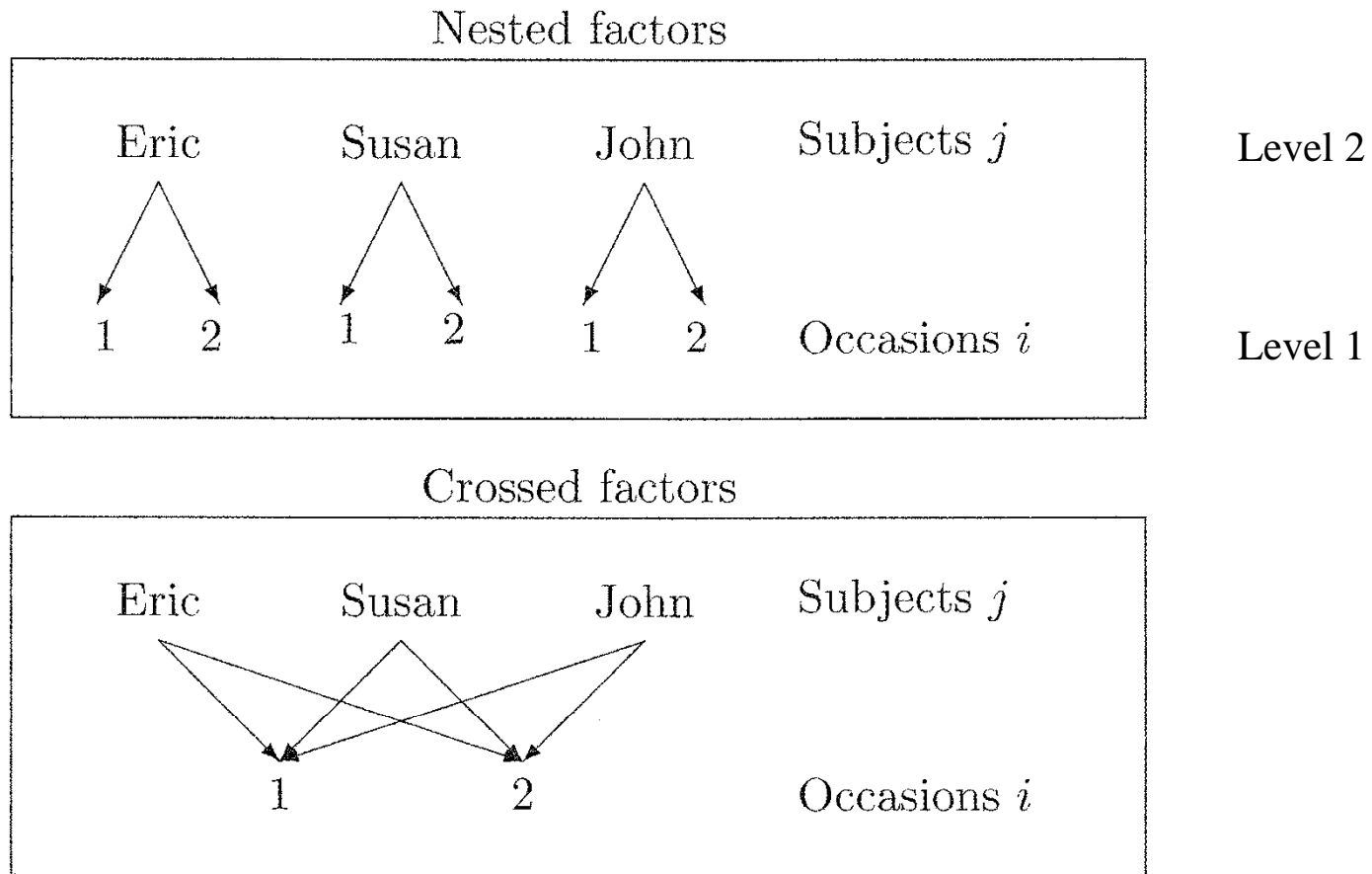


Figure : Illustration of nested and crossed factors



Variance components

$$y_{ij} = \beta + \underbrace{\zeta_j + \varepsilon_{ij}}_{\xi_{ij}}$$

i = occasions nested within subject (here: $i = 1, 2$)

j = subject (here: $j = 1, 2, \dots, 17$)

$$\zeta_j \sim N(0, \psi) \quad \psi : \text{between-subject variance; } SD(\zeta_j) = \sqrt{\psi}$$

