A consumer's guide to nestedness analysis

Werner Ulrich, Mário Almeida-Neto and Nicholas J. Gotelli

W. Ulrich (ulrichw@umk.pl), Dept of Animal Ecology, Nicolaus Copernicus Univ. in Toruń, Gagarina 9, PL–87-100 Toruń, Poland. – M. Almeida-Neto, Univ. de Brasilia, IB-Dept Ecologia, CEP 70910-900, Asa Norte, Brazil. –N. J. Gotelli, Dept of Biology, Univ. of Vermont, Burlington, VT 05405, USA.

Nestedness analysis has become increasingly popular in the study of biogeographic patterns of species occurrence. Nested patterns are those in which the species composition of small assemblages is a nested subset of larger assemblages. For species interaction networks such as plant–pollinator webs, nestedness analysis has also proven a valuable tool for revealing ecological and evolutionary constraints. Despite this popularity, there has been substantial controversy in the literature over the best methods to define and quantify nestedness, and how to test for patterns of nestedness against an appropriate statistical null hypothesis. Here we review this rapidly developing literature and provide suggestions and guidelines for proper analyses. We focus on the logic and the performance of different metrics and the proper choice of null models for statistical inference. We observe that traditional 'gap-counting' metrics are biased towards species loss among columns (occupied sites) and that many metrics are not invariant to basic matrix properties. The study of nestedness should be combined with an appropriate gradient analysis to infer possible causes of the observed presence–absence sequence. In our view, statistical inference should be based on a null model in which row and columns sums are fixed. Under this model, only a relatively small number of published empirical matrices are significantly nested. We call for a critical reassessment of previous studies that have used biased metrics and unconstrained null models for statistical inference.

The basic concept

In biogeography, the concept of nestedness was proposed independently by Hultén (1937; cited in Hausdorf and Hennig 2003), Darlington (1957) and Daubenmire (1975), to describe patterns of species composition within continental biotas and among isolated habitats such as islands and landscape fragments (Fig. 1A). In a nested pattern, the species composition of small assemblages is a nested subset of the species composition of large assemblages.

Even though the concept dates from the first half of the past century, nestedness analyses became popular among ecologists only after Patterson and Atmar (1986) and Patterson (1987) proposed that nestedness patterns reflected an orderly sequence of extinctions on islands and in fragmented landscapes. Afterwards, they introduced an intuitive 'matrix temperature' metric to quantify the pattern of nestedness. The matrix temperature could be easily calculated with a freely distributed software package (The Nestedness Temperature Calculator), which included a bundled set of 294 presence–absence matrices that were compiled from the literature (Atmar and Patterson 1993, 1995). These innovations were largely responsible for the initial popularity of nestedness analysis and its continued application during the past 20 years.

Nestedness data are usually organized as a familiar binary, presence–absence matrix: each row is a species, each column is a site (or a sampling time), and the entries indicate the presence (1) or absence (0) of a species in a site (McCoy and Heck 1987). Typically, the matrix is ordered according to the marginal row and column sums, with common species placed in the upper rows, and species-rich sites placed in the left-hand columns (Fig. 1). When the data are organized this way, nestedness is expressed as a concentration of presences in the upper left triangle of the matrix (Fig. 1A).

Another distinct application of nestedness analysis has been the description of bipartite networks involving two sets of potentially interacting species (Fig. 1B). In bipartite networks, the rows of the matrix represent one set of species (such as predators or herbivores) and the columns represent the other set of species (such as prey or plants). Nestedness is particularly common in mutualistic networks, such as those involving plants species and their pollinators or seed dispersers (Bascompte et al. 2003, Dupont et al. 2003, Ollerton et al. 2003; but see also Guimarães et al. 2006, 2007, Ollerton et al. 2007 for other mutualistic systems). Some antagonistic and commensal bipartite networks also have exhibited nested patterns (host–parasite: Valtonen et al. 2001, plant–herbivore: Lewinsohn et al. 2006, plant–epiphyte: Burns 2007, tree–fungus: Vacher et al. 2008). In such nested interaction networks, specialists from both sets of species interact preferentially with generalists (Bascompte and Jordano 2007).

Figure 1. Nested geographical (A) and interaction (B) matrices. The isocline in (A) separates the occupied and empty regions of a maximally nested matrix. According to the temperature concept the marked absences and presences have the greatest distances to the isoclines and are therefore less probable (contain more information) than respective absences and presences nearer the isoclines. The upper left square in (B) marks the group of generalist core species (Bascompte et al. 2003), whereas the bottom right rectangle depicts possible forbidden species combinations.

The distinction between geographical and bipartite matrices is not strict, but instead marks the ends of a continuum. For example, nestedness analysis has been applied to describe the distribution of species among resource patches, such as parasite species among hosts (Guégan and Hugueny 1994, Poulin 1996, Worthen and Rhode 1996, Rhode et al 1998, Timi and Poulin 2008) and scavenger species among carrion patches (Selva and Fortuna 2007). Such systems can be viewed both as occupied resource patches and as bipartite interaction networks. Patches correspond to the biogeographical islands, but differ from them because patches represent both habitats and food resources. In addition, they are dynamical islands capable of responding to their 'guests' both in ecological time (population dynamic responses) and in evolutionary time (coevolutionary responses).

Nestedness analysis has been a widely used ecological tool to describe patterns of species occurrences and their underlying mechanisms. A search in Scopus within the subject 'environmental sciences' returned more than 120 papers published since 2000 with the keyword 'nestedness'. This interest has been accompanied by a growing number of studies of the statistical properties of nestedness metrics, the performance of null models in nestedness analysis, and the inference of mechanism from a pattern of nestedness (Cook and Quinn 1998, Wright et al. 1998, Jonsson 2001, Fischer and Lindenmayer 2002, 2005, Maron et al. 2004, Greve and Chown 2006, Higgins et al. 2006, Rodríguez-Gironés and Santamaría 2006, Burgos et al. 2007, Hausdorf and Hennig 2007, Moore and Swihart 2007, Nielsen and Bascompte 2007, Ulrich and Gotelli 2007a, 2007b, Almeida-Neto et al. 2007, 2008, Santamaria and Rodríguez-Gironés 2007, Timi and Poulin 2008).

