

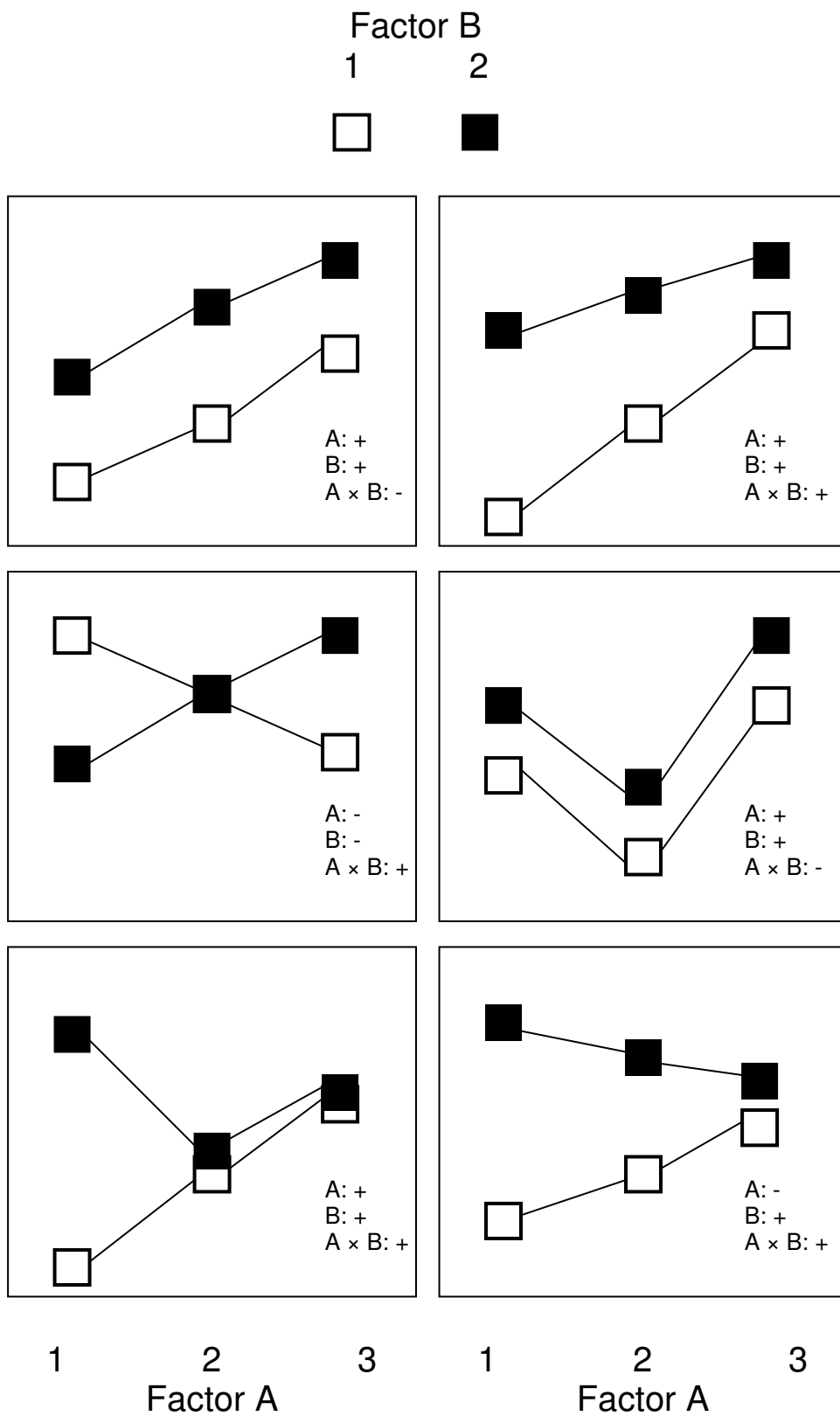
Analysis of variance (II)

- In many cases, we are interested in the effects of more than one factor on our response variable → more complex ANOVAs.
- There is a multitude of different ANOVA models, and which one to apply depends on the structure of the data at hand. Sometimes very complex! I will concentrate on the most commonly used models here.