Stata *xtreg*: sigma_u

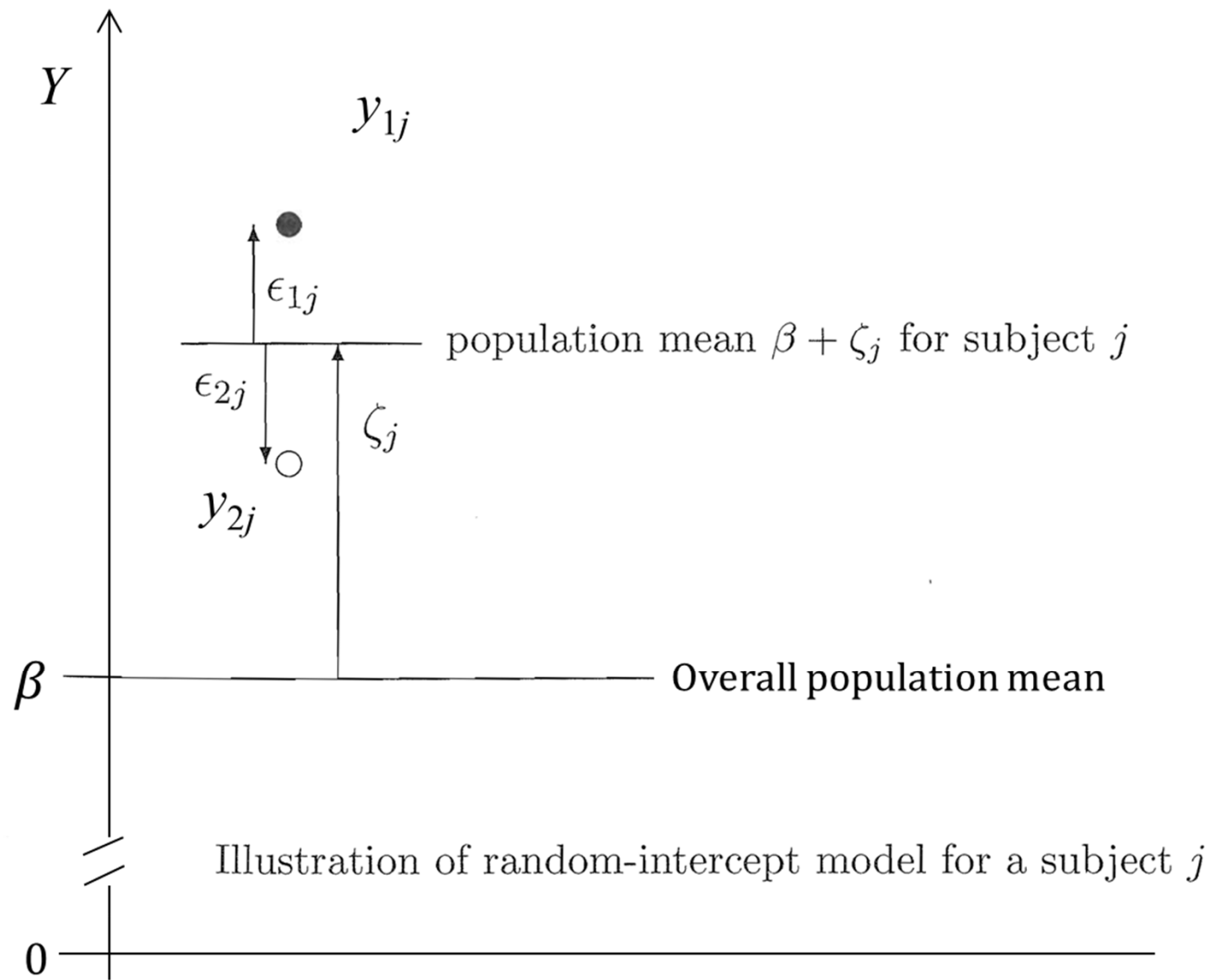
$$\varepsilon_i \sim N(0, \theta) \quad \theta : \text{within-subject (residual) variance; } SD(\varepsilon_i) = \sqrt{\theta}$$