There are three steps in a nestedness analysis: 1) calculation of a metric to quantify the pattern of nestedness in a matrix; 2) comparison with an appropriate null model or randomization test to assess the statistical significance of the metric; 3) inference of the mechanism that generated the pattern of nestedness. On all three points, no consensus has yet been reached among ecologists, which has hindered a general understanding of the frequency, causes, and consequences of nestedness (Ulrich and Gotelli 2007a, Almeida-Neto et al. 2008, Timi and Poulin 2008). The growing number of applied and theoretical studies of nestedness calls for a critical review of the state of art and for perspectives for future research. In this paper we concentrate on the different nestedness metrics that have been proposed and the randomization algorithms with which they are tested. Our aim is to provide a review of the basic statistical issues and provide some recommendations for future analyses. We will focus on nestedness patterns in biogeography and community ecology, which are the most popular form of analysis and rely on a species occurrence matrix (Fig. 1A). However, many of the suggestions and guidelines proposed here can also be applied to the analysis of bipartite networks (Fig. 1B).

Causes of nested subsets

Nestedness was first described for insular faunas (Darlington 1957, Patterson and Atmar 1986) and was attributed to differential rates of extinction and colonization. However, a variety of different mechanisms can lead to a nested pattern, some of which are deterministic and some of which are stochastic. Table 1 and 2 briefly review the different mechanisms that have been proposed for nestedness in species occurrence matrices and networks of interacting species. For the latter, there has been much recent debate on the mechanisms underlying nestedness (see Santamaría and Rodríguez-Gironés 2007 and responses by Vázquez 2007, Devoto 2007 and Stang et al. 2007a), and Table 2 should be treated as an initial compilation of hypotheses.

All of the explanations for nested subsets within geographic matrices can be seen as variations of ordered colonisations or extinctions along environmental or biological gradients (area, isolation, quality) of the target patches. In most cases, these mechanisms cannot be distinguished

Table 1. Causes of nested subset patterns in metacommunities.

Hypothesis	Assumption/precondition		Predictions	Examples
	Site properties – gradient of:	Species properties – gradient of:		
Passive sampling	carrying capacities of sites	regional abundance	power function relationship between richness and area (SAR). Regional abundance predicts occupancy	Andrén 1994a, 1994b, Cutler 1994, Fischer and Lindenmayer 2002, Higgins et al. 2006, Ulrich and Gotelli 2007a
Neutrality	carrying capacities of sites	dispersal ability	regional log-series distributions. Extinction inversely proportional to local abundance	Ulrich and Zalewski 2007
Selective colonization	isolation	dispersal ability	selective occupancy of sites according to isolation	Darlington 1957, Patterson 1990, Cook and Quinn 1995, Honnay et al. 1999, McAbendroth et al. 2005
Selective extinction	carrying capacities of sites	extinction susceptibility (faunal relaxation)	selective occupancy of sites according to area of sites	Patterson and Atmar 1986, 2000, Wright and Reeves 1992, Bruun and Moen 2003, Wethered and Lawes 2005
Nested habitats	habitat heterogeneity	degrees of specialization	higher proportion of generalist species in smaller and/or resource poor patches	Wright and Reeves 1992, Honnay et al. 1999, Fleishman and Mac Nally 2002, Hausdorf and Hennig 2003, Hylander et al. 2005
Selective environmental tolerances	environmental harshness	environmental tolerances	selective occupancy of sites according to tolerance to environmental stress	Blake 1991, Worthen et al. 1998, Smith and Brown 2002, Greve et al. 2005, Driscoll 2008
Habitat quality	environmental harshness	–	site occupancy in accordance to the ideal free distribution model. Abundances tend to increase in the same direction	Simberloff and Martin 1991, Fernández-Juricic 2002, Hylander et al. 2005, Bloch et al. 2007

merely by establishing the statistical pattern of nestedness. Pinning down the different mechanisms usually will require additional data beyond the original presence–absence matrix, such as the sequence of extinctions that have led to the distribution of species on islands, or the spatial pattern of nesting of habitats and resources. To that extent, null models based on different orderings of the same matrix may allow for some inferences about simple colonization mechanisms and their contribution to nestedness patterns. Ideally, nestedness analyses should be accompanied by appropriate gradient analyses (Leibold and Mikkelson 2002). An ordering of sites in species × sites matrices according to such one-dimensional gradients (which could be generated through ordination and other multivariate methods) should result in different degrees of nestedness and allow for an identification of the strongest gradient that generates the nested pattern (Lomolino 1996).

We note that certain mechanisms, such as passive sampling, are embedded in some of the null model procedures used to test for patterns of nestedness. The passive sampling effect is widespread because metacommunities are typically characterized by species with highly unequal regional abundances that are distributed among patches (or isolates sensu Preston 1962) of different sizes. If the probability that a species colonizes a site is proportional to its regional abundance, abundant species have a better chance of colonizing many patches than do low-density species, a 'mass effect' (Leibold et al. 2004). Similarly, larger sites tend to harbour more species because they receive effectively larger samples than smaller ones (Connor and McCoy 1979, Cam et al. 2000). Passive sampling should not be confused with a similar 'collecting artefact' that results from disproportional sampling of rare species or small assemblages (Cam et al. 2000). Collecting artefacts introduce undesirable bias that needs to be controlled for (Gotelli and Colwell 2001), whereas passive sampling only needs to be controlled for if the aim is to test whether nestedness results from some biological process apart from the mass effect. Following the lead of all other nestedness analyses, we assume that the presence-absence data matrix is accurate and does not reflect major collecting artefacts.

