Multivariate ANOVAs

- *Objectives:*

Discuss general approach of multivariate experiments

Go over the settings / results of multivariate ANOVAs

Go over the settings / results of blocked MRPP

ANOVA approach

- Advantages:

• Ideal for evaluating specific hypotheses – Effect sizes (e.g., differences between groups of samples)

• Address multiple factors at once

• Investigates interaction terms

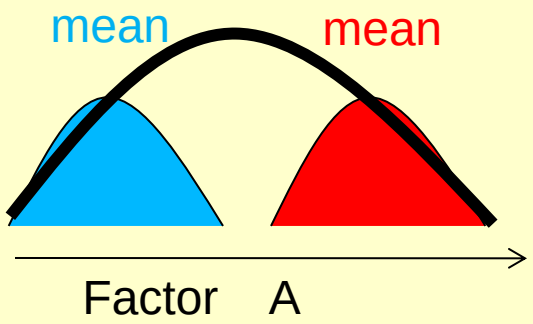
- Disadvantages:

• Requires careful blocked design / replication

• Relies on assumptions of normality and equal variances

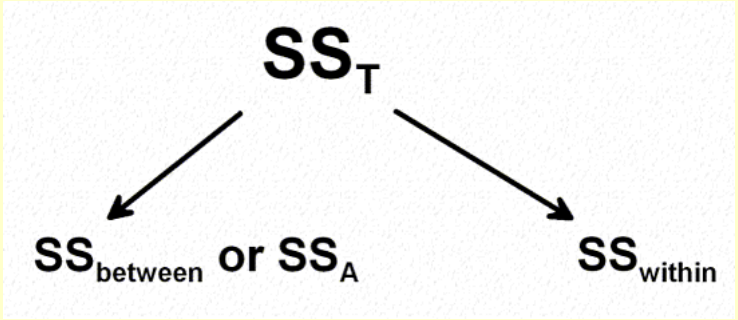
ANOVA – One-Way

- Conceptual Approach:



- Consider one Factor

- Calculate amount of variance explained by this factor



- F test quantifies the ratio of variance between / within:

$$F = \frac{\text{between-group variability}}{\text{within-group variability}}.$$

- Pseudo-f statistic:

$$F = \frac{\text{explained variance}}{\text{unexplained variance}},$$

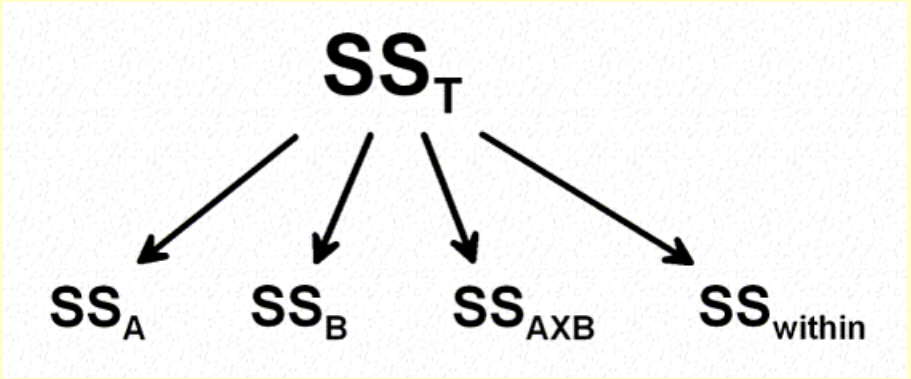
ANOVA – Two-Way

- Conceptual Approach:

- Define two or more Factors

- Calculate means for the different factor combinations

- Calculate amount of variance explained by each factor, and by their interaction



		Factor B				A Marginals
		b_1	b_2	b_k	b_q	
Factor A	a_1	X_{i11}	X_{i12}	X_{i1k}	X_{i1q}	
		X_{n11}	X_{n12}	X_{n1k}	X_{n1q}	
		$\bar{X}_{11}$	$\bar{X}_{12}$	$\bar{X}_{1k}$	$\bar{X}_{1q}$	$\bar{X}_{1.}$
	a_2	X_{i21}	X_{i22}	X_{i2k}	X_{i2q}	
		X_{n21}	X_{n22}	X_{n2k}	X_{n2q}	
		$\bar{X}_{21}$	$\bar{X}_{22}$	$\bar{X}_{2k}$	$\bar{X}_{2q}$	$\bar{X}_{2.}$
	a_j	X_{ij1}	X_{ij2}	X_{ijk}	X_{ijq}	
		X_{nj1}	X_{nj2}	X_{njk}	X_{njq}	
		$\bar{X}_{j1}$	$\bar{X}_{j2}$	$\bar{X}_{jk}$	$\bar{X}_{jq}$	$\bar{X}_{j.}$
	a_p	X_{ip1}	X_{ip2}	X_{ipk}	X_{ipq}	
		X_{np1}	X_{np2}	X_{npk}	X_{npq}	
		$\bar{X}_{p1}$	$\bar{X}_{p2}$	$\bar{X}_{pk}$	$\bar{X}_{pq}$	$\bar{X}_{p.}$
B Marginals		$\bar{X}_{.1}$	$\bar{X}_{.2}$	$\bar{X}_{.k}$	$\bar{X}_{.q}$	
					Grand Mean	$\bar{X}_{..}$

Multivariate ANOVA

Traditional MANOVA has two properties that render it inappropriate for analysis of ecological communities:

- Euclidean distance is assumed model of the relationships among data points; yet this distance measure performs poorly with community data (McCune & Grace 2002)

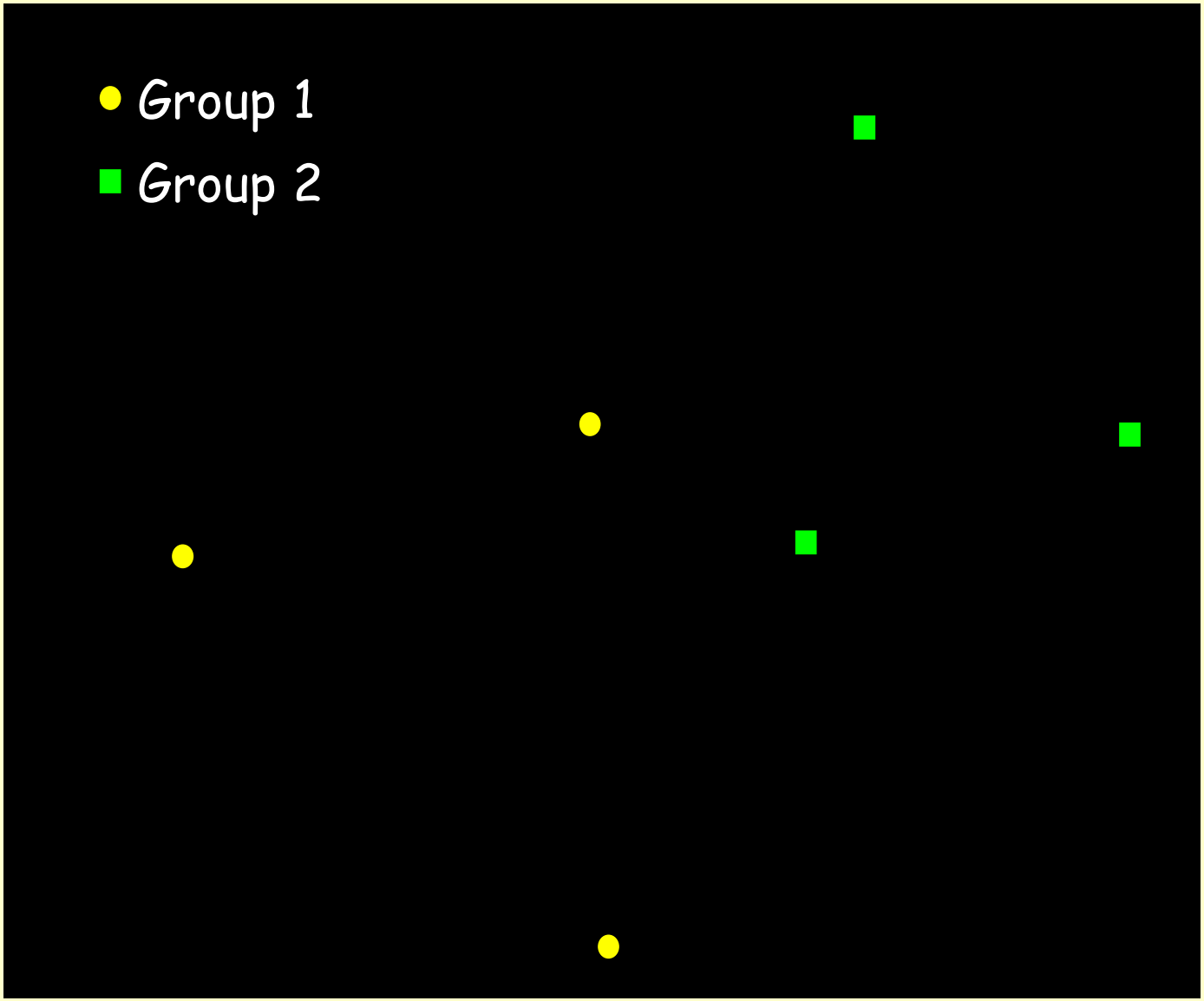
- Calculation of p values assumes multivariate normality, though this assumption is infrequently reasonable for community data (McCune & Grace 2002)