Stata *xtreg*: sigma_e

Stata *xtmixed* uses different notation:

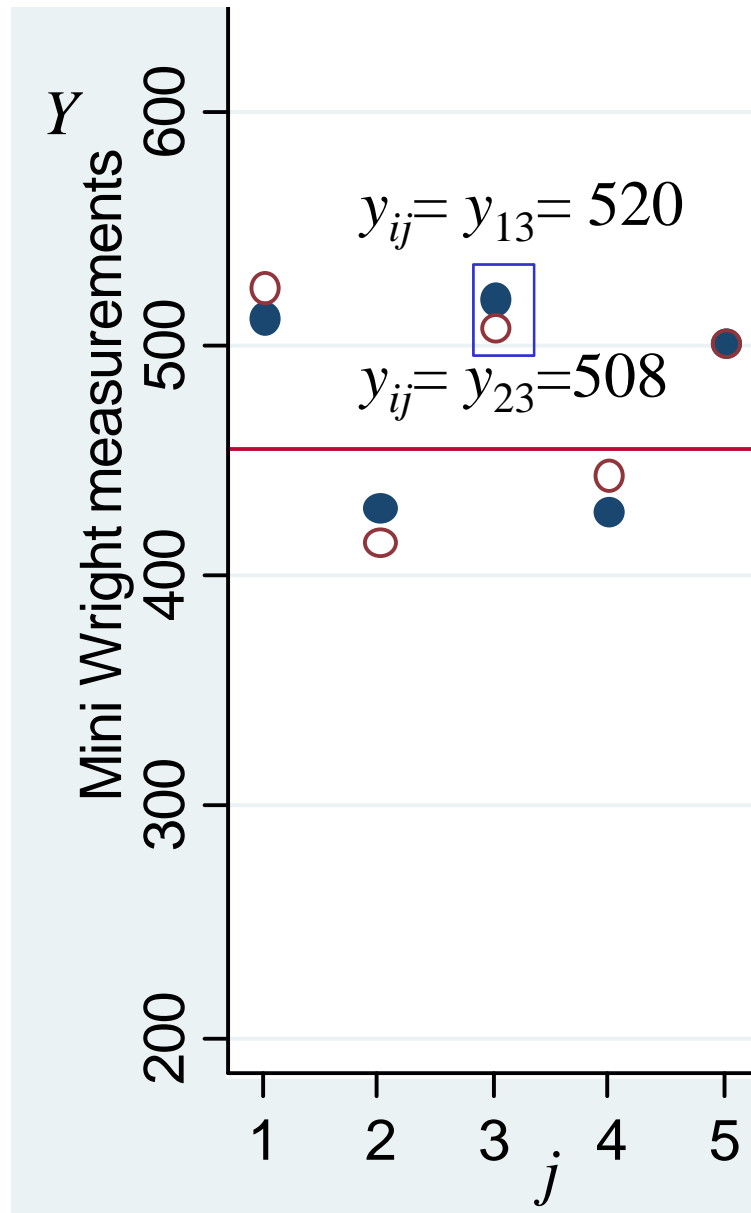
i indicate clusters (here: subjects)

j indicate residuals (here occasions)