Although habitat fragmentation is not a direct cause of nestedness, it is expected to generate a nested pattern because fragmented landscapes are characterized by patches that differ in size and relative isolation. According to Patterson and Atmar (2000), nestedness within fragmented landscapes is caused mostly by ordered extinction sequences. That means that smaller fragments selectively lose species that are habitat specialists with low abundance; these same species have a better chance of persistence in larger and/or less isolated fragments. Similarly, disturbance can also produce a nested pattern if ordered sequences of absences along disturbance gradients occur due to distinct disturbance susceptibilities (Worthen et al. 1998, Fernández-Juricic 2002, Bloch et al. 2007). This mechanism reduces to the nested habitat quality hypothesis because disturbance can be seen as one aspect of habitat quality.

Some mechanisms that cause nestedness in geographical matrices also apply to interaction networks. For instance, Ollerton et al. (2003) found abundant insect species visited a wider range of plant species than did rare insect species, and Olesen et al. (2008) reported that new species entering

Table 2. Causes of nested subset patterns in networks of interacting species.

Hypothesis	Assumption/precondition	Predictions	Examples
	Species properties – gradient of:		
Passive sampling	abundance and/or ubiquity	abundance and/or ubiquity predict occupancy	Ollerton et al. 2003, Medan et al. 2007
Asymmetric interaction strength	ecological specialization	ecological specialization leads to forbidden interactions. species are ecologically not equivalent (not neutral)	Bascompte et al. 2003, Thompson 2005, Jordano et al. 2006, Ollerton et al. 2007, Olesen et al. 2007
Phenotypic complementary	morphological specialization	different tempo of phenotypic change should result in a generalist/specialist gradient	Rezende et al. 2007

a pollination network preferentially interact with already well-connected (abundant) species. These patterns suggest that passive sampling explanation might account for nestedness patterns in pollinator networks: abundant pollinators and plant species simply have higher chances to visit and to be visited by their more abundant counterparts (Table 2). Furthermore, the aforementioned selective extinction and colonization models apply to interaction networks as well.

Jordano et al. (2003, 2006) drew attention to 'forbidden interactions' within interaction networks (Fig. 1B). These are interactions that are impossible due to physical or biological constraints, such as phenological asynchrony or morphological mismatching. For instance, imagine an interaction matrix of seed dispersers and plants. If both groups exhibit morphological gradients of body size and seed size, bipartite combinations of large seed size and small seed-disperser body size and vice versa might be less probable than interactions of pairs of more similar sizes. Recently Stang et al. (2006, 2007a) showed that nectar-holding depth of flowers produces asymmetric interactions between plants and their insect pollinators. Although they found a significant nested patter under an equiprobable null model (Table 4), their analysis did not evaluate directly whether nestedness might arise simply from mismatches of pollinator proboscis length and nectar holding depth.

Nested interaction networks would also arise from the complementarity and convergence of phenotypic traits between both sets of interacting species (Thompson 2005). In a simulation study, Rezende et al. (2007) found that highly nested networks can emerge from phenotypic complementarity. Their analysis of empirical mutualistic networks supported the hypothesis that co-evolution of several phenotypic traits results in strongly nested matrices. This finding directly links nestedness to evolutionary processes.

Getting started

Two questions need to be answered before performing a nestedness analysis: (1) What type of nestedness pattern is of interest: nestedness of species incidence, of species composition, or both? (2) What is the criterion used to order the rows and/or columns of the matrix? Answers to these questions will affect both the choice of the nestedness metric, and the choice of the null model.

The first question can be translated as 'is nestedness being tested for matrix rows (species incidence), for matrix columns (species composition), or for both?' If the hypothesis being tested predicts nestedness both for species composition and species incidence, then we should use a metric capable of quantifying both components of nestedness. Conversely, if one wishes to test whether differences in environmental variables or, alternatively, in life-history traits, promote nestedness, we should use a metric that is able to measure nestedness independently among columns and among rows.

For example, the matrix temperature metric (Atmar and Patterson 1993, Rodríguez-Gironés and Santamaría 2006) is based on distances of unexpected presences and absences from a diagonal isocline of perfect nestedness. Therefore, this metric is actually testing for an aggregate pattern that reflects both species incidence and species composition. Although some authors have subsequently tried to correlate the temperature metric with environmental factors or species traits in the packed matrix (Meyer and Kalko 2008), this approach cannot discriminate patterns of nestedness in composition from the patterns of nestedness in incidence.

Nestedness analysis requires an ordering of rows and/or columns of the incidence matrix according to some predefined criterion. This will be an ordering either according to species richness and incidence or according to an environmental gradient hypothesized to be controlling the observed distribution of species (Lomolino 1996, Fernández-Juricic 2002, Bruun and Moen 2003). Patch area and the degree of isolation are commonly used for ordering matrices (Lomolino 1996, Bruun and Moen 2003), in which case the pattern is linked to the common species – area and isolation – diversity relationships (MacArthur and Wilson 1963). Different outcomes after sorting according to area and isolation can be used to judge whether the system is colonization- or extinction-driven (Bruun and Moen 2003). If the area-sorted matrix is nested but the isolation-sorted not, the system should be extinction-driven because colonization does not seem to be sufficiently strong to generate nestedness. Whether the opposite argument holds is less clear because either selective immigration or extinction can generate nested patterns for isolation-sorted matrices. However, passive sampling can be ruled out because nestedness should appear after either method of sorting (Driscoll 2008). Hence differential

sorting according to independent environmental or biological gradients is a way to falsify at least the passive sampling hypothesis.

