Introduction to Nested (hierarchical) ANOVA

Partitioning variance hierarchically

Two factor nested ANOVA

- Factor A with *p* groups or levels
  - fixed or random but usually fixed
- Factor B with *q* groups or levels within each level of A
  - usually random
- Nested design:
  - different (randomly chosen) levels of Factor B in each level of Factor A
  - often one or more levels of subsampling

Sea urchin grazing on reefs

- Andrew & Underwood (1997)
- Factor A - fixed
  - sea urchin density
  - four levels (0% original, 33%, 66%, 100%)
- Factor B - random
  - randomly chosen patches
  - four (3 to $4m^2$) within each treatment

Sea urchin grazing on reefs

- Residual:
  - 5 replicate quadrats within each patch within each density level
- Response variable:
  - % cover of filamentous algae

Worked example

Density	0	33	etc.
Patch	1 2 3 4	5 6 7 8	
Reps	n = 5 in each of 16 cells		

p = 4 densities, q = 4 patches

Data layout

Factor A	1	2	i
A means	$\bar{y}_1$	$\bar{y}_2$	$\bar{y}_i$
Factor B	1…j….4	5...j….8	9...j….12
B means (q=4)	$\bar{y}_{11}$		$\bar{y}_{ij}$
Reps	y_{111}		y_{ij1}
	y_{112}		y_{ij2}
	…		...
	y_{11k}		y_{ijk}

Linear model

$$y_{ijk} = \mu + \alpha_i + \beta_{j(i)} + \varepsilon_{ijk}$$

where

μ	overall mean
α_i	effect of factor A (μ_i - μ)
$\beta_{j(i)}$	effect of factor B within each level of A (μ_{ij} - μ_i)
ε_{ijk}	unexplained variation (error term) - variation within each cell

Linear model

(% cover algae)$_{ijk}$ = μ + (sea urchin density)$_i$ + (patch within sea urchin density)$_{j(i)}$ + ε_{ijk}

Worked example

Density	0	33	etc.
Patch	1 2 3 4	5 6 7 8	
Reps	n = 5 in each of 16 cells		

p = 4 densities, q = 4 patches

Effects

- Main effect:
  - effect of factor A
  - variation between factor A marginal means
- Nested (random) effect:
  - effect of factor B within each level of factor A
  - variation between factor B means within each level of A

Null hypotheses

- H_0: no difference between means of factor A
  - $\mu_1 = \mu_2 = \ldots = \mu_i = \mu$
- H_0: no main effect of factor A:
  - $\alpha_1 = \alpha_2 = \ldots = \alpha_i = 0$
  - $\alpha_i = (\mu_i - \mu) = 0$

Sea urchin example

- No difference between urchin density treatments
- No main effect of density

Null hypotheses

- H_0: no difference between means of factor B within any level of factor A
  - $\mu_{11} = \mu_{12} = \ldots = \mu_{1j}$
  - $\mu_{21} = \mu_{22} = \ldots = \mu_{2j}$
  - etc.
- H_0: no variance between levels of nested random factor B within any level of factor A:
  - $\sigma_\beta^2 = 0$

Sea urchin example

- No difference between mean filamentous algae cover for patches within any urchin density treatment
- No variance between patches within each density treatment

Residual variation

- Variation between replicates within each cell
- Pooled across cells if homogeneity of variance assumption holds

$$\sum (y_{ijk} - \bar{y}_{ij})^2$$

Partitioning total variation

SS_{Total}

SS_A + $SS_{B(A)}$ + $SS_{Residual}$

SS_A	variation between A marginal means
$SS_{B(A)}$	variation between B means within each level of A
$SS_{Residual}$	variation between replicates within each cell

Nested ANOVA table

Source	SS	df	MS
Factor A	SS_A	p-1	$\frac{\text{SS A}}{p-1}$
Factor B(A)	$SS_{B(A)}$	$p(q$-1)	$\frac{\text{SS B(A)}}{p(q-1)}$
Residual	$SS_{Residual}$	$pq(n$-1)	$\frac{\text{SS Residual}}{pq(n-1)}$

Expected mean squares

A fixed, B random:

- MS_A $\quad \sigma^2 + n\sigma_\beta^2 + \frac{nq\sum\alpha_i^2}{p-1}$
- $MS_{B(A)}$ $\quad \sigma^2 + n\sigma_\beta^2$
- $MS_{Residual}$ $\quad \sigma^2$