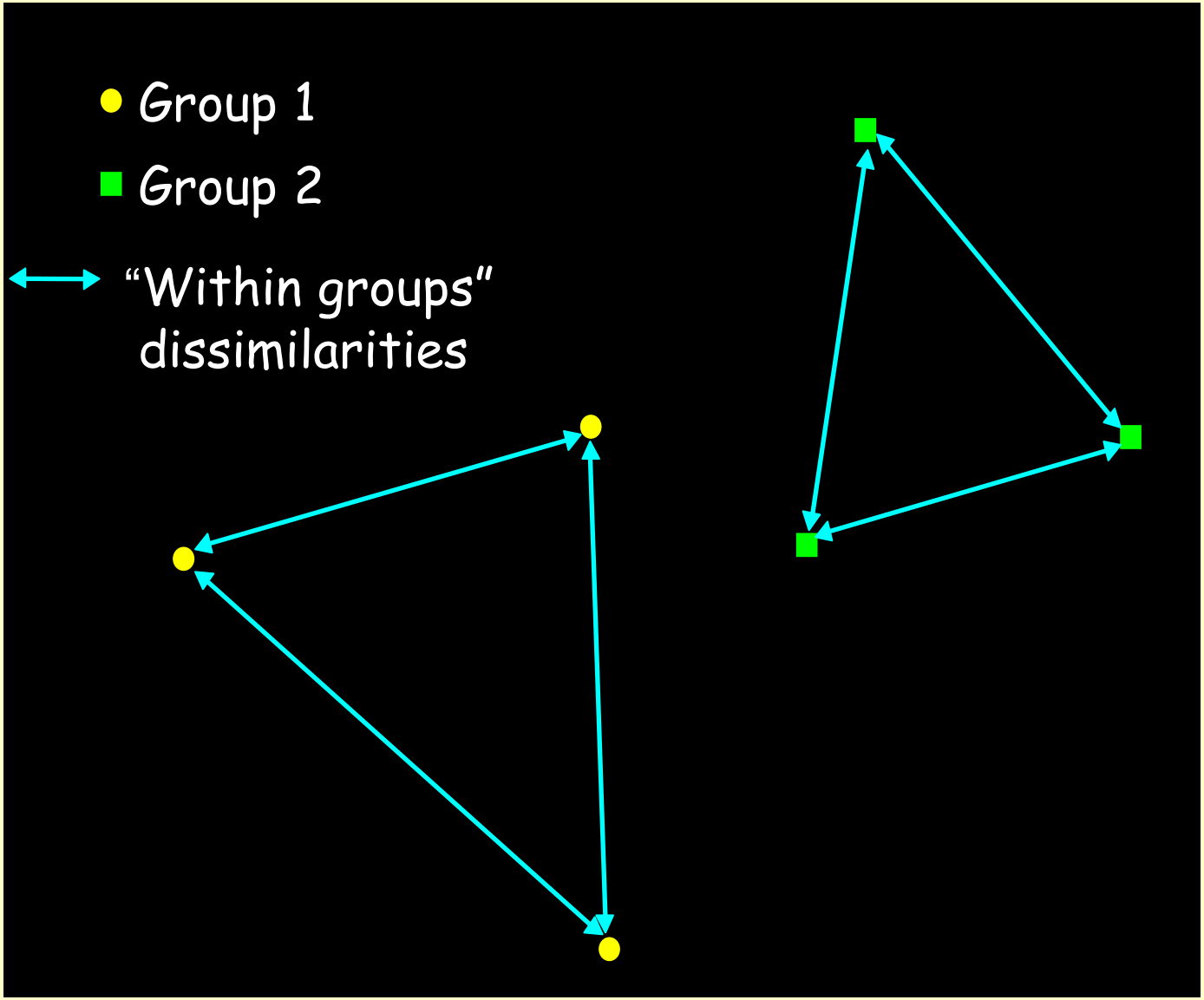
Multivariate ANOVA

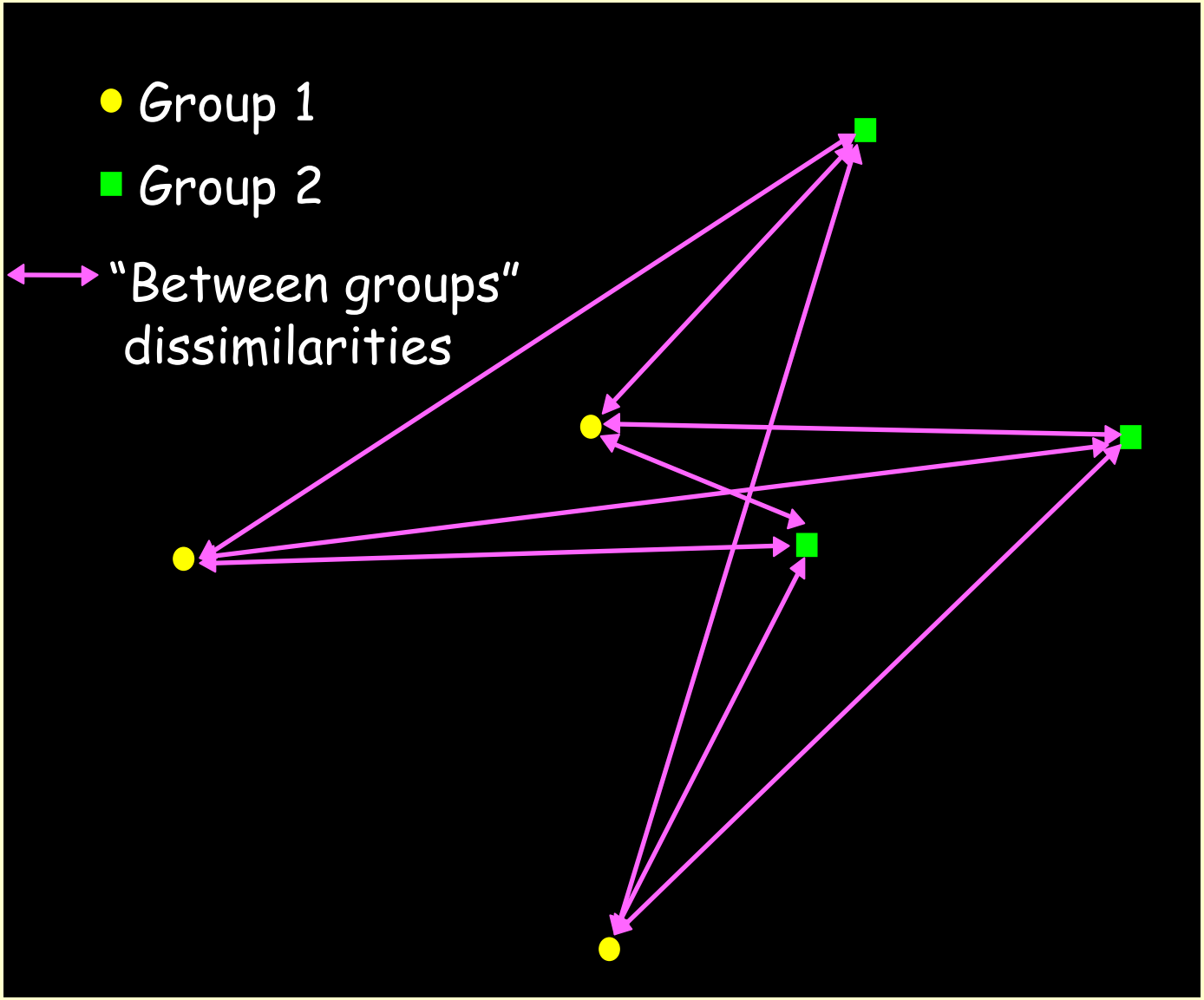
- Multivariate ANOVA (MANOVA): An analysis of variance where the response consists of two or more potentially interrelated variables. In contrast, an univariate analysis of variance has only one response variable.