Analyses of interaction networks have simply used matrix row and column totals of the matrix, and have not addressed alternative orderings. However, sorting according to morphological gradients or measures of resource specialization should be explored to provide further tests of the 'forbidden interactions' hypothesis (Jordano et al. 2006, Ollerton et al. 2007).

The choice of a metric that is based on incidences, species composition, or both addresses whether presences and absences in the matrix differ in information content. For instance, in a colonization-driven assemblage of species with high dispersal ability, absences might be more informative than presences, and a metric should put more weight on unexpected absences (holes) than on unexpected presences (outliers). In contrast, if extinctions dominate the system, local presences might contain more information. In the absence of a priori expectations, presences and absences in the matrix should receive equal weight. This important point has been overlooked in nearly all previous studies on nestedness.

Quantifying nestedness

Several studies have quantified nestedness by ordering columns on the basis of area, isolation, or other criteria, and then testing whether the ranks of occupied cells versus unoccupied cells are significantly different by a U-, t-, or χ^2-test (Schoener and Schoener 1983, Patterson 1984, Simberloff and Levin 1985, Fernández-Juricic 2002). This method is potentially valuable because it may reveal which variables lead to strong or weak nestedness patterns. However, this method necessitates many tests of individual species performed on the same data matrix and requires a correction of probability levels for multiple tests. Most researchers now use the sequential Bonferroni correction for dependent or independent data sets (Holm 1979, Benjamini and Yekutieli 2001), although Moran (2003) has cautioned that these procedures may inflate type II error rates (incorrectly accepting a null hypothesis that is false). Perhaps a more fundamental issue is that these tests treat each species in isolation, whereas most nestedness studies are concerned with community-level patterns.

The first community-wide metric for nestedness was developed by Patterson and Atmar (1986), and since then, several metrics have been proposed (Table 3). The aim of any metric is to quantify the extent to which a given arrangement of presences and absences approximates or deviates from a perfectly nested pattern. However, some of the metrics differ because they quantify distinct matrix properties (e.g. unexpected absences or holes, unexpected presences or outliers, and overlaps), and/or put different weights to these properties (Table 3). Recently, Almeida-Neto et al. (2008) showed that most metrics do not measure nestedness according to what most authors have defined as a nested pattern for metacommunities or bipartite networks.

Any nestedness metric should be invariant to simple algebraic re-arrangements of the matrix that do not reflect different biological hypotheses. Moreover, the null model

analysis used to test for statistical significance should ideally have good power to detect nestedness when the pattern is generated by a non-random process (low type II error), but also should not detect nestedness when applied to matrices generated by a random process (low type I error). The nestedness metric and its statistical performance ideally should not be affected by matrix size and shape. Matrix fill (percent of 1s in the matrix) also affects the detection of nestedness, but comparison with a proper null model should control in part for the effects of matrix fill. Moreover, there is no a priori reason for a nestedness metric to treat absences and presences differentially. If both contain the same amount of information, a metric should be unaffected by occurrence inversion and give the same degree of nestedness after all of the matrix presences have been converted to absences and vice versa. Further, the placement of species in rows or columns is arbitrary, so that a metric that measures nestedness in both rows and columns should be invariant to a matrix transpose. Violations of these requirements might increase the risk of type I and type II errors (Wright et al. 1998, Ulrich and Gotelli 2007a).

Gap metrics

Given an incidence matrix whose columns and rows have been sorted by their marginal totals, a 'gap metric' quantifies nestedness by counting the number of absences followed by presences according to some predefined rule (first seven metrics in Table 3). Gap metrics evaluate whether species in species-poor columns are proper subsets of the species in richer columns. This popular definition of nestedness implies that gap metrics are column oriented and therefore not independent of a matrix transpose. To make a gap metric transpose invariant, we can calculate the metric separately for rows and columns and take the smaller value as the final score. The original definition of the Brualdi and Sanderson (1999) discrepancy index (BR) even implies such a calculation, and below we use BR in this way (Ulrich 2006, Ulrich and Gotelli 2007a).

Gap-counting metrics are inherently correlated with matrix size and fill (Wright et al. 1998, Ulrich and Gotelli 2007a, Almeida-Neto et al. 2008) so it has been common for researchers to transform them before analysis and comparison (Wright and Reeves 1992, Lomolino 1996, Wright et al. 1998, Brualdi and Sanderson 1999, Brualdi and Shen 1999, Greve and Chown 2006). However, these algebraic transformations will not address the problem that all statistical tests have low power at small sample size (=small matrix size). Moreover, algebraic standardization will not account for differences in pattern that are affected by matrix row and column sums.

One of the primary motivations for null model analysis has been that null model tests may potentially control for basic influences of matrix size and shape because all of the simulated matrices have the same size and shape as the empirical matrix (Gotelli and Graves 1996). This accomplishes much of the desired standardization, although differences in the strength of the pattern among matrices may still be correlated with matrix size and shape in null model analyses, and a Z-transform (Gotelli and

Table 3. An overview over existing nestedness metrics. The seven first are gap counting metrics and the two last ones are metrics based on overlap among columns and/or rows.