Testing null hypotheses

- If no main effect of factor A:
  - H_0: $\mu_1 = \mu_2 = \mu_i = \mu$ ($\alpha_i = 0$) is true
  - *F*-ratio MS_A / $MS_{B(A)} \leq 1$
- If no nested effect of random factor B:
  - H_0: $\sigma_\beta^2 = 0$ is true
  - *F*-ratio $MS_{B(A)}$ / $MS_{Residual} \leq 1$

MS_A

$$\sigma^2 + n\sigma_\beta^2 + \frac{nq\sum \alpha_i^2}{p-1}$$

$MS_{B(A)}$

$$\sigma^2 + n\sigma_\beta^2$$

$MS_{Residual}$

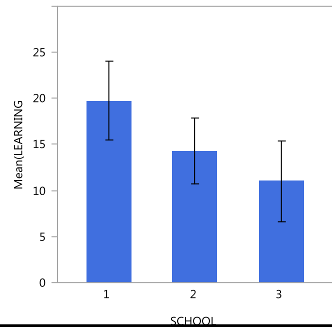
$$\sigma^2$$

Additional tests

- Main effect:
  - planned contrasts & trend analyses as part of design
  - unplanned multiple comparisons if main *F*-ratio test significant
- Nested effect:
  - usually random factor
  - Sometimes little interest in further tests
  - *Often can provide information on the characteristic spatial signal of a population*

Worked example

- What is the effect of schools on standardized tests?
- Is the effect of school driven in part by differences in teachers



Collected data (three schools, two teachers at each schools, two scores per teacher

	Teacher 1	Teacher 2
School 1	25	14
	29	11
School 2	11	22
	6	18
School 3	17	5
	20	2

True data matrix, accounts for teachers not being the same at each school

	Teacher 1	Teacher 2	Teacher 3	Teacher 4	Teacher 5	Teacher 6
School 1	25	14				
	29	11				
School 2			11	22		
			6	18		
School 3					17	5
					20	2

Data format for statistics

School	Teacher	Score
1	1	25
1	1	29
1	2	14
1	2	11
2	3	11
2	3	6
2	4	22
2	4	18
3	5	17
3	5	20
3	6	5
3	6	2

No obvious effect of School

- After accounting for teacher effect

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	5	724.00000	144.800	20.6857
Error	6	42.00000	7.000	**Prob > F**
C. Tota	11	766.00000		0.0010 *

Variance Component Estimates

Component	Var Comp Est	Percent of Total
TEACHER[SCHOOL]&Rando	91.08333	92.863
Residual	7	7.137
Total	98.08333	100.000

These estimates based on equating Mean Squares to Expected Value.

Tests wrt Random Effects

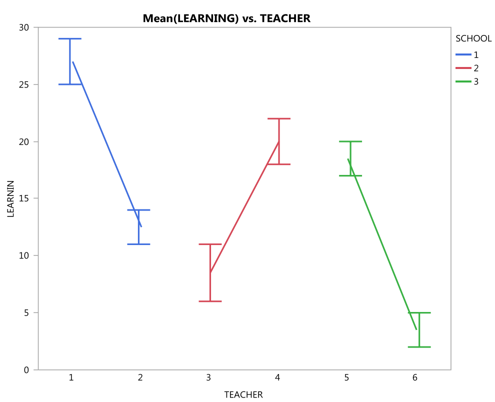
Source	SS	MS Num	DF Num	F Ratio	Prob > F
SCHOOL	156.5	78.25	2	0.4137	0.6940
TEACHER[SCHOOL]&Rando	567.5	189.167	3	27.0238	0.0007 *

Test Denominator Synthesis

Source	MS Den	DF Den	Denom MS Synthesis
SCHOOL	189.167	3	TEACHER[SCHOOL]&Rando
TEACHER[SCHOOL]&Rando	7	6	Residual

Big effect of teacher!!

- What about school effect
  - Test MS $_{\text{school}}$ / MS $_{\text{teacher(school)}}$



Spatially nested designs

- Used to provide information on the characteristic spatial signal of populations
- Other techniques (geostatistical models) also can do this but nested models are very efficient
- Variance component models (part of nested) can provide the percent of variation that is associated with particular spatial scales.