. list wm1 wm2 in 1/5

	wm1	wm2
1.	512	525
2.	430	415
3.	520	508
4.	428	444
5.	500	500



$$\hat{\xi}_3 = \frac{508 + 520}{2} - 453.9$$

$$= 514 - 453.9 = 60.1$$

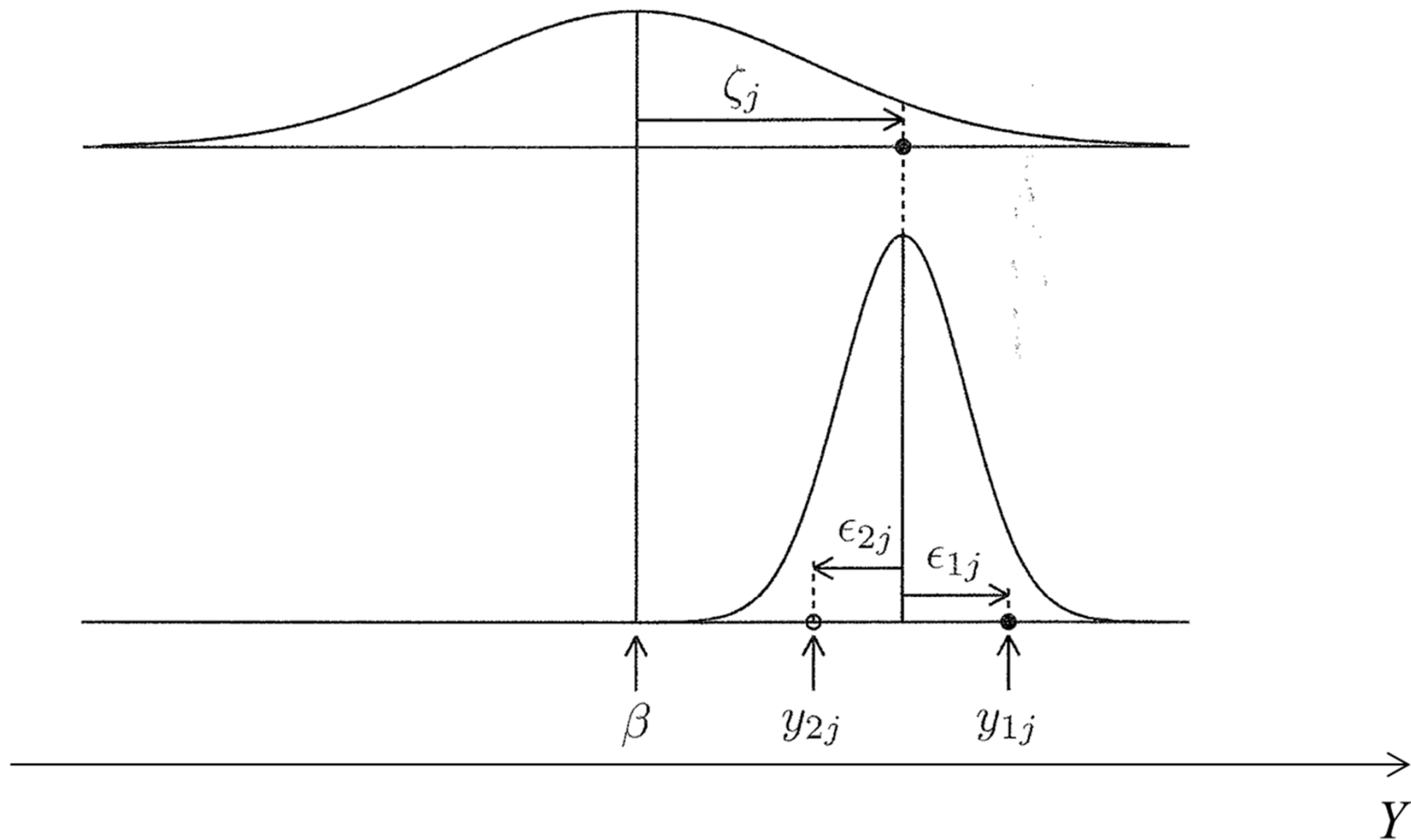
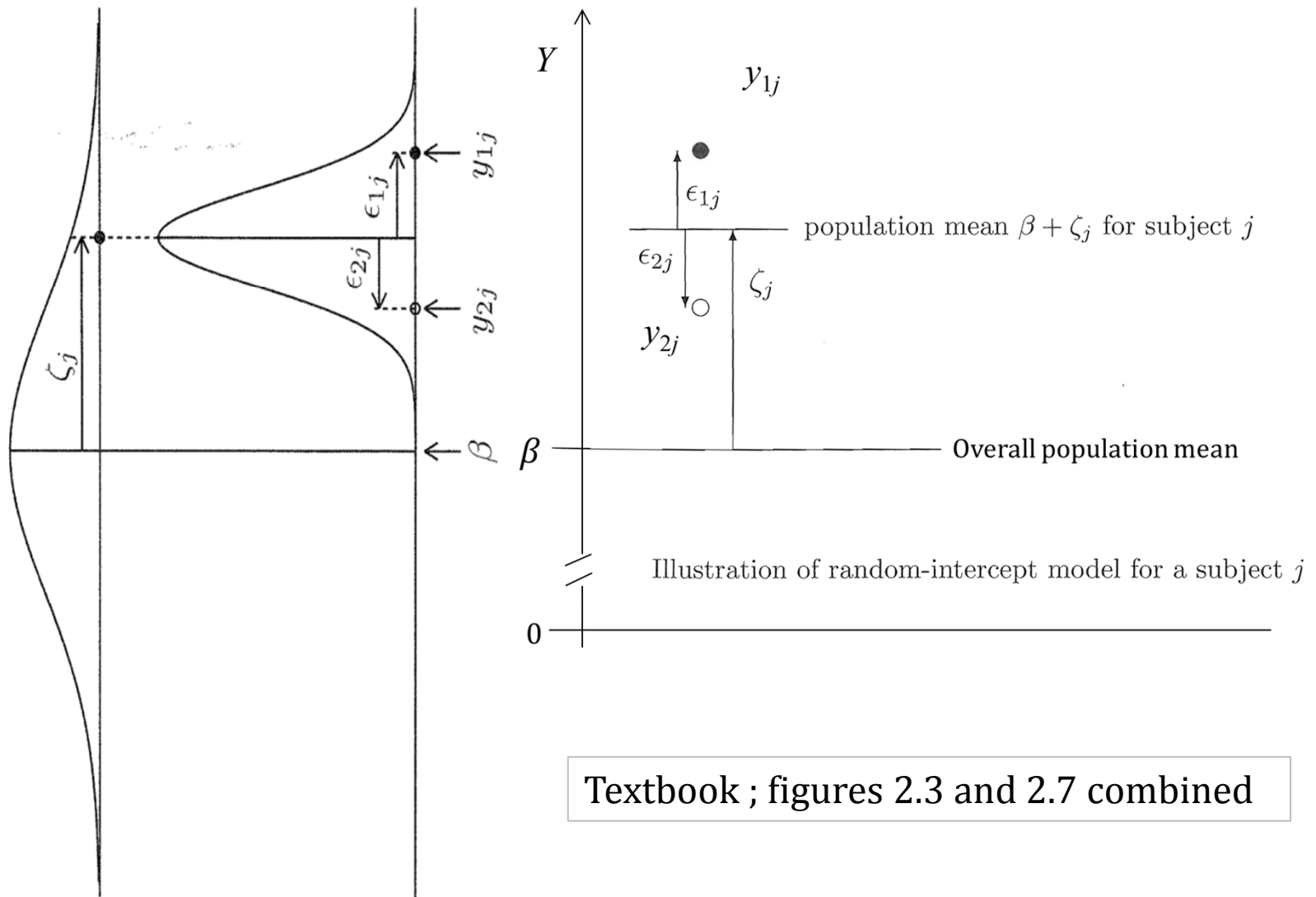