Nestedness metric	Author(s)	Aim to quantify whether a metacommunity:	Description
N0 (no. of absences)	Patterson and Atmar 1986	deviates from a nested pattern due to non-ordered extinctions from the poorest to the richest sites in which each species occurs	a count of how often a species is absent from a site with greater species richness than the most impoverished site in which it occurs and sums across all species
N1 (no. of presences)	Cutler 1991	deviates from a nested pattern due to non-ordered colonizations from the richest to the poorest sites in which each species occurs	a count of the number of occurrences of a species at sites with fewer species than the richest site lacking it and sums across all species
Ua (no. of unexpected absences)	Cutler 1991	deviates from a nested pattern due to non-ordered extinctions from an intermediate richness to the richest sites in which each species occurs	a count of unexpected absences of species from more species-rich sites for which the sum of unexpected absences and presences is minimal
Up (no. of unexpected presences)	Cutler 1991	deviates from a nested pattern due to non-ordered colonizations from an intermediate richness to the poorest sites in which each species occurs	a count of unexpected presences of species from more species-poor sites for which the sum of unexpected absences and presences is minimal
Ut (no. of unexpected transformations)	Cutler 1991	deviates from a nested pattern due to both factors explained for Ua and Up	the sum of Ua and Up
Nc (nestedness index)	Wright and Reeves 1992	approximates from a nested pattern by evaluating if presences of species in poorer sites are correctly predicted by their presences in equally rich or richer ones	a count of the number of species shared over all pairs of sites
D (no. of departures)	Lomolino 1996	deviates from a nested pattern by means of sequences of unexpected absences followed by presences	the number of times the absence of a species is followed by its presence on the next site
BR (discrepancy measure)	Brualdi and Sanderson 1999	deviates from a nested pattern by means of minimum number of replacements of presences to produce a new nested matrix	counts of the minimum number of discrepancies (absences or presence) for rows and columns that must be erased to produce a perfectly nested matrix
T (matrix temperature)	Atmar and Patterson 1993	deviates from a nested pattern due to unexpected extinctions and colonizations, respectively, in more and less ''hospitable'' sites	a normalized sum of squared relative distances of absences above and presences below the hypothetical isocline that separates occupied from unoccupied areas in a perfectly nested matrix
HH (the number of supersets)	Hausdorf and Hennig 2003	to quantify whether less frequent species are found in subsets of the sites where the most widespread occur	counts the cases in which the occurrence of a species form a subset of the occurrence of another species
NODF (nestedness measure based on overlap and decreasing fills)	Almeida-Neto et al. 2008	to quantify independently (1) whether depauperate assemblages constitute subsets of progressively richer ones and (2) whether less frequent species are found in subsets of the sites where the most widespread occur	the percentage of occurrences in right columns and species in inferior rows which overlap, respectively, with those found in left columns and upper rows with higher marginal totals for all pairs of columns and of rows

McCabe 2002) is still needed to meaningfully compare the strength of the pattern for different matrices.

Wright et al. (1998) reported the 'percent metric' values of the gap-counting metrics N1, UA, UT and UC (abbreviations in Table 3) to be positively correlated with matrix fill using a null model with equiprobable cells and no constraints on row and column sums (Table 4). Ulrich and Gotelli (2007a) found that matrix fill also affected the N0, N1, UA, UP metrics even with a more constrained null model with fixed row and column sums. Among the gap metrics, only BR and NC appeared to be largely independent of matrix fill, shape and size, irrespective of null model (Ulrich and Gotelli 2007a, Almeida-Neto et al. 2008).

To test whether the gap metrics are invariant to transposition and occurrence inversion we used a subset of 286 matrices of the 294 presence–absence matrices provided by Atmar and Patterson (1995) and generated for each matrix its transpose and occurrence inverse. Of the gap-counting metrics we found only BR and UT to be invariant to both transformations.

Overlap metrics

Hausdorf and Hennig (2003) devised a metric based on the numbers of species that form a subset of other species, i.e. the number of supersets (HH, Table 3). In contrast to gap metrics, HH quantifies nestedness for species incidence instead of species composition. Unfortunately, the metric is excessively affected by outliers and is not standardized.

Recently Almeida-Neto et al. (2008) introduced a new metric (NODF) based on standardized differences in row and column fills and paired matching of occurrences (Table 3). An appealing feature of NODF is that it decomposes total nestedness into a sum of the nestedness introduced by columns and by rows. Both its absolute values and its Z-transform are size invariant (Almeida-Neto et al. 2008). NODF is also invariant to transpose but not to occurrence inversion.

The temperature metric

By far, the most popular metric for quantifying nestedness has been the matrix temperature T introduced by Atmar and Patterson (1993, 1995) and its recent modifications (Rodríguez-Gironés and Santamaría 2006, Ulrich and Gotelli 2007a). T is a normalized sum of squared relative distances of absences above and presences below the hypothetical isocline that separates occupied from unoccupied areas in a perfectly nested matrix (Fig. 1A, Table 1). Thus, T evaluates simultaneously whether rows and columns are nested with respect to species incidence and species composition. In our test with the Atmar and Patterson (1995) matrices, T appeared to be to invariant to both matrix transpose and occurrence inversion after sorting according to marginal totals. However, due to its weighing algorithm, T is positively correlated with matrix size (Wright et al. 1998, Almeida-Neto et al. 2008).

The temperature concept differs from the gap-counting metrics because it is based on the specific biogeographic hypothesis that absences in predominately occupied areas of the matrix and occurrences in predominately empty areas are less probable (more informative) than respective occurrences and absences. This argument is motivated by the theory of island biogeography, which implies that absences at species-rich sites and presences at species-poor sites demand particular attention. In contrast to the gap metrics, T weights the cells of the matrix in proportion to their distance from the isocline (Greve and Chown 2006). Atmar and Patterson's (1993) original T uses quadratic weights, but other weighting functions are possible. Because of this weighting, one prominent shortcoming of the temperature metric is its dependence on matrix size and fill that increases type I error rates for larger and more filled matrices (Rodríguez-Gironés and Santamaría 2006, Greve and Chown 2006, Ulrich and Gotelli 2007a).

The different philosophies behind gap metrics, overlap metrics, and matrix temperature have often been overlooked in comparative studies, which has led to some confusion

Table 4. Common null models used to infer expected nestedness (species in rows, sites in columns).