Kelp Forests

Giant kelp's range
CA to the south
Santa Cruz
~1450 km
Asunción
N
Pacific Ocean

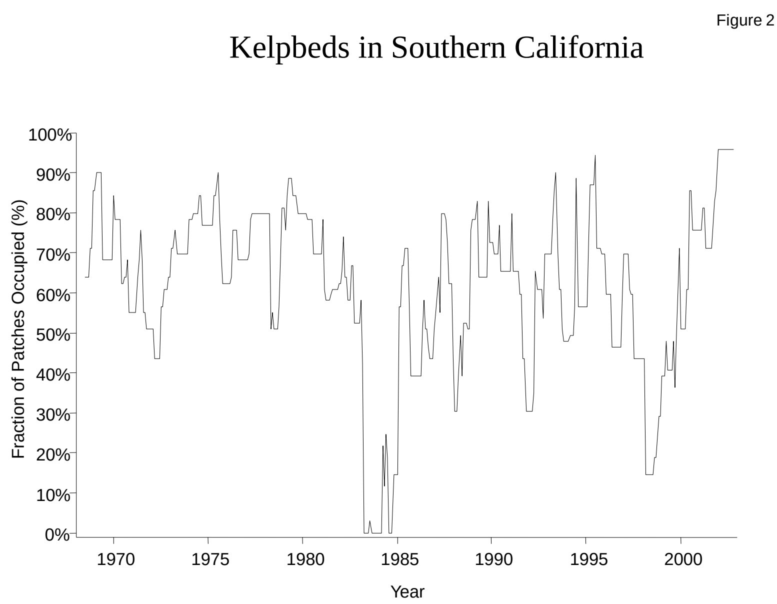


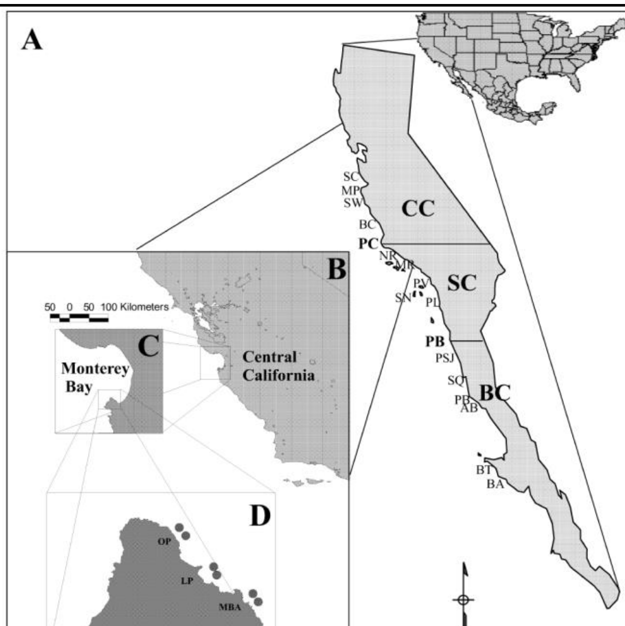
Figure 2
Kelpbeds in Southern California
Fraction of Patches Occupied (%)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1970
1975
1980
1985
1990
1995
2000
Year

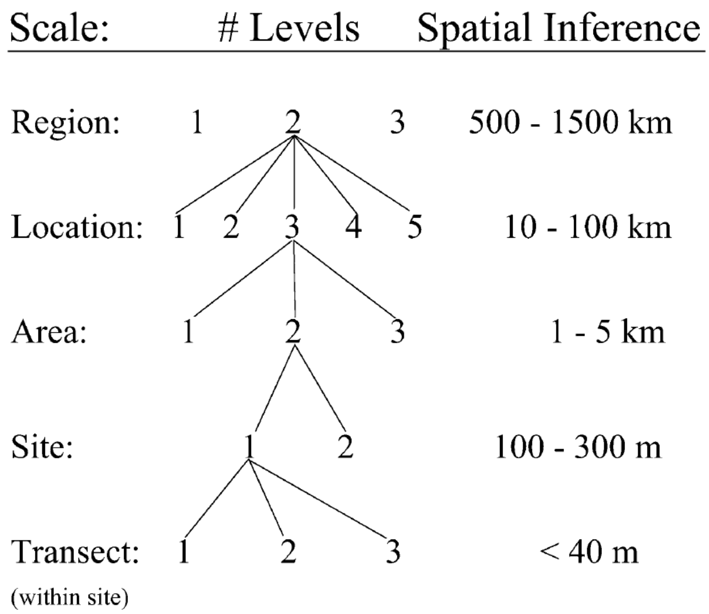
Oecologia (2004) 138: 436–447
DOI 10.1007/s00442-003-1452-8