Illustration of distributions of error components for a subject j



Textbook ; figures 2.3 and 2.7 combined

Reshape from wide to long format (usually necessary to run regression models)

. list

	id	wm1	wm2
1.	1	512	525
2.	2	430	415
3.	3	520	508
4.	4	428	444
5.	5	500	500
6.	6	600	625
7.	7	364	460
8.	8	380	390
9.	9	658	642
10.	10	445	432
11.	11	432	420
12.	12	626	605
13.	13	260	227
14.	14	477	467
15.	15	259	268
16.	16	350	370
17.	17	451	443



. reshape long wm, i(id) j(occ) // slide 33
(note: j = 1 2)

Data	wide	->	long
Number of obs.	17	->	34
Number of variables	3	->	3
j variable (2 values)		->	occ
xij variables:	wm1 wm2	->	wm

. list //slide 33

	id	occ	wm
1.	1	1	512
2.	1	2	525
3.	2	1	430
4.	2	2	415
5.	3	1	520
6.	3	2	508
7.	4	1	428
8.	4	2	444
9.	5	1	500
10.	5	2	500
11.	6	1	600
12.	6	2	625
13.	7	1	364
14.	7	2	460
15.	8	1	380
16.	8	2	390

```
. xtreg wm, i(id)
```

```
Random-effects GLS regression
Group variable: id
```

```
Number of obs   =      34
Number of groups =      17
```

```
R-sq:  within = 0.0000
       between = 0.0000
       overall = 0.0000
```

```
Obs per group:  min =      2
                  avg =     2.0
                  max =      2
```

```
corr(u_i, X) = 0 (assumed)
```

```
Wald chi2(0) = .
Prob > chi2 = .
```

wm	$\hat{\beta}$	Coef.	Std. Err.	z	P> z	[95% Conf. Interval]
_cons		453.9118	26.99208	16.82	0.000	401.0083 506.8153
sigma_u		110.39704				
sigma_e		19.910831				
rho		.96849628				(fraction of variance due to u_i)

