1.1 General description

Key references

- **Method**: Anderson (2001a) , McArdle & Anderson (2001)
- **Permutation techniques**: Anderson (2001b) , Anderson & ter Braak (2003)

PERMANOVA is a routine for testing the simultaneous response of one or more variables to one or more factors in an analysis of variance (ANOVA) experimental design on the basis of any resemblance measure, using permutation methods. It is assumed that the user has relevant knowledge of multi-factorial ANOVA, which has the same basic logic in multivariate as in univariate analysis (see Underwood (1981) , Underwood (1997) and Quinn & Keough (2002)), and an understanding of what it means to test a multivariate hypothesis (see Clarke (1993)). A more complete description of the method is given in Anderson (2001a) and McArdle & Anderson (2001) . These papers merely elaborate the essential idea of *partitioning for dissimilarity matrices* which was originally (to our knowledge) presented by Brian McArdle (McArdle (1990) , McArdle (1994)) and which has also been articulated by Pillar & Orloci (1996) , Legendre & Anderson (1999) and Gower & Krzanowski (1999) .

In essence, the routine performs a partitioning of the total sum of squares[4] according to the full experimental design specified by the user, including appropriate treatment of factors that are fixed or random, crossed or nested (hierarchical), and all interaction terms. The routine will correctly calculate an appropriate distance-based pseudo-*F* statistic for each term in the model, based on the expectations of mean squares (EMS), in a fashion that is directly analogous to the construction of the *F* statistic for multi-factorial univariate ANOVA models (Cornfield & Tukey (1956) , Hartley (1967) , Rao (1968)). *P*-values are obtained using an appropriate permutation procedure for each term, and the user can specify whether permutation of raw data or residuals under either a full or reduced model are to be used (Anderson (2001b) , Anderson & ter Braak (2003)). Correct *P*-values may also be obtained through Monte Carlo random draws from the asymptotic permutation distribution (Anderson & Robinson (2003)) in the event that too few permutations are available for a given test.

In addition to the main overall PERMANOVA partitioning and tests, the routine will also perform *a posteriori* pair-wise comparisons among levels of factors, including within individual levels of other factors (or cells) in the case of significant interaction terms. Other important features of the PERMANOVA routine include: (i) catering for unbalanced designs, including a choice regarding the type of sum of squares to be used for the partitioning; (ii) pooling or the exclusion of individual terms from a model; (iii) choice to include one or more quantitative covariates in the model; (iv) contrasts; (v) analysis of designs lacking replication; and (vi) analysis of asymmetric designs.

Before using PERMANOVA, the user should have a fairly solid grasp of the basic issues and logic in experimental design (some good reference textbooks on this topic include Mead (1988) , Snedecor & Cochran (1989) , Winer, Brown & Michels (1991) , Underwood (1997) and Quinn & Keough (2002)). *Know your hypotheses and know your design*! It is also best to store and import the factors and their levels along with the data in order to avoid making mistakes. Otherwise, use the tools available in PRIMER to either create or import factors and their levels (see chapter 2 in Clarke & Gorley (2006)).

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[4] The *total sum of squares* is understood here to be defined by reference to the distance/dissimilarity measure of choice. It will only correspond to the traditional univariate total sum of squares when one variable is being analysed and Euclidean distance has been chosen as the basis of the analysis.

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