Name	Other names used in the literature	Row constraint	Column constraint	Author(s)
Equiprobable–equiprobable	SIM1, R00	equiprobable	equiprobable	Atmar and Patterson 1993, Gotelli 2000
Fixed–equiprobable	SIM2, R0, Random0	fixed	equiprobable	Patterson and Atmar 1986, Gotelli 2000
Equiprobable–fixed	SIM3	equiprobable	fixed	Gotelli 2000
Fixed–fixed	SIM9	fixed	fixed	Connor and Simberloff 1979, Diamond and Gilpin 1982, Gotelli 2000
Fixedincidence proportional	SIM5, R1, Random1	fixed	proportional to species incidences	Patterson and Atmar 1986, Gotelli 2000
Abundance–proportional	Randnest	none	proportional to species relative abundances	Jonsson 2001
Incidence–proportional	SIM8, Model 2	proportional to species incidences	proportional to species incidences	Gotelli 2000, Bascompte et al. 2003
Equiprobable–proportional		proportional to species incidences	equiprobable	Fischer and Lindenmayer 2002
Proportional	R_{ecol}	proportional to species relative abundances	proportional to carrying capacities	Moore and Swihart 2007

Questions	Answers	Metrics	Observations
What is being tested?	(1) composition (2) incidence (3) both	BR$_{BS}$ and NODFc HH and NODFr T, BR and NODF	composition is a property of sites incidence is a property of species the focus is both differences among sites (e.g. size, isolation) and among species (e.g. dispersal ability)
How are columns and/or rows sorted?	(1) MT	BR, HH, and NODF	columns and rows of expected matrices created by null models should also be sorted by MT
	(2) LHT (columns) and MT (rows)	T, BR, HH, and NODF	only rows of expected matrices should be sorted by MT
	(3) MT (columns) and EV (rows)	BR, and NODF	only columns of expected matrices should be sorted by MT
	(4) EV (columns) and LHT (rows)	T, BR and NODF	columns and rows of expected matrices should not be sorted by MT

BR$_{BS}$ =Brualdi and Sanderson's (1999) original algorithm for BR that only replaces 0–1's within rows.

about the interpretation of nestedness patterns. The temperature metric was designed specifically for insular floras and faunas, in which ordered sequences of colonization and extinction can be reasonably associated with unexpected gaps in the incidence matrix. Thus, Atmar and Patterson's (1995) data compilation – on which many subsequent tests were based – contains nearly exclusively island matrices. However, it is unclear whether the temperature concept should be applied to interaction networks (Bascompte et al. 2003, Dupont et al. 2003, Ollerton et al. 2003). In networks of interacting species, there is no a priori reason to assume that certain species pairs are less probable than others and to weight the cells by their distance to the isocline. None of the three explanations for nestedness in such matrices (Table 2) explicitly refers to differential occurrence and absence probabilities. More appropriate metrics for interaction networks are NODF (Almeida-Neto et al. 2008), BR (Brualdi and Sanderson 1999), and HH (Hausdorf and Hennig 2003). Nevertheless, these points deserve further attention and a critical meta-analysis and re-analysis of published networks of interacting species is needed.

Unexpected presences and absences

A perfectly nested matrix contains no absences (holes or unexpected absences) within its filled part and no presences (outliers or unexpected presences) within its empty part. The gap and temperature metrics use the numbers of holes and outliers to quantify the degree of nestedness. However, neither holes nor outliers have been defined consistently in the literature (Wright et al. 1998, Bird and Boecklen 1998). For example, in Fig. 2, numbers of holes and outliers defined by N0 and N1 differ from those defined by T. This inconsistency in definition has consequences for the use of holes and outliers in subsequent analyses, for instance in the identification of idiosyncratic species and sites in biogeographic analysis (below). Gap metrics define holes and outliers based on rows and columns only. However, in our view, the position of holes and outliers in the matrix intimately depends on the number and distribution of absences and presences within the whole matrix (Arita et al.
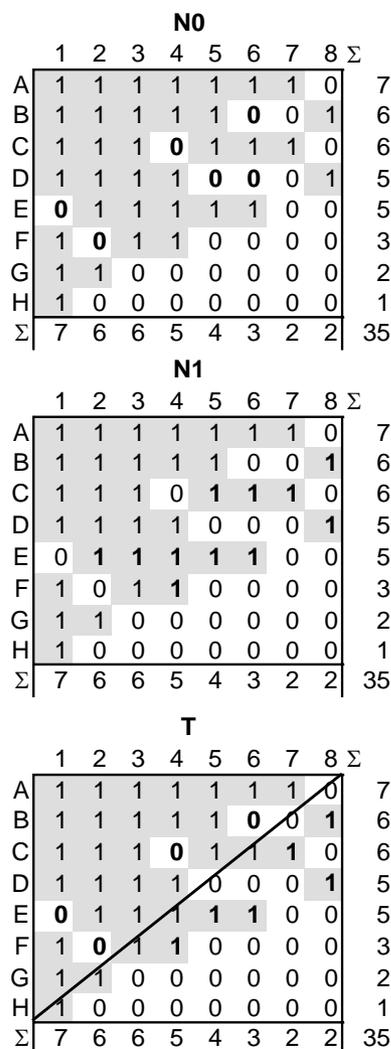
Figure 2. The gap metrics N0 and N1 and the temperature metric T define holes and outliers (the bold numbers) different. Using the definitions of N0 and N1 we would identify six holes and 11 outliers. T identifies four holes and six outliers.

2008). To date, only the temperature concept uses a clear matrix-wide definition of holes and outliers, although there is some vagueness in the original definition of the isocline that separates filled and empty parts of the matrix (Rodríguez-Gironés and Santamaría 2006, Ulrich and Gotelli 2007a).