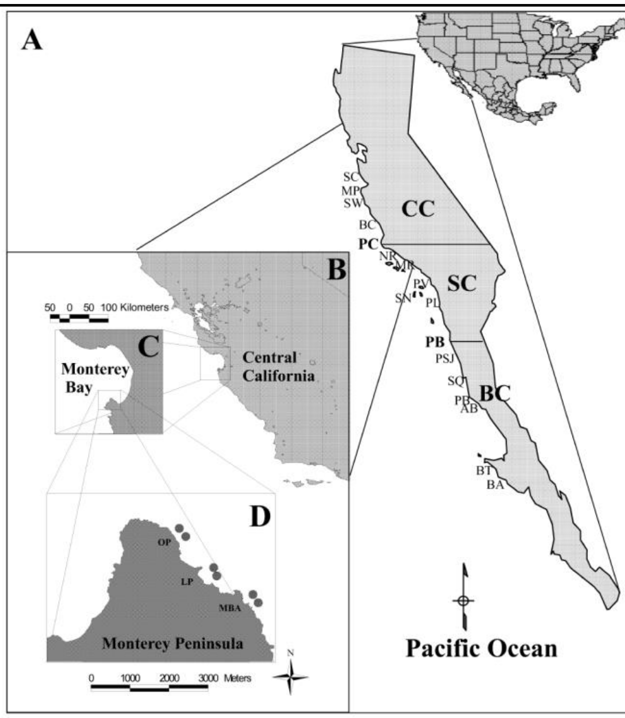
ECOSYSTEM ECOLOGY

Matthew S. Edwards

Estimating scale-dependency in disturbance impacts: El Niños and giant kelp forests in the northeast Pacific

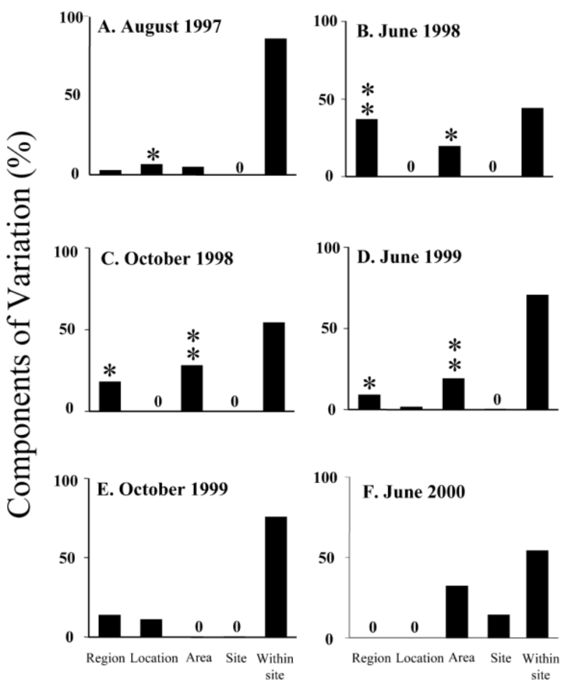


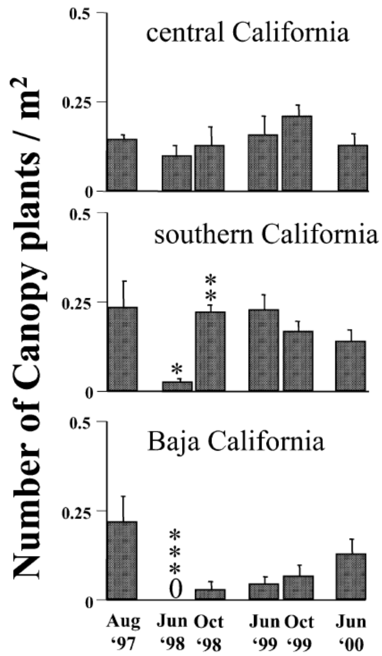

Scale: # Levels Spatial Inference
Region: 1 2 3 500 - 1500 km
Location: 1 2 3 4 5 10 - 100 km
Area: 1 2 3 1 - 5 km
Site: 1 2 100 - 300 m
Transect: 1 2 3 < 40 m
(within site)


A
SC
MP
SW
CC
BC
PC
NP
MB
PV
SN
PL
SC
PB
PSJ
SQ
PB
AB
BC
BT
BA
Pacific Ocean
B
50 0 50 100 Kilometers
C
Monterey Bay
Central California
D
OP
LP
MBA
Monterey Peninsula
0 1000 2000 3000 Meters
N

