**A novel perspective on nestedness and a theory-oriented procedure for the use of null models.** Pinheiro R. B. P.\*, Dormann C.F., Felix G.M.F., and Mello M.A.R.

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# Commented scripts

To reproduce the analysis performed in our study, please keep the actual organization of folders and set as working directory the main folder containing the files “simulations.R” and “case\_study.R”.

Packages required: sads, bipartite, betapart, and doParallel. All required packages are available at CRAN repository (<https://CRAN.R-project.org>).

## simulations.R

This script runs simulations to compare the capacity of several nestedness indices in distinguishing between matrices with random topologies (equiprobable null model) and nested matrices (proportional null model).

The script performs 3 types of simulations: 1) weighted matrices with fixed dimensions and total weights, 2) binary matrices with fixed connectance, 3) binary matrices with fixed connectance but with probabilities derived from binary marginal totals (see main text). Simulated matrices are produced, indices are calculated, and results are saved within the folder “simulations”. For each type of simulation, 720 files are saved, each one containing 1000 simulations.

Parallel processing is used on the script. The easiest way to run the code without parallelizing is by registering only 1 core in “registerDoParallel (cores=1)”, but be prepared because the simulations may take a LONG time.

After performing the simulations and calculating the indices, the last part of the script summarizes the results, producing the files “simulations1.RData”, “simulations2.RData”, and “simulations3.RData” within the folder “results”. Those files are used in the R markdown files to produce Appendix S2 and S3.

## R Markdown scripts: Appendix S2.Rmd and Appendix S3.Rmd

These scripts are used to generate the pdf Appendices. They use the results from “simulations.R” and produce plots to compare the ability of nestedness indices in distinguishing nested and non-nested matrices.

## case\_study.R

In this script we illustrate our new procedures and the use of our new functions in a weighted empirical plant-pollinator network. This network was downloaded from the Web of Life database ([www.web-of-life.es](http://www.web-of-life.es), network: M\_PL\_060\_17), and originally described by Kaiser-Bunbury [1].

# Manual for the new functions

## Function nestnulls

Description: **nestnulls** is an R function to calculate matrix nestedness, generate nullmodels, and visualize results following the procedure proposed in the main text.

Table 1 - Nestedness metrics implemented in the R function *nestnulls*.

|  |  |  |
| --- | --- | --- |
| Metric | Code | Reference |
| Binary | | |
| Temperature | temperature | [2] |
| Manhattan distance | MD | [3] |
| NODF | nodf | [4] |
| βNES | betaNES | [5] |
| βNES / βSOR | betaNES2 | [5] |
| Binary spectral radius | binSR | [6] |
| Weighted | | |
| WNODF | wnodf | [7] |
| WNODA | wnoda | [8] |
| Spectral radius | SR | [6] |
|  |  |  |

Usage: nestnulls (M, index="wnoda", equi.null=T, prop.null=T, n.null=1000, calc.at=NULL, print.at.each=NULL, wprob=F, wsamp=F, density.plot=T, sampling.plot=T, diag.rm=F, lower.rm=F)

Requirements:

R packages *vegan*, *bipartite*, and *betapart*. All required packages are available at CRAN repository (<https://CRAN.R-project.org>).

Arguments:

M = an adjacency matrix (binary or weighted).

index = the nestedness (or overlap) metric to be applied. See codes in Table 1 for each metric implemented in the function.

equi.null = False or True. Indicates whether you want to generate an equiprobable null model.

prop.null = False or True. Indicates whether you want to generate a proportional null model.

n.null = the number of randomized matrices in each null model.

calc.at = a vector indicating the different sampling intensities at which nestedness should be calculated on the null models. If NULL, nestedness is only calculated at the actual sampling intensity. Only meaningful if total weights instead of connectance is conserved in the null models (weighted indices or binary indices in weighted network with wsamp=T).

print.at.each = the number of null matrices produced for the function to print the progress. If this is not necessary, set to NULL.

wprob = False or True. If a weighted matrix is provided but a binary nestedness index is selected, indicates whether the weighted marginal sums should be used to produce the null models. If False and a binary index is selected, probabilities follow the null model 2 of Bascompte *et al.* [9].

wsamp = False or True. If a weighted matrix is provided but a binary nestedness index is selected, indicates whether total weights instead of connectance should be fixed in the null matrices.

density.plot = False or True. Indicates whether a density plot for comparison between the observed and the null models is produced after the analysis.

sampling.plot = False or True. Indicates whether a plot for comparison between the observed and the null models, under different sampling intensities, is produced after the analysis.

diag.rm = False or True. Indicates whether interaction on the diagonal of the matrix should be removed and forbidden in the null models. Ideal for unipartite networks without self-connections. NA’s on the diagonal of the input matrix are allowed if diag.rm is True. If True, the matrix must be square.

lower.rm = False or True. Indicates whether the lower triangular of the matrix should be removed and forbidden in the null models. Ideal for non-directed unipartite networks. If True, the input matrix must be square and symmetrical.

Outputs:

A list with

$observed = observed nestedness for the input matrix

$significance = significance of nestedness compared to the null models

$summary.equi = mean, standard deviation, and upper and lower limits for 95% of values for the equiprobable null model, for each sampling intensity defined in the argument calc.at.

$summary.prop = mean, standard deviation, and upper and lower limits for 95% of values for the proportional null model, for each sampling intensity defined in the argument calc.at.

$equinull = nestedness values for each matrix in the equiprobable null model.

$propnull = nestedness values for each matrix in the proportional null model.

$nsampled = sampling intensity in the input matrix

$parameters = a list with the parameter values applied

Plots

a density plot for comparison between the observed and the null models (if density.plot = T). Red line: equiprobable null model. Blue line: proportional null model based on weighted marginal sums; Yellow line: proportional null model based on binary marginal sums (node degrees).

a plot for comparison between the observed and the null models, under different sampling intensities (if sampling.plot = T). It can only be produced when total weights instead of connectance is fixed on random matrices (weighted index applied or wsamp = T). Red line: equiprobable null model. Blue line: proportional null model based on weighted marginal sums;

Notes:

The code used to calculate Spectral Radius and Manhattan Distance was adapted from the FALCON package developed by Stephen Beckett. FALCON is freely available at GitHub: <https://github.com/sjbeckett/FALCON>.

## Function plot1.nestnulls

Description: **plot1.nestnulls** creates a density plot for comparison between the observed and the null models from an output of **nestnulls** function. Identical to the plot produced when the argument density.plot = T in nestnulls.

Usage: plot1.nestnulls (x)

Arguments:

x = an output of **nestnulls** function.

## Function plot2.nestnulls

Description: **plot2.nestnulls** creates a plot for comparison between the observed and the null models, under different sampling intensities, from an output of **nestnulls** function. Identical to the plot produced when the argument sampling.plot = T in nestnulls.

Usage: plot2.nestnulls (x)

Arguments:

x = an output of **nestnulls** function.

## References:

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