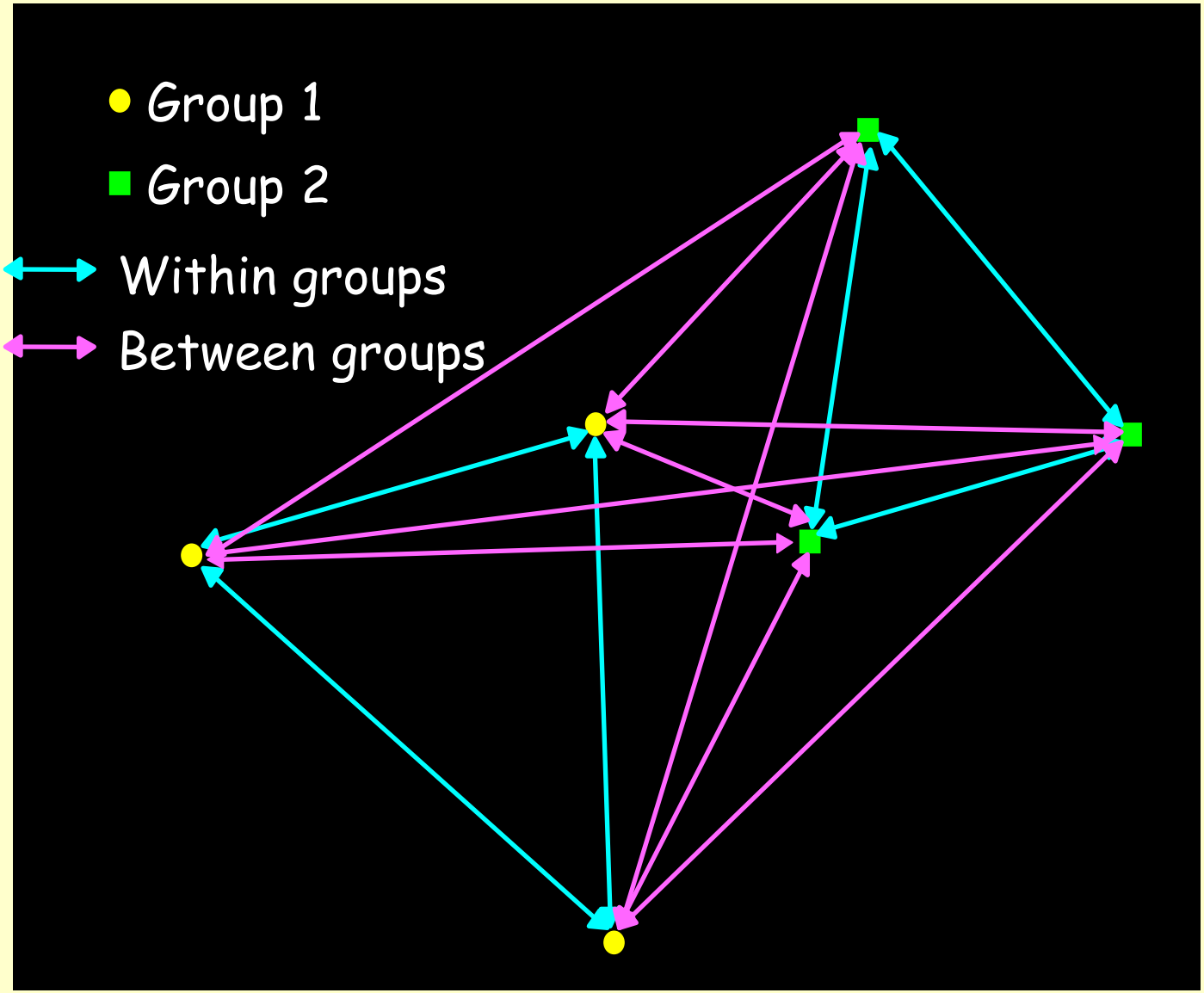
- PerMANOVA performs distance-based multivariate analysis of variance, also known as nonparametric MANOVA or npMANOVA.

- Hypotheses are evaluated with permutation tests, rather than by reference to an assumed (normal) distribution.


Group 1
Group 2


Group 1
Group 2
"Within groups" dissimilarities

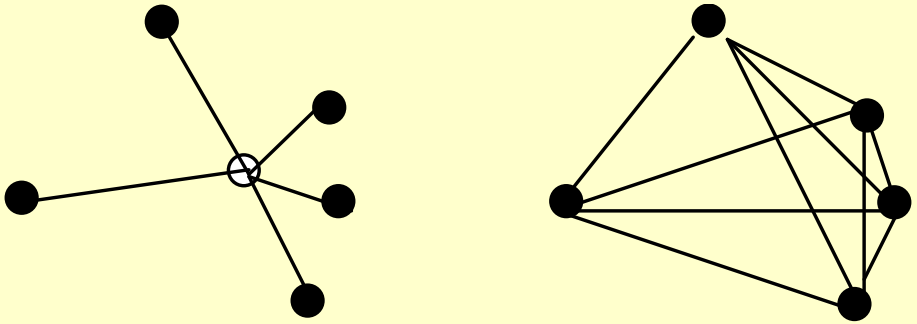

Group 1
Group 2
"Between groups" dissimilarities


Group 1
Group 2
Within groups
Between groups

Multivariate ANOVA

Key to development of PerMANOVA was Anderson's (2001) recognition that sums of squares could be calculated directly using distances among data points, rather than the distances from the data points to the mean. She explains the problem:

"In the case of an analysis based on Euclidean distances, the average for each variable across the observations within a group constitutes the measure of central location for the group in Euclidean space, called a centroid. For many distance measures, however, the calculation of a central location may be problematic"



Sums of distances from points to centroid (left) calculated from average squared interpoint distance (right).