Staistical inference

Normalization

The pattern of nestedness in matrices that differ in size, shape, or fill cannot be directly compared if the metric used is not invariant to these properties. Further, if for a given metric some of these matrix properties inflate type I and II error rates, we cannot compare the significance of nestedness. One way to overcome these difficulties is standardization. T and NODF are already normalized within the range of 0 to 100. To account for the dependence of T on matrix size, Greve and Chown (2006) proposed a modified standardization of T according to matrix fill instead of size. However, this allows for T-values larger than 100 and potentially alters the performance of T. The performance of the modification remains unclear.

The other metrics can be standardized (E_{ST}) by the following transformation (Wright and Reeves 1992)

$$E_{ST} = \frac{E_{obs} - E_{exp}}{E_{max} - E_{exp}} \qquad (1)$$

This transform still requires a null expectation E_{exp} (below) and the expected value E_{max} for a perfectly nested matrix. For metrics with $E_{max} = 0$ Eq. 1 is identical to the standardization proposed by Lomolino (1996). Equation 1 is a linear transform and does not change the response of the metric along the nestedness gradient. Other possible transformations such as the arc tangent transform that squeezes a metric within the range from −1 to 1 are non-linear and might influence the behaviour of the test. In the case of BR, Greve and Chown (2006) proposed a simple transformation independent of any null expectation

$$BR_{ST} = \frac{BR}{fill} \qquad (2)$$

where fill is the number of 1s in the matrix. UT can be transformed in the same way. Both transforms range between 0 and 1 (for the majority of data sets between 0 and 0.5). However, the transforms Eq. 1 and 2 do not fully eliminate dependencies on matrix properties. Although these kinds of transforms are mathematically convenient, they are not a good substitute for a proper null model analysis.

The most common standardization in nestedness analysis and in null model analysis in general is the Z-score

$$Z = \frac{E_{obs} - E_{exp}}{StDev_{exp}} \qquad (3)$$

which often gives an approximately normally distributed metric with mean $= 0$ and standard deviation (StDev) $= 1$. This transformation is based on a similar measure of 'effect size' in meta-analysis (Gurevitch et al. 1992). Unlike the previous algebraic transformations, the Z-score is a standardization that quantifies the position of the observed metric within the simulated distribution from the null model in common units of standard deviation. For this reason, in most cases the metric performs well and allows for valid statistical inference due to the well-known properties of the standard normal distribution. The Z-transforms of UT, BR and NODF appear to be independent of matrix properties (Ulrich and Gotelli 2007a, Almeida-Neto et al. 2008), but the Z-transform of T decreases with fill and size (Ulrich and Gotelli 2007a). Of course, in most cases the distribution of Z-transforms will be skewed. Therefore, estimation of probabilities for individual matrices should always be made directly from the distribution of simulated values, rather than from the normal approximation of the Z-transform.

Null models

The most controversial part of statistical inference in nestedness analysis is surely the choice of the appropriate null distribution to get E_{exp}. Starting from Connor and Simberloff (1979) there is a growing number of models that generate null expectations of matrix incidences (reviewed by Gotelli and Graves 1996). These ecological null models differ in their treatment of incidences (fill and marginal totals, Table 4). Most models constrain matrix fill to observed values (for an exception see Moore and Swihart 2007) but there is a gradient from liberal models that put no constraints on marginal totals (equiprobable null: Table 4) to restrictive models that fix numbers of occurrences within rows and columns (fixed − fixed null). Restrictive models incorporate more elements that are observed in the empirical matrix, although the precise way these constraints translate into biological assumptions is not always clear. For instance, the fixed − fixed model (Gotelli 2000) constrains column and row totals in the null model to match the observed values in the matrix. This generically preserves differences among rows and among columns, but does not specify which traits of species or resources are preserved. Constrained models often reduce the effect sizes ($E_{obs} - E_{exp}$) and potentially decrease type I and increase type II error levels. Because type I error levels have traditionally played a major role in statistical inference, more constrained null models are preferable because they are conservative and will not reject the null hypothesis unless the generating process is strong.

Another argument that speaks against the use of unconstrained null models is the potential dependence of the variance on sample size. The equiprobable null model is prone to give similar values of E_{exp} in larger matrices and therefore lowered variances due to statistical averaging (Ulrich unpubl.). This effect increases the respective Z-values and the associated type I error rates. This problem should not be so severe in the fixed-fixed model because much of the variability in the metric scores is associated with variability in row and column totals of the matrix. The fixed − fixed model is not applicable to matrices that are almost filled or almost empty because in these cases, there are very few matrix re-arrangements that will preserve all the row and column totals. However, such matrices are unusual and in the vast majority of cases, the sampling space should

be large enough for the fixed – fixed model to be applicable. The effect of matrix size on the power of a statistical test has been largely ignored in nestedness analysis (Fischer and Lindenmayer 2005).

The performance of different nestedness metrics is also influenced by the choice of the null model. For the Patterson and Atmar dataset, Fig. 3 illustrates regressions of Z-scores versus matrix size for the best-performing gap metrics (UT, BR,), and the temperature and NODF metrics under the equiprobable (A, B) and the fixed – fixed (C, D) models. Irrespective of metric, the equiprobable null model gave for most matrices extraordinarily large Z-scores and identified them as being highly nested. The fixed – fixed appeared to be much more conservative and identified only a small fraction of the matrices as being nested. Under the equiprobable null model, Z-scores of all four metrics were highly positively correlated, and thus exhibited similar statistical power. Under the fixed – fixed model, these correlations vanished and different metrics identified different matrices as being nested (Fig. 3C–D): of the 286 empirical matrices, 113 were identified by at least one of the metrics as being nested. However, only 10 matrices were jointly identified as being not random by all four metrics. Most similar in performance were NODF and T, with 29 joint significances. Moreover, the ranking of matrices according to the degree of nestedness depended on the null model used, and the ranks of Z-scores obtained from the equiprobable and the fixed – fixed nulls were only weakly correlated. The metrics that behaved most similarly with the different null models were UT (Spearman's rank $r = 0.33$, $p < 0.01$) and BR ($r = 0.29$, $p < 0.01$), whereas the respective ranks of T and NODF were not significantly correlated.