Complex nested designs

Source	Expected mean square	Variance Component	F-ratio
Region: A	$\sigma_\varepsilon^2 + n\sigma_\delta^2 + nk\sigma_\gamma^2 + nkr\sigma_\beta^2 + nkrq\sigma_\alpha^2$	$\frac{MS_A - MS_{B(A)}}{nkrq}$	$\frac{MS_A}{MS_{B(A)}}$
Location: B(A)	$\sigma_\varepsilon^2 + n\sigma_\delta^2 + nk\sigma_\gamma^2 + nkr\sigma_\beta^2$	$\frac{MS_{B(A)} - MS_{C(B(A))}}{nkr}$	$\frac{MS_{B(A)}}{MS_{C(B(A))}}$
Area: (C(B(A))	$\sigma_\varepsilon^2 + n\sigma_\delta^2 + nk\sigma_\gamma^2$	$\frac{MS_{C(B(A))} - MS_{D(C(B(A)))}}{nk}$	$\frac{MS_{C(B(A))}}{MS_{D(C(B(A)))}}$
Site: D(C(B(A)))	$\sigma_\varepsilon^2 + n\sigma_\delta^2$	$\frac{MS_{D(C(B(A)))} - MS_{Residual}}{n}$	$\frac{MS_{D(C(B(A)))}}{MS_{Residual}}$
Transect: Residual E(D(C(B(A))))	σ_ε^2		





Introduction to Repeated Measures designs

Two major types of repeated measures ANOVA

- Subjects used repeatedly but performance is unlikely to be linked to order (timing)
  - Same subjects used for a series of treatments, treatment order randomized among subjects
- Subjects used repeatedly and performance is likely to be linked to order (timing)
  - Performance = growth, size, etc

Subjects used repeatedly but performance is unlikely to be linked to order (timing)

- Example: the effect of four types of drugs on blood pressure compared between men and women
  - Gender is fixed effect (consider between subject effect)
  - Each subject (within a gender) receives all four drugs (within subject effects)
  - Drug order is:
    - Random and
    - Separation between drugs is assumed to be long enough that there are no carryover effects

Subjects used repeatedly and performance is likely to be linked to order (timing)

- Example: effect of 4 hormones on individual size of fish. Measurements taken repeatedly over time
  - Hormone effect is ‘between subject’ effect
  - Time and Time*Hormone levels are ‘within subject’ effects
  - Separate error terms for between and within subject effects
    - Between subject effects are estimated using (eq.) of means of all temporal measurements (one estimate per individual)
    - Within subject effects are estimated using all measurements (temporal replicates within individuals are used)

Repeated measures designs

- Each whole plot (or individual) measured repeatedly under different treatments and/or times
- Within plots (individual) factor often time, or at least treatments applied through time
- Plots (individuals) termed “subjects” in repeated measures terminology

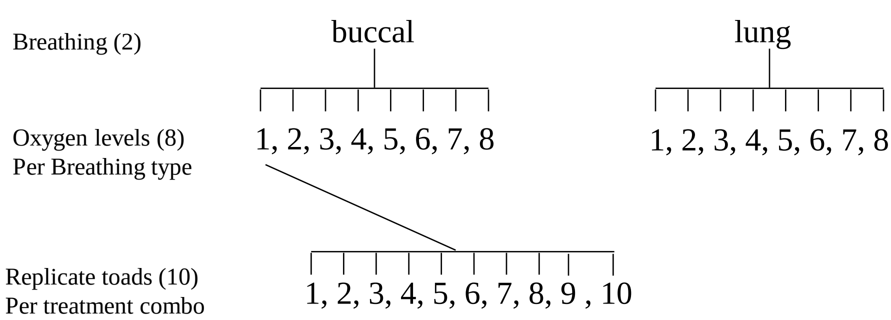
Cane toad breathing and hypoxia

Cane toad breathing and hypoxia

- How do cane toads respond to conditions of hypoxia?
  - Mullens (1993)
- Two factors:
  - Breathing type (buccal vs lung breathers)
  - O_2 concentration (8 different $[O_2]$)
- 10 replicates per breathing type and $[O_2]$ combination
- Response variable is breathing rate

Completely randomized design

- 2 factor design (2 x 8) with 10 replicates
  - total number of toads = 160
- Toads are expensive
  - reduce number of toads?
- Lots of variation between individual toads
  - reduce between toad variation?