PerMANOVA – How it Works

The total sum of squares of a distance matrix **D** with *N* rows and *N* columns is:

$$SS_T = \frac{1}{N}\sum_{i=1}^{N-1}\sum_{j=i+1}^{N} d_{ij}^2$$

The residual (within-group) sum of squares for a one-way classification is:

$$SS_R = \frac{1}{n}\sum_{i=1}^{N-1}\sum_{j=i+1}^{N} d_{ij}^2 \varepsilon_{ij}$$

where *n* is the number of observations per group,
N is the number of sample units,
ε_{ij} = 1 if *i* and *j* in same group; ε_{ij} = 0 if in different groups.

PerMANOVA – How it Works

Thus, the sum of squares between groups is:

$$SS_A = SS_T - SS_R$$

This allows user to calculate a pseudo-*F*-ratio:

$$F = \frac{SS_A / (a-1)}{SS_R / (N-a)}$$

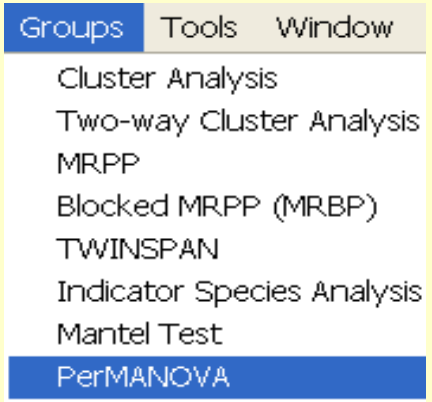
Explained Variance

Unexplained Variance

where *a* is group number and N is sample number.

Note: If distance matrix contains Euclidean distances, then pseudo-F = parametric univariate *F* ratio.

PerMANOVA – Limitations



- Limitations and non-limitations

- Maximum number of levels is 500 for each grouping variable.

- Maximum number of factors is 2.

- Design must be balanced, with equal numbers of observations in each cell.

- Each cell must have replication (more than one observation), except for a randomized complete block design, which has only one level of a factor in each block

- All cells in the design must be filled (no empty cells)

PerMANOVA - Output

- PerMANOVA writes a result file. No graphics produced.

- To explore the relationships among the data points and groups, use ordination to provide a visual summary.

- NOTE: Univariate vs. multivariate ANOVA:

  You can use perMANOVA in PC-ORD to perform either univariate or multivariate permutation-based ANOVA.

  If you have only one column in your main matrix, then the analysis is univariate. If you use Euclidean distance and the analysis is univariate, then the results will match a textbook ANOVA example, except that the p-value is estimated by randomization.

PerMANOVA - Example

- Indian Ocean Seabirds: Equal Replication (15 samples)
- Group Definitions – Relate back to Hypothesis

3 Watermasses:

- 1) Tropical:
  SST > 20 deg. C.

- 2) Subtropical:
  20 < SST < 18

-3) Transition:
  18 > SST

Main - WORK.WK1

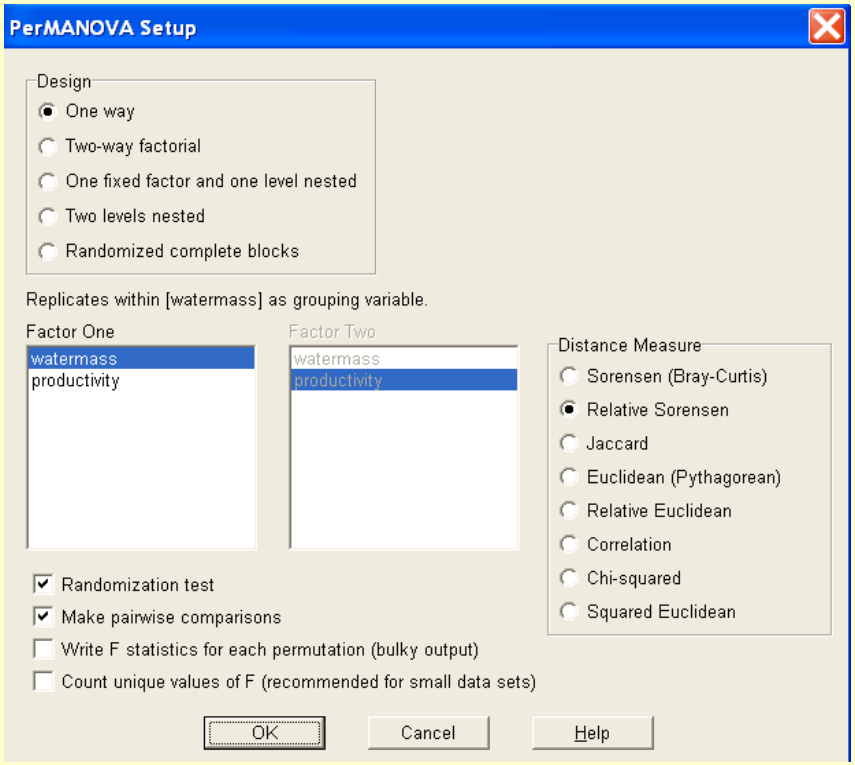
15				
42				
	Q	Q	Q	Q
	SPPT	YNAL	LISH	LTJA
plot1	0	0	0	0
plot2	0	0	12.5	0
plot3	28.21	0	0	0
plot4	23.98	2.04	3.06	1.02
plot5	15.63	3.23	2.16	0
plot6	2.03	0.08	0	0
plot7	1.64	0	0	0
plot8	0.19	0	0	0
plot9	2.29	0.08	0	0
plot10	0.67	69.02	0	0
plot11	0	6.1	0	0
plot12	0	0	0	0
plot13	0	0	0	1.25
plot14	0	0	3.37	2.25
plot15	0	0	1.57	0.2

Second - WORK2.WK1

15	plots	
2	variabl	
	C	C
	waterma	product
plot1	1	1
plot2	1	1
plot3	1	1
plot4	2	2
plot5	3	3
plot6	3	2
plot7	3	3
plot8	3	3
plot9	3	2
plot10	2	3
plot11	2	2
plot12	2	2
plot13	2	3
plot14	1	1
plot15	1	1

PerMANOVA - Example

- One-Way Test: One Factor (watermass)
- Distance Metric Used (Relative Sorensen)
- Randomizations
- Pair-wise Comparisons



PerMANOVA - Example

- Pseudo-F
- Df: 14 (12, 2)

IndianOceanBirds Groups

Groups were defined by values of: watermass

Main matrix has: 15 plots by 42 species

Distance measure = Relative Sorensen

Evaluation of differences in species between groups.
Design: One-way
Randomization test of significance of pseudo F values
Number of randomizations: 4999
Random number seed: 4553 selected by time.

Source	d.f.	SS	MS	F	p *
watermas	2	2.1219	1.0609	3.9684	0.001000
Residual	12	3.2082	0.26735		
Total	14	5.3301			

- Variance explained

Variance components estimated for random effects model (Model II)
Ignore variance components if you consider the factor to have fixed effects.
COMPONENTS OF VARIANCE

Source	Variance	% of variation
watermas	0.15872	37.252
Residual	0.26735	62.748
Total	0.42607	100.000

PerMANOVA - Example

- P values calculated with permutations

Statistics from randomizations

Source	F Observed	F from randomized groups: Mean	Maximum	S.Dev	Number observed F	p *
watermass	3.96840	1.02572	5.45999	0.00563	4	0.001000

* proportion of randomized trials with indicator value equal to or exceeding the observed indicator value.
p = (1 + number of runs >= observed) / (1 + number of randomized runs)

- Pair-wise comparisons

PAIRWISE COMPARISONS for factor watermas
Note: p values are not corrected for multiple comparisons.