Two-way factorial ANOVA

- Factorial designs common and powerful in **manipulative experimental studies**.
- Two (or more!) factors are **crossed** in an orthogonal manner – *(preferably same numbers of) observations occur of every level of every factor in combination with every level of all other factors*.
 - * Both factors most often have fixed effects – “Model 1 ANOVA”
 - * Rare that both are random effects factors – “Model 2 ANOVA”
 - * Sometimes a mix between fixed and random effects factors (mixed model ANOVA) – “Model 3 ANOVA”

These models differ quite a bit – important to choose the right model...
- Two distinct types of effects...**main effects** of each factor and **interaction effects**...very important to understand...
 - * **Main effects** are “independent” of other factors (marginal means)
 - * **Interaction effects** occur when the effects of one factor depends on the level(s) of the other factor(s) (i.e.: *lines in effect plots are not parallel!*)

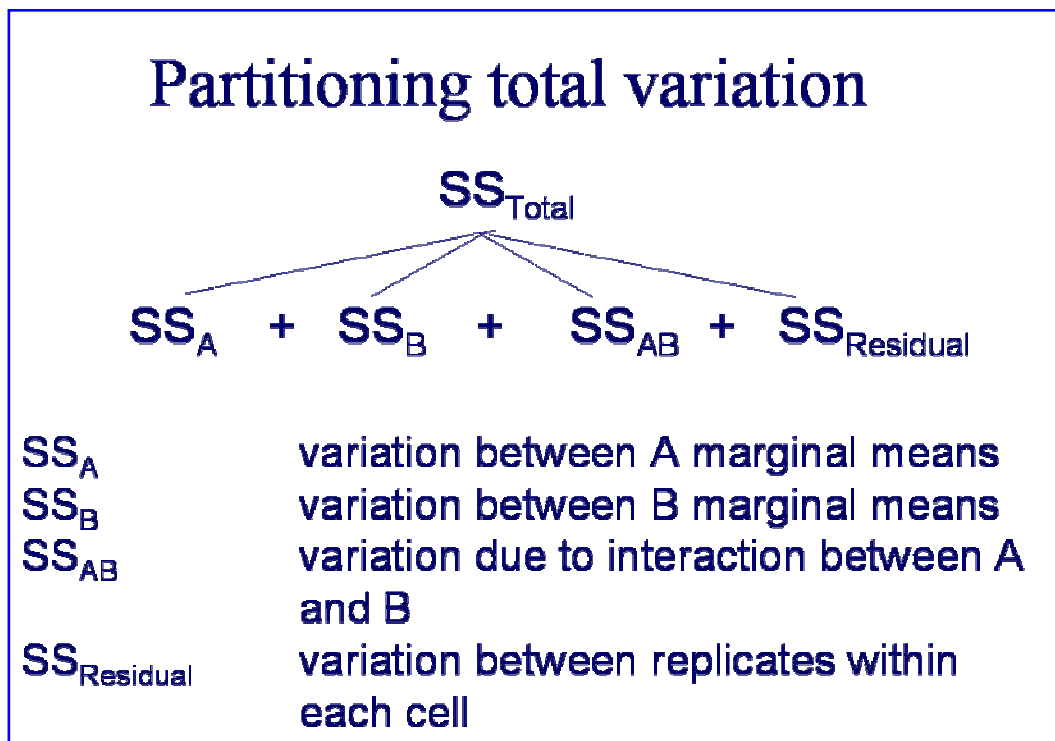


- Consider a two-way design of factor A with p levels and factor B with q levels, k replicates of each “cell” and a total sample size of n .
- The effect model for the two-way factorial ANOVA is then:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

where μ is overall mean, α_i is effect of factor A pooling over all levels of B, β_j is effect of factor B pooling over all levels of A, $(\alpha\beta)_{ij}$ is the effect of the interaction between A and B (i.e., the product) and ε_{ijk} is unexplained residual variation (error). The *marginal mean* is the mean of a treatment level for a given factor averaging over all levels of other factors.