$\hat{\beta}$

$\sqrt{\hat{\psi}}$

$\sqrt{\hat{\theta}}$

ICC = $\hat{\rho}$

```

. xtreg wm, i(id) mle
Iteration 0:   log likelihood = -187.89003
Iteration 1:   log likelihood = -184.95979
Iteration 2:   log likelihood = -184.76189
Iteration 3:   log likelihood = -184.5855
Iteration 4:   log likelihood = -184.5784
Iteration 5:   log likelihood = -184.57839

```

Random-effects ML regression
Group variable: id

Number of obs = 34
Number of groups = 17

Random effects u_i ~ Gaussian

Obs per group: min = 2
avg = 2.0
max = 2

Log likelihood = -184.57839

Wald chi2(0) = 0.00
Prob > chi2 = .

	$\hat{\beta}$	Coef.	Std. Err.	z	P> z	[95% Conf. Interval]	
$\sqrt{\hat{\psi}}$ wm							
_cons		453.9118	26.18616	17.33	0.000	402.5878	505.2357
/sigma_u		107.0464	18.67858			76.0406	150.6949
/sigma_e		19.91083	3.414659			14.2269	27.8656
rho		.9665602	.0159494			.9210943	.9878545

Likelihood-ratio test of sigma_u=0: $\chi^2(01)=$ 46.27 Prob>=chi bar2 = 0.000

$\sqrt{\hat{\theta}}$

ICC = $\hat{\rho}$

Intraclass correlation – ICC

- ICC may take on values [0,1]
- A measure of the extent the observations within a cluster are closer than the clusters are

Example, pefr.dta

$$\text{ICC} = \rho = \frac{\psi}{\psi + \theta}$$
$$\hat{\rho} = \frac{\hat{\psi}}{\hat{\psi} + \hat{\theta}} = \frac{107.05^2}{107.5^2 + 19.91^2} = 0.97$$