Level vs. Level	t	p
1 vs. 2	1.1737	0.188400
1 vs. 3	2.3813	0.007800
2 vs. 3	2.4737	0.007000

********** PerMANOVA finished **********

Multivariate Experiments

- Investigators can use careful sampling design to address gradients / changes in community composition.
- For example: outgroup poles / replicate samples

Natural Experiments

- Investigators can take advantage of natural events in nature that are similar to manipulative treatments.
- For example: one hillside burned and another one did not

Experiments vs Exploration

- Traditionally, ecologists face a dichotomy between experimental and exploratory scientific approaches: pattern description versus experimental manipulation

- For example: PCA / NMDS vs PO / MRPP

- What is an Experiment?

  Change one or more variables in a consistent way

  Contrast multiple treatments (& interactions)

  Contrast response against controls (unmanipulated)

- Take Home: Investigator controls the allocation of samples to treatments (replicates)

ANOVA Designs

➢ Definitions following Searle et al. 1992

• Factor: A classification that assigns each observation to one level of the classification. In PC-ORD, the factors are chosen from individual columns in the second matrix.

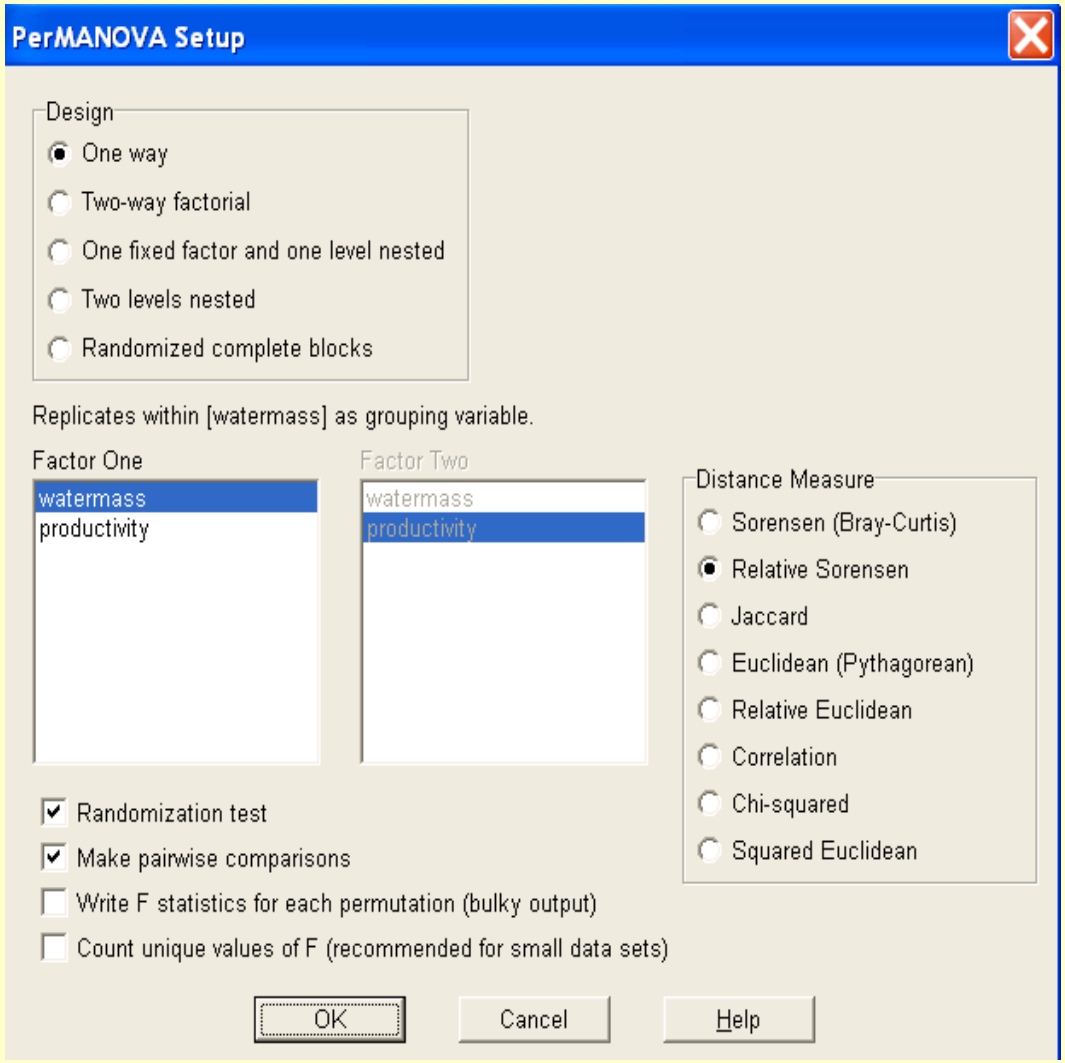
• Level: Individual classes of a classification. For example, factor "sex" has 2 levels: "male" and "female." In PC-ORD different levels are assigned integer numerical values. The actual value chosen to represent the levels is unimportant.

• Cell: A subset of data occurring at the intersection of one level of every factor being considered. Every data point in a design belongs to one and only one cell of the design.

• Balanced Design: Every cell with equal sample size.

Expanded ANOVA Designs

- Two-factors (interaction)
- Blocking
- Nestedness



PerMANOVA – Experimental Design

- You must choose one of the following designs.
- For each design, specify the design variables (factors), by choosing them from a list of variables in second matrix.

One way:

Replicates within [_____] as grouping variable

Two-way factorial:

Replicates within [_____] and [_____] as grouping variables

PerMANOVA – Experimental Design

One fixed factor and one level nested (Mixed Model):

Replicates within [_____] nested within [_____] as grouping variable

Two levels nested (Model II):

Replicates nested within [_____], nested within [_____]

Randomized complete blocks:

Blocks are [_____] , fixed factor [_____] is grouping variable

Nestedness

Nested layouts needed when constraints prevent the crossing every level of one factor with every level of another factor. Thus, fewer than all levels of one factor occur within each level of the other factor.

If Factor B is nested within Factor A, then some level of Factor B can only occur within some other level of Factor A and there can be no interaction of Factor A and Factor B.

For example:

We are studying demographic rates in different countries and continents. Yet, countries are only represented within their continents. Thus, countries are nested within continents.

Blocking

Blocking to "remove" the effect of nuisance factors

For block designs, one factor or variable is of primary interest. However, there are also several other uncontrolled factors.

Blocking can be used to reduce or eliminate the contribution to experimental error contributed by these nuisance factors.

The basic concept is to create homogeneous blocks in which the nuisance factors are held constant and the factor of interest is allowed to vary.

Within blocks, it is possible to assess the effect of the factor of interest without worrying about variations due to changes of the block factors, which are accounted for in the analysis.