- Note the linear structure of the model and the similarity with multiple regression models.



Factorial ANOVA table			
Source	SS	df	MS
Factor A	SS_A	$p-1$	$\frac{SS_A}{p-1}$
Factor B	SS_B	$q-1$	$\frac{SS_B}{q-1}$
Interaction A X B	SS_{AB}	$(p-1)(q-1)$	$\frac{SS_{AB}}{(p-1)(q-1)}$
Residual	$SS_{Residual}$	$pq(n-1)$	$\frac{SS_{Residual}}{pq(n-1)}$

- Factor A with p levels; factor B with q levels, n replicates of each “cell”.
- SS’s can be calculated in three slightly different ways; called Type I, II and III SS. **Type III SS is *strongly preferable***, especially when data is unbalanced. Type III SS is default in most packages, but **not in R** – watch out! When empty cells occur, an entirely different ANOVA model (means model) is normally most appropriate...
- ***Strive to get balanced data.....!!!!!!!!!!!!!!!!!!!!!!!!!!!!***
- The expected MS (i.e., variance components estimated – or what the estimated MS “represents”) differs importantly depending on whether both factors are fixed (model 1), both are random (model 2) or one is fixed and the other random (model 3; mixed model).
- For this reason, effects are tested with **different F-ratios and df’s** depending on type of model/factors:

“Default” in most software packages!

Standard mixed model version
– generally preferable!

Table 9.11 | F-ratios used for testing main effects and interactions in a two factor ANOVA model for different combinations of fixed and random factors

Source	A and B fixed	A and B random	A fixed, B random	A fixed, B random
			Restricted version	Unrestricted version
A	$\frac{MS_A}{MS_{Residual}}$	$\frac{MS_A}{MS_{AB}}$	$\frac{MS_A}{MS_{AB}}$	$\frac{MS_A}{MS_{AB}}$
B	$\frac{MS_B}{MS_{Residual}}$	$\frac{MS_B}{MS_{AB}}$	$\frac{MS_B}{MS_{Residual}}$	$\frac{MS_B}{MS_{AB}}$
AB	$\frac{MS_{AB}}{MS_{Residual}}$	$\frac{MS_{AB}}{MS_{Residual}}$	$\frac{MS_{AB}}{MS_{Residual}}$	$\frac{MS_{AB}}{MS_{Residual}}$

Example of a typical **fixed factor** output:

Dep Var: LOG_LSP N: 76 Multiple R: 0.4084448 Squared multiple R: 0.1668271

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
VAR00001\$	0.6536209	1	0.6536209	5.1585953	0.0263015
VAR00002\$	0.5611530	3	0.1870510	1.4762693	0.2287519
VAR00001\$					
*VAR00002\$	0.5346707	3	0.1782236	1.4066001	0.2484011
Error	8.6159541	68	0.1267052		

- If random or mixed models, you can use the MS to construct your own ANOVA table and F-values (but keep track of df's for your F ratios!).
- More complex factorial designs also exists; e.g., three-way factorial, fractional factorial, etc. Often special models are implemented for the latter type of designs (limited possibility to test interactions).