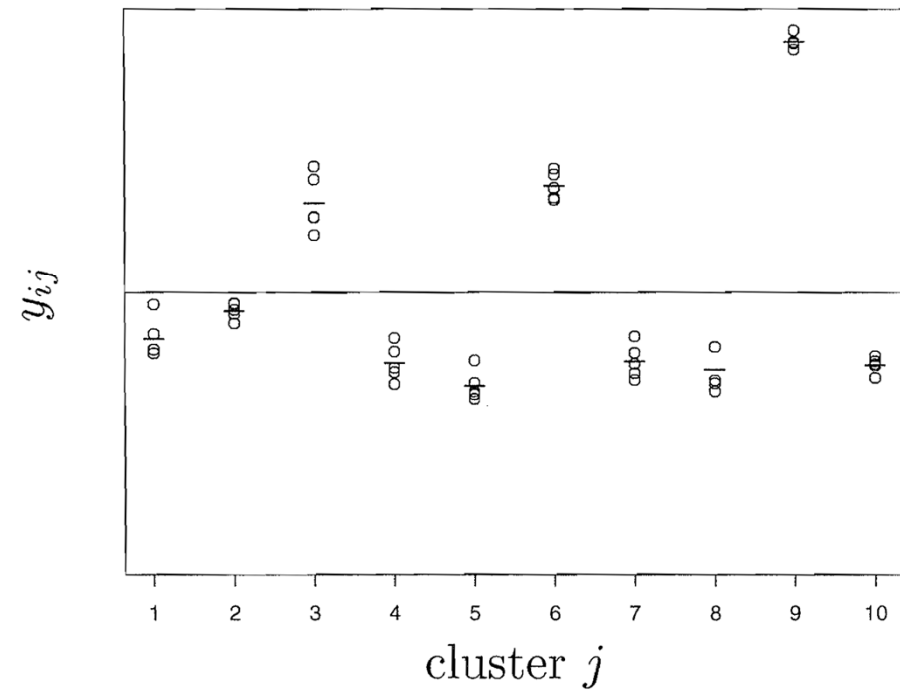
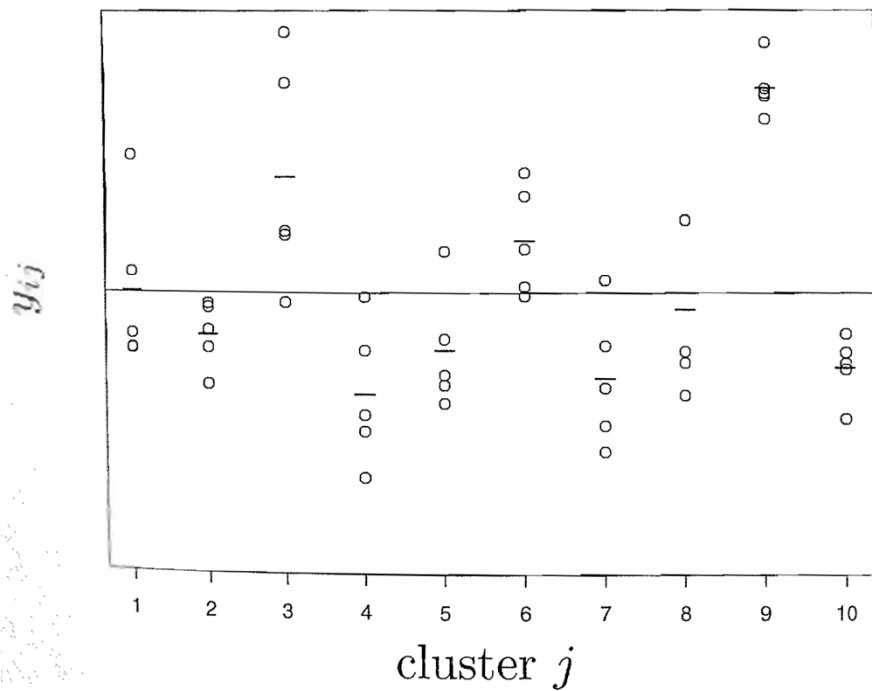


Figure : Illustration of lower intraclass correlation (left) and higher intraclass correlation (right)

General tool in Stata for mixed models¹: mixed

¹“mixed” means that both fixed effects and random effects are present in the model

```
. mixed wm ||id:, stddev mle // slide 38
```

Performing EM optimization:

Performing gradient-based optimization:

Iteration 0: log likelihood = -184.57839

Iteration 1: log likelihood = -184.57839

Computing standard errors:

Mixed-effects ML regression
Group variable: id

Number of obs = 34
Number of groups = 17
Obs per group: min = 2
 avg = 2.0
 max = 2

Log likelihood = -184.57839

Wald chi2(0) = .
Prob > chi2 = .

wm $\hat{\beta}$	Coef.	Std. Err.	z	P> z	[95% Conf. Interval]
_cons	453.9118	26.18617	17.33	0.000	402.5878 505.2357

Random-effects Parameters	Estimate	Std. Err.	[95% Conf. Interval]
id: Identity			
sd(_cons)	107.0464	18.67858	76.04062 150.695
sd(Residual)	19.91083	3.414678	14.22687 27.86564

LR test vs. linear regression: $\chi^2(0) = \sqrt{\hat{\theta}}$ 46.27 Prob >= χ^2 = 0.0000

Obtaining predictions of the random effects (ζ_j)

```
. predict u, reffects // slide 38  
. list id u // slide 38
```

	id	u
1.	1	63.48996
2.	1	63.48996
3.	2	-30.87763
4.	2	-30.87763
5.	3	59.06648
6.	3	59.06648
7.	4	-17.60719
8.	4	-17.60719
9.	5	45.30454
10.	5	45.30454
11.	6	155.8916
12.	6	155.8916
13.	7	-41.19909
14.	7	-41.19909
15.	8	-67.73997
16.	8	-67.73997
17.	9	192.7539
18.	9	192.7539
19.	10	-15.1497
20.	10	-15.1497
21.	11	-27.43715
22.	11	-27.43715
23.	12	158.8406
24.	12	158.8406
25.	13	-206.8339