Blocking

A nuisance factor can be used as a blocking factor if every level of the primary factor occurs the same number of times with each level of the nuisance factor. The analysis will focus on varying the primary factor within each experiment block.

subplot	plot	region
1	1	1
2	1	1
1	2	1
2	2	1
1	3	1
2	3	1
1	1	2
2	1	2
1	2	2
2	2	2
1	3	2
2	3	2

subplot	plot	region
1	1	1
2	1	1
1	2	1
2	2	1
1	3	1
2	3	1
1	4	2
2	4	2
1	5	2
2	5	2
1	6	2
2	6	2

Examples:

- Seasonal vegetation at multiple sites (samples linked by location)
- Paired comparisons (morning / night samples at same location)

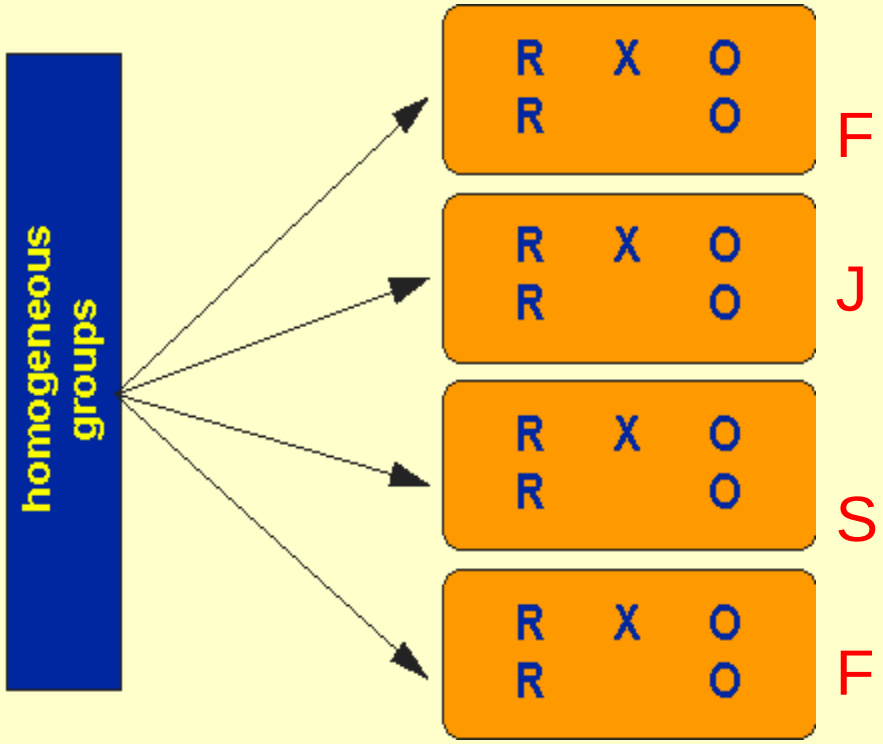
Blocked MRPP (MRBP)

Randomized block or paired-sample data can be analyzed with a variant of MRPP called MRBP or blocked MRPP.

Blocking is the arranging of experimental units in homogeneous groups (blocks) different from others

A blocking factor is a source of variability that is not of primary interest to the experimenter, but may affect the result. Blocking controls it.

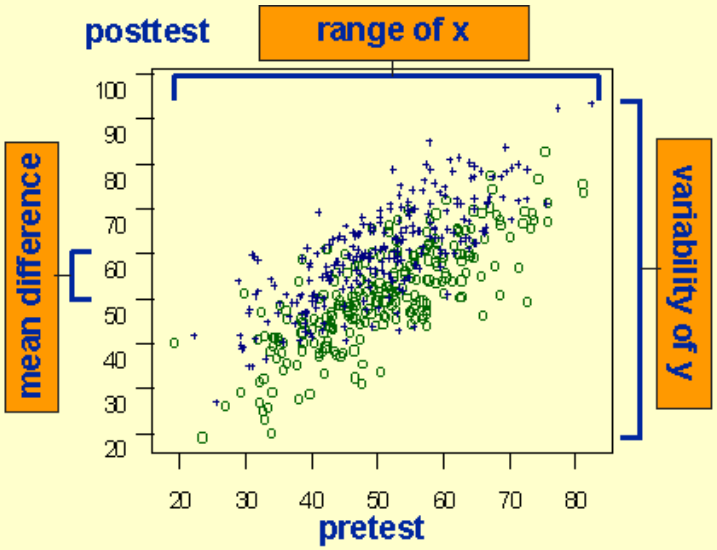
Example: does software use (R / O) affect stats skills, after controlling for grade level?

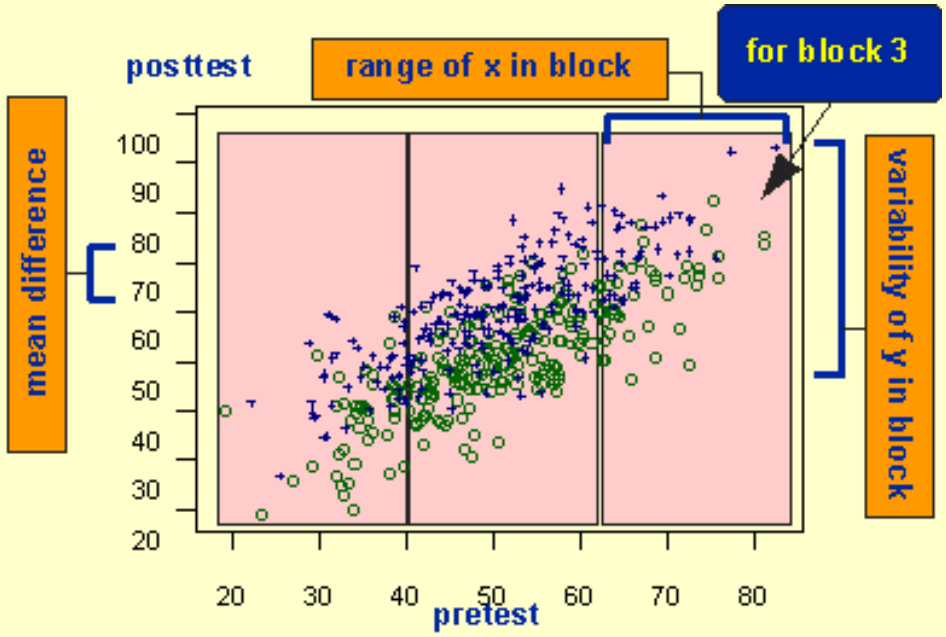


Blocked MRPP (MRBP)

The factor of interest is depicted by crosses / circles

A blocking factor controls a source of variability not of primary interest to the experiment (e.g., grade level)

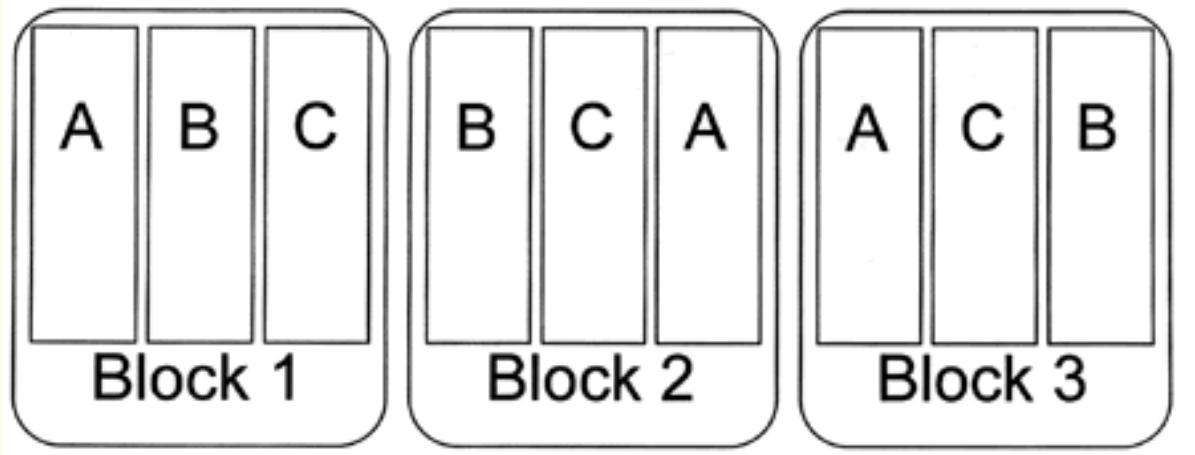




The general rule is to:

"Block what you can, randomize what you cannot"

Blocking in MRPP



Limitations: this analysis **requires a balanced design**

- One sample unit for each block / treatment combination.
- Number of treatments must be equal among blocks.
- Each treatment must be present in each block.

(NOTE: PC-ORD allows up to 1000 blocks and 100 groups).