Nested ANOVA

- Nested designs are different from crossed factorial designs, in that the *actual factor levels of the nested factor are different within each level of the main factor.*

Crossed design:

Factor A	1			2			i		
Factor B	1	2	j	1	2	j		1	2	j
Reps	y_{111}							y_{ij1}		
	y_{112}							y_{ij2}		
	y_{11k}							y_{ijk}		
Cell means	y_{11}							y_{ij}		

Nested design:

Factor A	1			2			i		
Factor B	A	B	jj	C	D	jk	E	F	jl	
Reps	y_{111}							y_{ij1}		
	y_{112}							y_{ij2}		
	y_{11k}							y_{ijk}		
Cell means	y_{11}							y_{ij}		

- Nested factor **typically random**; often some level of spatial or temporal subsampling or replication. Expressed in tables as e.g. B(A) or B[A]; meaning that the nested factor B is nested within the main factor A.
- Main factor/s can be either fixed or random.
- Interactions cannot normally be tested in nested designs → because levels of one factor are unique to (i.e., is not replicated over) levels of the other, we can not tell whether one level depends on levels of the other...

Table 9.3 ANOVA table for two factor nested linear model with factor A (p levels), factor B (q levels) nested within A, and n replicates within each combination of A and B

Source	SS	df	MS
A	$nq \sum_{i=1}^p (\bar{y}_i - \bar{y})^2$	$p - 1$	$\frac{SS_A}{p - 1}$
B(A)	$n \sum_{i=1}^p \sum_{j=1}^q (\bar{y}_{j(i)} - \bar{y}_i)^2$	$p(q - 1)$	$\frac{SS_{B(A)}}{p(q - 1)}$
Residual	$\sum_{i=1}^p \sum_{j=1}^q \sum_{k=1}^n (y_{ijk} - \bar{y}_{j(i)})^2$	$pq(n - 1)$	$\frac{SS_{Residual}}{pq(n - 1)}$
Total	$\sum_{i=1}^p \sum_{j=1}^q \sum_{k=1}^n (y_{ijk} - \bar{y})^2$	$pqn - 1$	

Table 9.4 Expected mean squares and F-ratios for tests of null hypotheses for two factor nested ANOVA model

Source	A fixed, B random		A fixed, B fixed	
	Expected mean square	F-ratio	Expected mean square	F-ratio
A	$\sigma_\epsilon^2 + n\sigma_\beta^2 + nq \frac{\sum_{i=1}^p \alpha_i^2}{p - 1}$	$\frac{MS_A}{MS_{B(A)}}$	$\sigma_\epsilon^2 + nq \frac{\sum_{i=1}^p \alpha_i^2}{p - 1}$	$\frac{MS_A}{MS_{Residual}}$
B(A)	$\sigma_\epsilon^2 + n\sigma_\beta^2$	$\frac{MS_{B(A)}}{MS_{Residual}}$	$\sigma_\epsilon^2 + n \frac{\sum_{i=1}^p \sum_{j=1}^q \beta_{j(i)}^2}{p(q - 1)}$	$\frac{MS_{B(A)}}{MS_{Residual}}$
Residual	σ_ϵ^2	σ_ϵ^2		

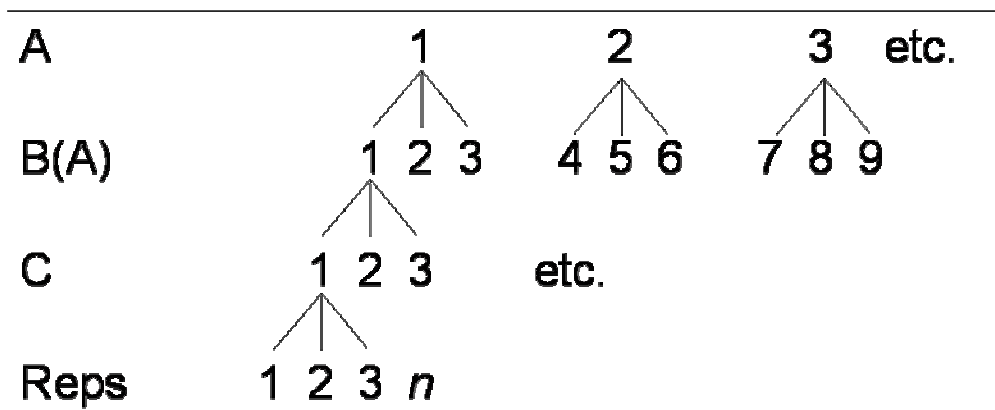
Note that both are treated as fixed factors as default in most programs –
WRONG in most cases – recalculate F-ratios and P-values “by hand”!