160 reps in a completely randomized design

Repeated measures designs

- Factor A:
  - units of replication termed “subjects”
- Factor B (subjects) nested within A
- Factor C:
  - repeated recordings on each subject

Repeated measures design

Breathing type	Toad	$[O_2]$ 1	2	3	4	5	6	7	8
Lung	1	x	x	x	x	x	x	x	x
Lung	2	x	x	x	x	x	x	x	x
…	…	…	…	…	…	…	…	…	…
Lung	9	x	x	x	x	x	x	x	x
Buccal	10	x	x	x	x	x	x	x	x
Buccal	12	x	x	x	x	x	x	x	x
…	…	…	…	…	…	…	…	…	…
Buccal	21	x	x	x	x	x	x	x	x

Repeated measures design

- Factor A is breathing type:
  - lung vs buccal
  - applied to toads = subjects = plots
- Factor B is subjects (i.e. toads) nested within A
- Factor C is $[O_2]$ treatment
  - 0, 5, 10, 15, 20, 30, 40, 50%
  - applied to toads (subjects) repeatedly

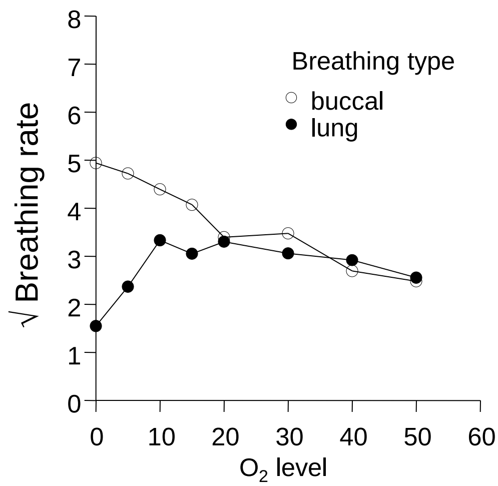
ANOVA

Source of variation	df
Between subjects (toads)	
Breathing type	1
Toads within breathing type (Residual 1)	19
Within subjects (toads)	
$[O_2]$	7
Breathing type x $[O_2]$	7
Toads (Breathing type) x $[O_2]$ (Residual 2)	133
Total	167

ANOVA toad example

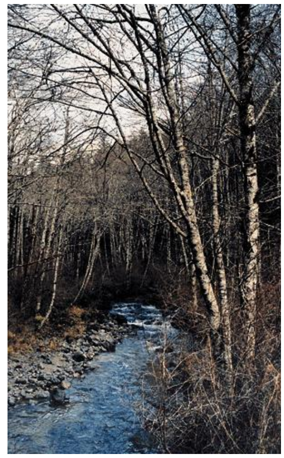
Source of variation	df	MS	*F*	*P*
Between subjects (toads)				
Breathing type	1	39.92	5.76	0.027
Toads (breathing type)	19	6.93		
Within subjects (toads)				
[O_2]	7	3.68	4.88	<0.001
Breathing type x [O_2]	7	8.05	10.69	<0.001
Toads (Breathing type) x [O_2]	133	0.75		
Total	167			

Mullens (1993)



Gange (1995)

- Factor A is tree species:
  - alder 1 vs alder 2
  - applied to trees = subjects = plots
- Factor B is subjects (i.e. trees) nested within A
- Factor C is date
  - 20 dates between May and September
  - recorded from trees (subjects) repeatedly
- Response variable is aphid abundance



Assumptions

- Normality & homogeneity of variance:
  - affects between-plots (between-subjects) tests
  - boxplots, residual plots, variance vs mean plots etc. for average of within-plot (within-subjects) levels

- No “carryover” effects:
  - results on one subplot do not influence results one another subplot.
  - time gap between successive repeated measurements long enough to allow recovery of “subject”

Sphericity

- Sphericity of variance-covariance matrix
  - variances of paired differences between levels of within-plots (or subjects) factor equal within and between levels of between-plots (or subjects) factor
  - variance of differences between [O_2] 1 and [O_2] 2 = variance of differences between between [O_2] 1 and [O_2] 3 etc.

Sphericity assumption

Toad	$O_21 – O_22$	$O_22 – O_23$	$O_21 – O_23$ etc.
1	y_{11}-y_{21}	y_{21}-y_{31}	y_{11}-y_{31}
2	y_{12}-y_{22}	y_{22}-y_{32}	y_{12}-y_{32}
3	y_{13}-y_{23}	y_{23}-y_{33}	y_{13}-y_{33}
etc.			