Blocked MRPP (MRBP)

Given *b* blocks and *g* groups (treatments), the MRPP statistic is modified to:

$$\delta = \sum_{i=1}^{g} C_i x_i$$

where $\Delta(xij, x ik)$ is the distance between points *xij* and *xik* in the *p* dimensional space.

$$\delta = \left[g\binom{b}{2} \right]^{-1} \sum_{i=1}^{g} \sum_{j<k} \Delta(x_{ij}, x_{ik})$$

Note that for paired-sample data, b is the number of linked observations (g = 2 in day / night example).

Delta is average distance between blocks within treatments.

Blocked MRPP (MRBP)

Approach:

Null hypothesis assigns equal probabilities to each of the $M = (g!)^b$ possible allocations of the g p-dimensional measurements to g treatments within each of b blocks.

In other words, the observed values are randomly reassigned to different treatments in each block.

Interpretation:

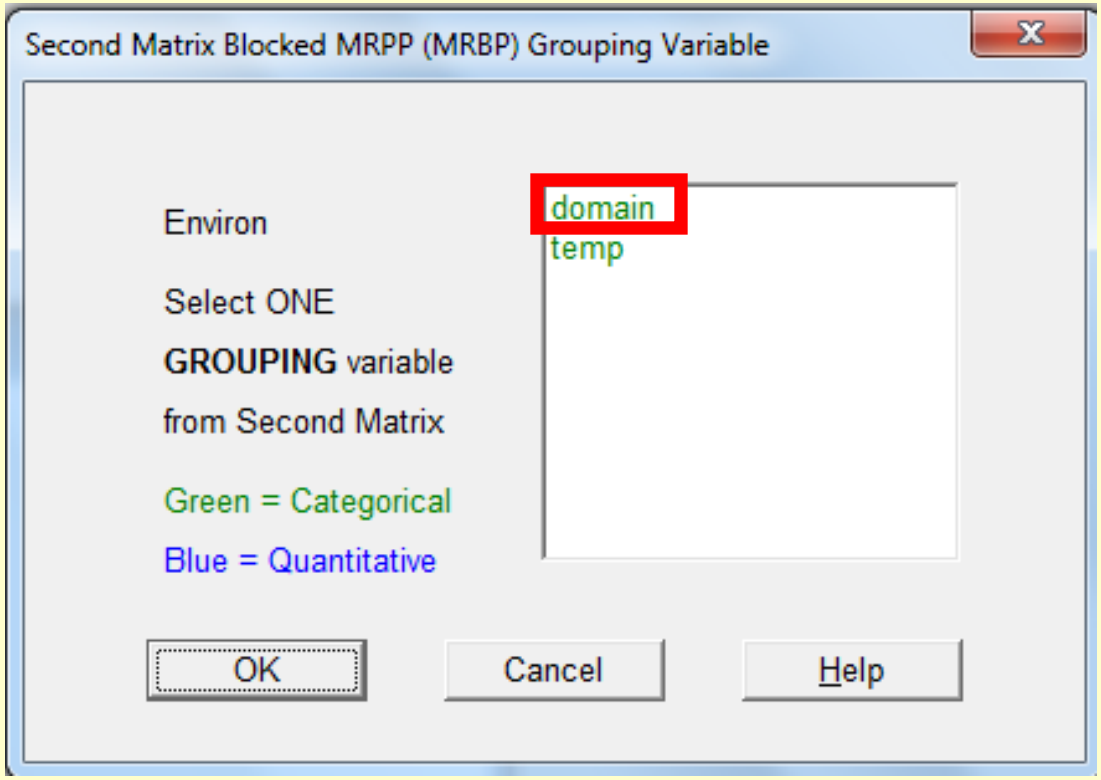
Like MRPP, small values of d imply a concentration of treatments in the p dimensional space.

The added features of MRBP are that:

- distances summed with respect to the blocks
- the user has the option of aligning blocks so that all treatments in a given block have a median of zero.

MRBP SetUp

- Select Grouping Variable
  - Matrix 2
  - Categorical



MRBP SetUp

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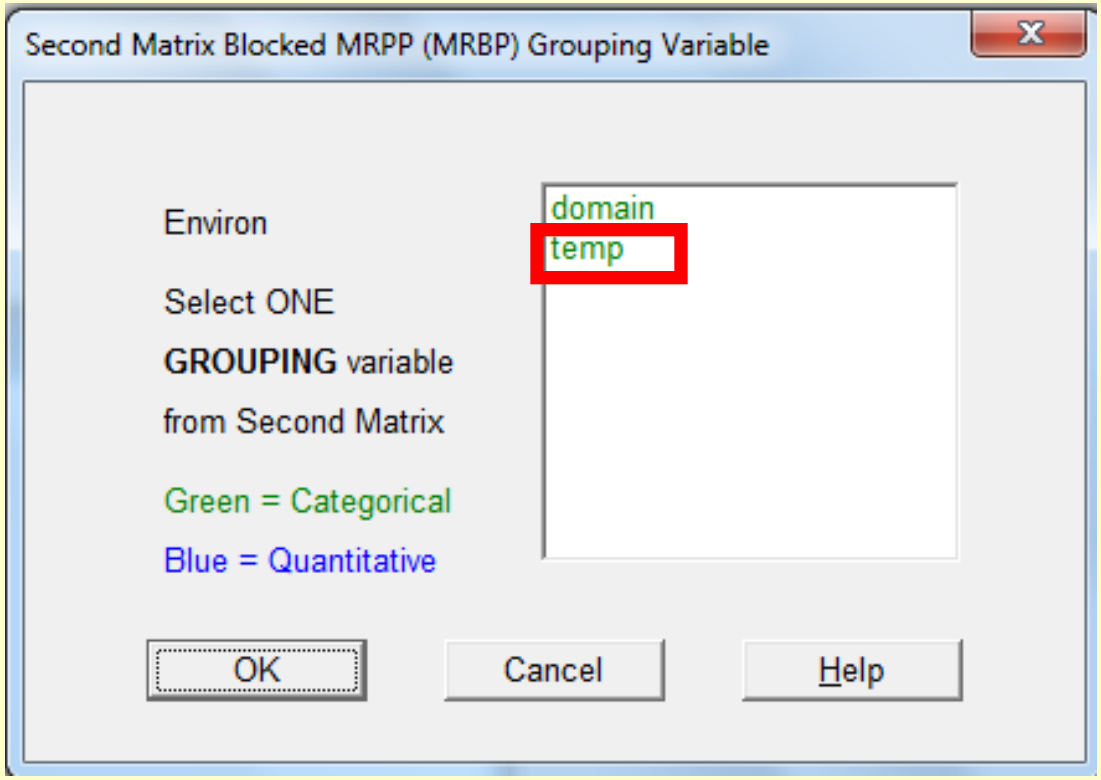
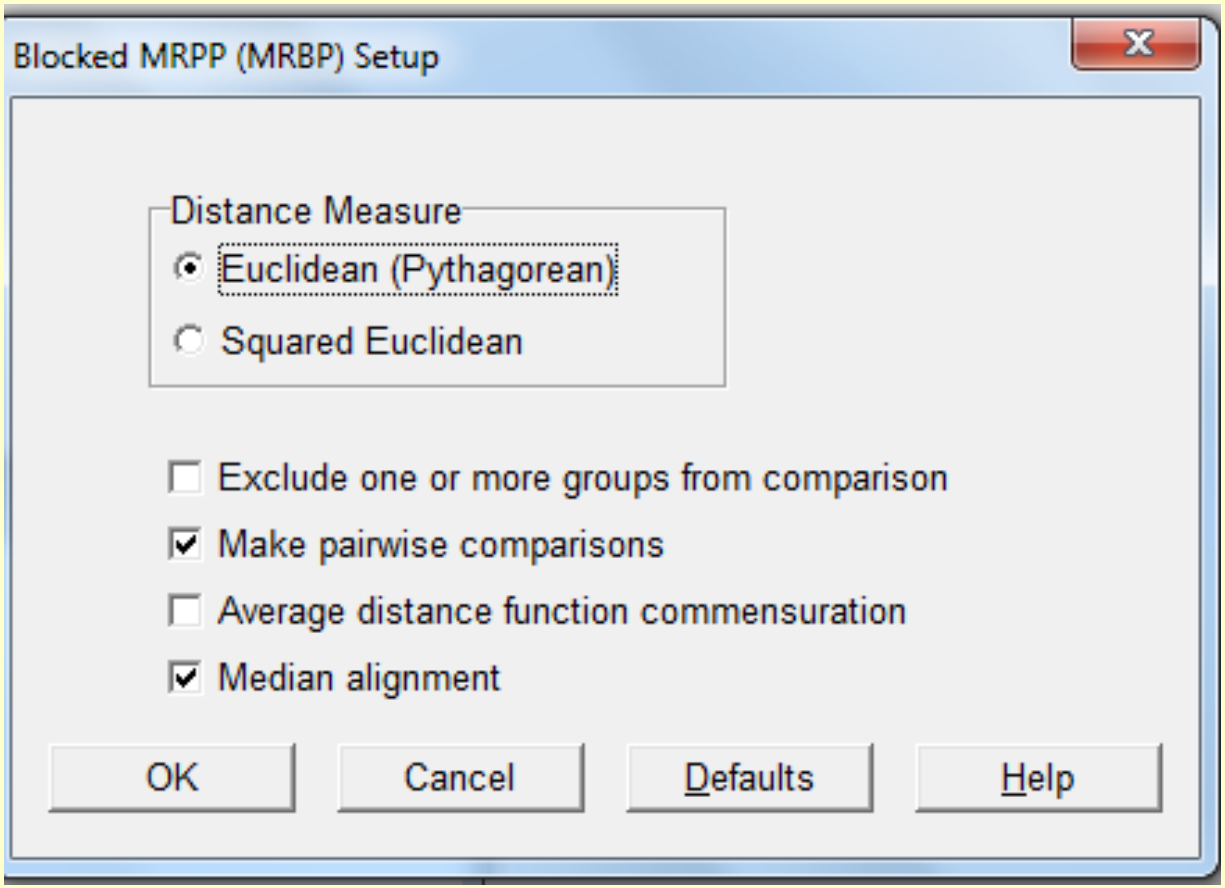