Example output two-way **nested** design:

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Dep Var: SON_SIZE   N: 102   Multiple R: 0.7113482   Squared multiple R: 0.5060163

Analysis of Variance
Source              Sum-of-Squares    df    Mean-Square    F-ratio    P
TREAT$              0.0674099        1      0.0674099      4.5802317  0.0365604
SIRE$(TREAT$)       0.8525990       42      0.0203000      1.3793016  0.1273081
Error               0.8536194       58      0.0147176
-----
```

- One can have more than one nested factor (e.g., $Y = A + B[A] + C[B[A]]$)
- More complex designs include **mixes of crossed and nested designs**; sometimes called “partly nested ANOVA”. For example, a factor B can be crossed with a factor C but nested within another factor A:



ANOVA table:

Source	df
A	$(p-1)$
B(A)	$p(q-1)$
C	$(r-1)$
A * C	$(p-1)(r-1)$
B(A) * C	$p(q-1)(r-1)$
Residual	$pqr(n-1)$

Because C is crossed with B(A), we can estimate the interaction between these

Randomized block designs and ANOVA

- In RB designs, we have one or more main factors of interest and a nuisance factor – a factor which introduces error that we wish to “control” for – often a result of design constraint...
- The nuisance factor is called a **blocking factor**, and treated as a **random effects factor** (often some sort of **spatial grouping** of replicates).
- Each main factor level (or combination of factors levels) is applied to **one observational/experimental unit per block** (i.e., design is **unreplicated** – if replicated, it is a factorial crossed mixed model...).
- Units **randomly** allocated to treatment levels **within blocks**!
- Can be powerful if replication is restricted (*"Block what you can, randomize what you cannot."*)

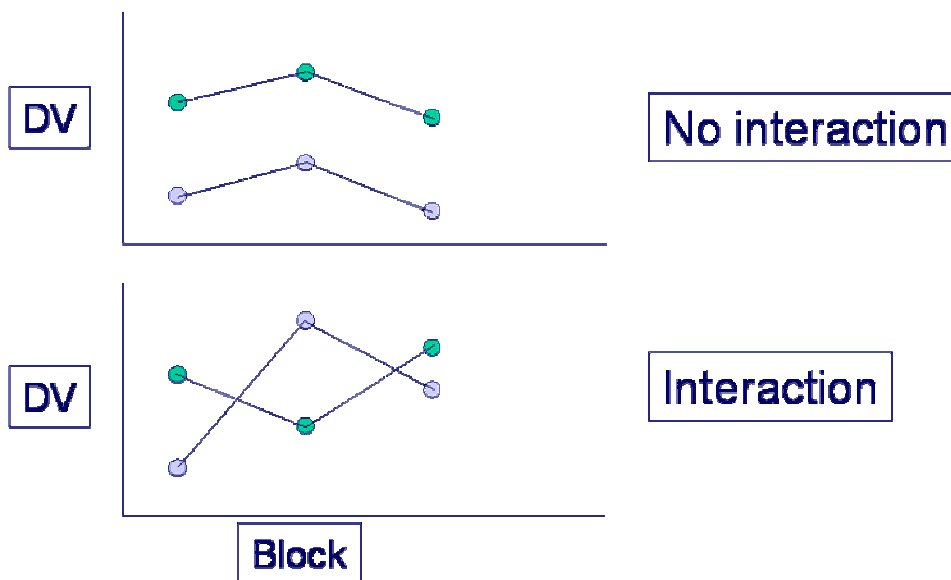
ANOVA df's (# of treatments or groups for factor A = p, # of blocks = q):

Source

Factor A	p-1
Blocks	q-1
Residual	(p-1)(q-1)
Total	pq-1

- In essence, randomised block ANOVA is 2 factor factorial design BUT with no replicates within each cell (treatment-block combination) - i.e. an unreplicated 2 factor design. This means:
 - * No measure of within-cell variation, so...
 - * No test for treatment by block interaction
- *Powerful*, but **only if** blocking factor explains much variation!
- *Weak* if blocking factor is unimportant – removing the blocking factor actually increases power...(denominator df differs).
- One *problematic and important assumption*: no block \times treatment interaction. Cannot be tested (se above) – at least do visual inspections!!