Var(diff(1-2)) = Var(diff(2-3)) = Var(diff(1-3))

Sphericity (compound symmetry)

- OK for split-plot designs
  - within plot treatment levels randomly allocated to subplots
- OK for repeated measures designs
  - if order of within subjects factor levels randomised
- Not OK for repeated measures designs when within subjects factor is time
  - order of time cannot be randomised

ANOVA options

- Standard univariate partly nested analysis
  - only valid if sphericity assumption is met
  - OK for some repeated measures designs (those where performance is not assumed to change with time)

ANOVA options

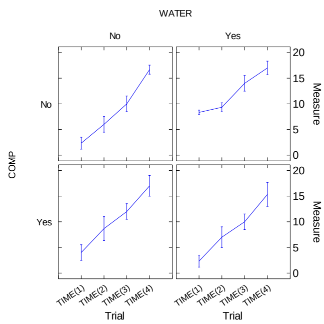
- Adjusted univariate *F*-tests for within-subjects factors and their interactions
  - conservative tests when sphericity is not met
  - Greenhouse-Geisser better than Huyhn-Feldt

ANOVA options

- Multivariate (MANOVA) tests for within subjects or plots factors
  - responses from each subject used in MANOVA
  - doesn't require sphericity
  - sometimes more powerful than GG adjusted univariate, sometimes not

Subjects used repeatedly and performance is likely to be linked to order (timing)

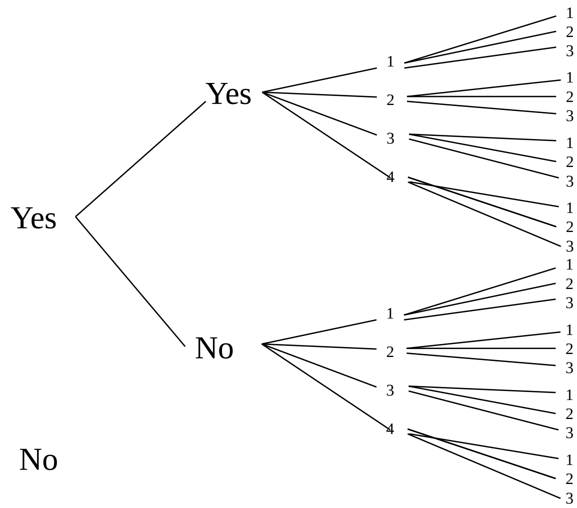
- Effect of Competition (2 levels),Watering (2 levels) and Time (4 levels) on growth of Oak Seedlings
- 3 replicate seedlings for each combination of competition and watering. Each seedling followed over time



Repeated measures seedling growth as a function of competition and watering

Completely randomized

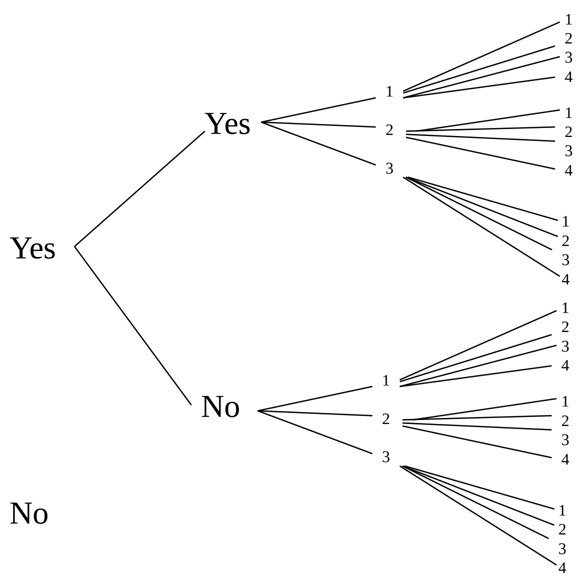
Competition (2) Watering (2) Time (4) Replicate (3)