MRBP SetUp



- Recommended Settings
  - Pairwise comparisons
  - Median alignment

Limitation: Euclidean Distance metric

MRBP

Median Alignment Within Blocks

If the median for each variable in each block is subtracted from the raw data for each block, then the medians are said to be aligned to zero for all blocks.

Usually alignment in a randomized block design is desirable.

But if the problem is conceptualized as paired agreement, say between model predictions and observed data, then alignment is not performed.

MRBP

Median Alignment Within Blocks

Table: Comparison of raw data and data aligned within blocks to zero as input to Blocked MRPP.

	Raw Data		Aligned Data	
	Block 1	Block 2	Block 1	Block 2
Group 1	4	9	1.5	1.5
Group 2	2	7	-0.5	-0.5
Group 3	3	8	0.5	0.5
Group 4	1	2	-1.5	-5.5
Median	2.5	7.5	0	0
Observed δ	5 = (5+5+5+1)/4		1 = (0+0+0+4)/4	
Expected δ	4.375		2.225	
Agreement (A)	0.086		0.556	
p	0.184		0.016	

MRBP

Median Alignment Within Blocks

In this case each event or observation represents a group and one block contains the observed data and one block contains the predicted data. Because the goal is an exact match of predicted and observed, rather than the two just being correlated, then the medians should not be aligned.

If, however, the goal is whether or not the two sets of numbers are correlated, apart from any exact agreement in value, then the blocks should be aligned.

MRBP

Average Distance Function Commensuration

This option equalizes the contribution of each variable to the distance function. For each variable *m* sum of deviations (Dev_m) is calculated:

$$Dev_m = \sum_{i=1}^{g} \sum_{j=1}^{\delta} \sum_{k=1}^{g} \sum_{l=1}^{\delta} |x_{mij} - x_{mkl}|^V$$

The exponent *V* is set to 2 for squared Euclidean distance or 1 for Euclidean distance. Then each element *x* of the data matrix is divided by the sum of the deviations for the corresponding variable to produce the transformed value *y*:

$$y_{mij} = x_{mij} / Dev_m$$

MRBP Example – from PC-ORD

SETUP

Comparison of response to thinning within blocks

Analysis of randomized block data with MRBP:

39 variables (species), 5 blocks, 4 groups

Groups were defined by values of: Treat

Blocks were defined by values of: StanBloc

Input data has: 20 plots by 39 attrib

OPTIONS

Distance measure: Euclidean

Median alignment performed

No average distance function commensuration

MRBP Example – from PC-ORD

QUESTION

Five forest patches – 39 total species

Test effect of time (succession), by sampling 4 times

Control the individual differences in the 5 locations

MRBP Example – from PC-ORD

GROUP: 1

Identifier: 1 Size: 5

Members: BEV1-1 GNO2-1 DLI3-1 SAN4-1 NWA5-1

GROUP: 2

Identifier: 2 Size: 5

Members: BEV1-2 GNO2-2 DLI3-2 SAN4-2 NWA5-2

GROUP: 3

Identifier: 3 Size: 5

Members: BEV1-3 GNO2-3 DLI3-3 SAN4-3 NWA5-3

GROUP: 4

Identifier: 4 Size: 5

Members: BEV1-4 GNO2-4 DLI3-4 SAN4-4 NWA5-4

MRBP Example – from PC-ORD

Test statistic: T = -4.1880177

Observed delta = 6.9528671
Expected delta = 7.4888375
Variance of delta = 0.16378141E-01

Skewness of delta = -0.48910187

Chance-corrected within-group agreement, A = 0.07156924
A = 1 - (observed delta/expected delta)
Amax = 1 when all items are identical within groups (delta=0)
A = 0 when heterogeneity within groups equals expectation by chance
A < 0 with more heterogeneity within groups than expected by chance

Probability of a smaller or equal delta, p = 0.00041511

Final Thoughts

- Features: Ideal for evaluating specific hypotheses
  - Address multiple factors at once
  - Investigates interaction terms
- Limitations:
  - Requires careful blocked design / replication
  - Blocking allows for controlling additional factors
  - Nesting allows for scenarios without full replication

PerMANOVA & ANOSIM – References

- MANOVA:

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26:32-46.

- ANOSIM (perMANOVA with ranked data):

Clarke, K. R. 1993. Non-parametric multivariate analyses of changes in community structure. Aust. J. Ecol. 18, 117-143.

MRBP – References

Mielke, P. W., Jr., and K.J. Berry. 1982. An extended class of permutation techniques for matched pairs. Commun. Statist.-Theor. Meth. 11:1197-1207.

Mielke, P. W. and H. K. Iyer. 1982. Permutation techniques for analyzing multiresponse data from randomized block experiments. Commun. Statist. A 11:1427-1437.

Biondini, M.E., C.D. Bonham, and E.F. Redente. 1985. Secondary successional patterns in a sagebrush (Artemisia tridentata) community as they relate to soil disturbance and soil biological activity. Vegetatio 60: 25-36.

Zimmerman, G.M., H. Goetz, and P. W. Mielke, Jr. 1985. Use of an improved statistical method for group comparisons to study effects of prairie fire. Ecology 66: 606-611.