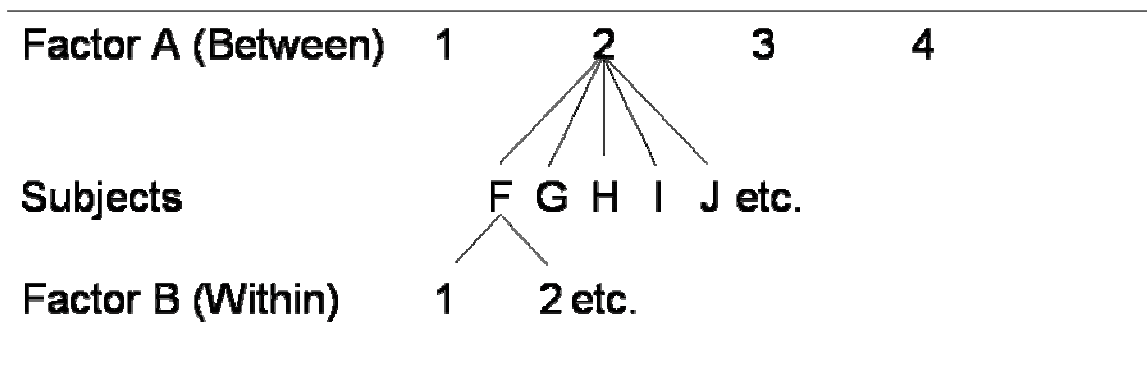
Interaction plots



Repeated measures designs and ANOVA

- In repeated measures designs (or “within-subjects designs”, or “split-plot designs”), two types of factors are involved:
 - * **Between subjects factors** – levels differ across subjects (plots)
 - * **Within subjects factors** – levels differ within subjects (plots)
- *Subjects is our experimental unit* (to which the between subjects factor is applied) and are typically individuals, plots, populations, etc.
- Within subjects factor is often, but does in no way not need to be, *time*.
- **Unreplicated** in the sense that there is only one observation for each between/within subject level combination!
- Appropriate if
 - 1) Number of subjects limited and we wish to evaluate several factors
 - 2) We suspect that units are different – we can “treat it away”...
 - 3) We are interested in following temporal trends

Example....



- In essence, “subject” is a nested random effects factor that we are not really interested in....

- In addition to the usual assumptions, randomized block designs and repeated measures designs make assumptions about the pattern of variance/covariance within blocks/subjects – **the sphericity assumption**.
- In reality, this is violated in many repeated measures data sets and quite commonly in randomized block data sets. Do two things (see output above!):
 - 1) Degree of violation should be estimated by epsilon (ϵ), which equals one under perfect sphericity.
 - 2) F-tests can then be adjusted for degree of non-sphericity: note that G-G often too conservative and H-F often too liberal....

Additional notes on (even more) complex ANOVA's

- No upper limit on the amount of complexity possible in an ANOVA framework – both for design and analysis!
- When running even more complex ANOVAs – at least **look in a stats book** and make sure that your variables are treated correctly (fixed/random), that the F-ratios are correct and that the df's for the tests are correct! This level of “understanding” is needed...software output can be “wrong” (i.e., inappropriate for your data).
- We make the same set of assumptions as for one-way ANOVA and regression - don't forget to at least **inspect/check the residuals**...(see earlier lecture)!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!
- If you do need to run post-hoc tests – post-hoc contrasts of all sorts are straightforward also for most more complex designs (see earlier lecture).