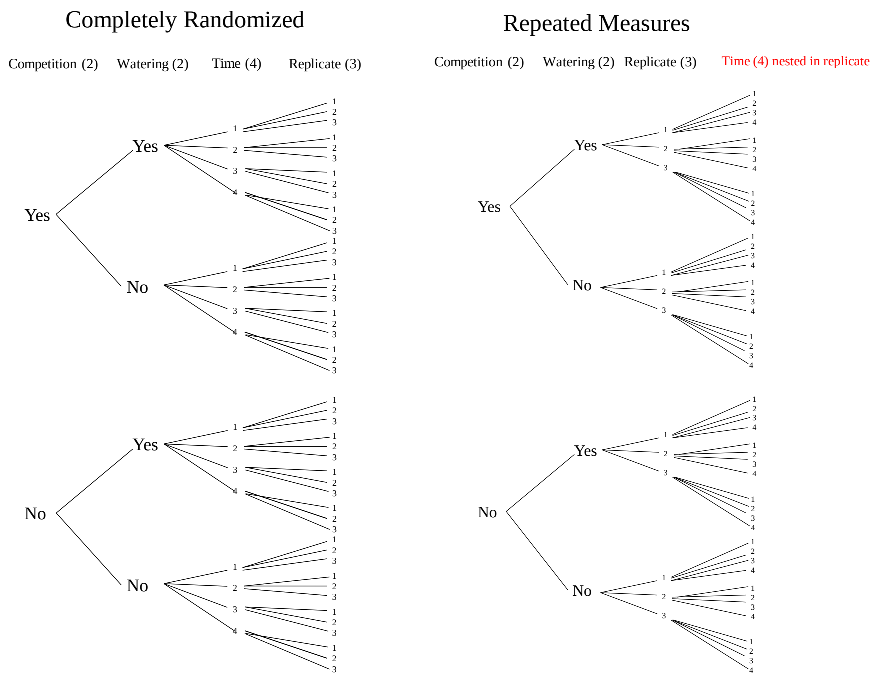
Replication
2x2x4x3 = 48
Each sampled 1 time

Repeated Measures

Competition (2) Watering (2) Replicate (3) Time (4) – note time is nested in replicate



Replication
2x2x3 = 12
Each sampled 4 times



Between subject effects (No time effects)

COMP$					
Test	Value	Exact F	NumDF	DenDF	Prob>F
F Tes	0.1222222	0.9778	1	8	0.3517

WATER$					
Test	Value	Exact F	NumDF	DenDF	Prob>F
F Tes	0.1010101	0.8081	1	8	0.3949

COMP$*WATER$					
Test	Value	Exact F	NumDF	DenDF	Prob>F
F Tes	0.9707071	7.7657	1	8	0.0237 *

Note degrees of freedom – the denominator DF does note include temporal replication

Within subject effects (includes time effects)

Time

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	63.843216	127.6864	3	6	<.0001 *
Univar unadj Epsilon	1	152.0511	3	24	<.0001 *
Univar G-G Epsilon	0.5361496	152.0511	1.6084	12.868	<.0001 *
Univar H-F Epsilon=	0.9023049	152.0511	2.7069	21.655	<.0001 *

Time*COMP$

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	3.0913235	6.1826	3	6	0.0289 *
Univar unadj Epsilon	1	1.2907	3	24	0.3003
Univar G-G Epsilon	0.5361496	1.2907	1.6084	12.868	0.3002
Univar H-F Epsilon=	0.9023049	1.2907	2.7069	21.655	0.3015

Time*WATER$

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	1.7731767	3.5464	3	6	0.0875
Univar unadj Epsilon	1	1.8658	3	24	0.1624
Univar G-G Epsilon	0.5361496	1.8658	1.6084	12.868	0.1967
Univar H-F Epsilon=	0.9023049	1.8658	2.7069	21.655	0.1693

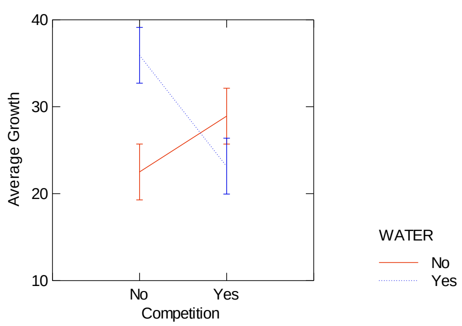
Time*COMP$*WATER$

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	2.0496341	4.0993	3	6	0.0669
Univar unadj Epsilon	1	1.9553	3	24	0.1477
Univar G-G Epsilon	0.5361496	1.9553	1.6084	12.868	0.1847
Univar H-F Epsilon=	0.9023049	1.9553	2.7069	21.655	0.1550

Note:

1) F-Test is Pillai Trace multivariate F test
2) Univar unadj – is regular F-test but subject to sphericity violations
3) Univar G-G and H-F are corrected univariate tests (account for sphericity)
4) Degrees of freedom for univar tests use temporal replication
5) G-G and H-F use adjusted degrees of freedom

Interaction between Competition and Watering



Effect of Time on Growth varies as a function of competition