The equiprobable–equiprobable null model assumes that both columns and rows are equivalent, so that the probability of a species occurrence is the same for any cell in the null matrix. However, species differ in abundance and therefore in colonization ability (the mass effect) and sites differ in carrying capacities. There is growing acceptance that null models that do not consider species-specific

differences and variability among sites should not be used in biogeographic studies (Jonsson 2001, Ulrich and Gotelli 2007a, 2007b, Moore and Swihart 2007) and even in analyses of interaction matrices (Vázquez and Aizen 2006, Vázquez 2007, Stang et al. 2007b). However, the equiprobable–equiprobable null model might be justified for the analysis of interaction matrices because there is no a priori reason to assume that certain interactions are less probable than others. More research is needed to determine the most appropriate null model to use for interaction matrices.

One possibility is to use a spatially explicit version of the ecological drift model (Hubbell 2001), which makes precise predictions about species occurrence patterns (Chave 2004, Ulrich and Zalewski 2007). However, estimating the parameters for the neutral model so it can be used as a null model is problematic (Gotelli and McGill 2006). Nevertheless, Ulrich and Zalewski (2007) found that neutrality predicted a degree of nestedness in a community of ground beetles on lake islands that was significantly greater than observed in the real data, whereas the fixed-fixed null model did not detect this departure from randomness.

Many studies have addressed whether particular null models are able to control for the effect of passive sampling and to discern between mass effects and other causes of nestedness (Andrén 1994b, Cook and Quinn 1998, Gotelli and Ulrich 2007a, Hausdorf and Hennig 2007, Moore and Swihart 2007). Ulrich and Gotelli (2007a) found the fixed – fixed model to control at least in part for passive sampling effects. They constructed artificial matrices from passive sampling of a log-normally distributed metacommunity and showed that the BR metric did not identify these matrices as being more nested than expected by chance. However, after the numbers of unexpected absences and occurrences were artificially decreased, the BR metric used with the fixed – fixed model correctly identified the modified matrices as exhibiting a significantly nested pattern.

Unseen species and sampling effects

Most null model analyses assume the binary presence-absence matrices do not contain errors. However, missing or unseen species can have large effects on patterns in interaction matrices (Nielsen and Bascompte 2007), although this problem has been ignored until recently in standard nestedness analyses (but see Cam et al. 2000). Greve and Chown (2006) showed that T incorrectly pointed to an increase in nestedness after a matrix was seeded with additional occurrences of rare, non-nested species. Although Greve and Chown (2006) were discussing the effects of endemics, the same problems would arise for unsampled or undersampled species as well. Adding rare species to incidence matrices might severely alter the detection probabilities of nestedness metrics. In contrast, Nielsen and Bascompte (2007) found sample size effects to be less important for the assessment of nestedness in interaction matrices than species richness and matrix fill. Because rare species are most sensitive to sampling effects, these errors deserve more study in nestedness and interaction matrix analysis.

Figure 3. Correlations of Z-scores [Z = (observed −expected)/ StdDev Expectation] between the gap metrics UT and BR and the distance weighing metrics T and NODF under the equiprobable (A, B) and the fixed – fixed (C, D) null model. The squares mark the non-significant Z-scores at the 5% error benchmarks. Data from 286 matrices compiled in Atmar and Patterson (1995).

The sampling problem has also a theoretical perspective. Do rare and endemic species strengthen the nested pattern and therefore conform to models of selective colonization and extinction (Patterson 1990, Patterson and Atmar 2000) or are their occurrences random, so that when they are properly censused, they contribute to a reduction in nestedness? Additional exploration of the effects of species rarity and sampling errors in nestedness analysis would be worthwhile.

Idiosyncratic species

Atmar and Patterson (1993) termed species that decrease the matrix wide nestedness 'idiosyncratic' (Fig. 1A). One goal of nestedness analysis was always to identify such 'deviating' species and to infer the causes of idiosyncrasy. Atmar and Patterson (1993) explained the existence of idiosyncratic species by post-isolation immigration, geographic barriers, and competitive exclusion. Idiosyncratic species can be described as running counter to ecological and geographic gradients of species occurrence. For diatom communities Soininen (2008) showed that idiosyncratic species had wider geographic range sizes than 'normal' species, a pattern that surely deserves attention. Moreover, assemblages dominated by idiosyncratic species appeared to have rather high local species turnover (Soininen 2008). This finding is consistent with the selective extinction hypothesis for nestedness (Patterson 1990).

From a statistical perspective, idiosyncratic species should be more common among species of intermediate occupancy simply because these species have more potential combinations of unexpected absences and presences. At intermediate occurrence frequencies, statistical tests for idiosyncratic distributions will have maximum power, whereas at very high or low occurrence frequencies, the tests will be very weak. For example, a species that occurs only at one island (an endemic) has only one possibility for a gap. The same holds for a widespread species, which is absent from only one island. In contrast, a species that occurs at four of ten islands has six possibilities for gaps. Existing null model protocols cannot easily control for such factors because they are inherent in the occurrence frequencies of each species.

In conservation ecology, the identification of idiosyncratic sites has been discussed with regard to the single-large-or-several-small (SLOSS) debate (Atmar and Patterson 1993, Boecklen 1997, Patterson and Atmar 2000, Fischer and Lindenmayer 2005, Fleishman et al. 2007). Atmar and Patterson (1993) argued that the widespread occurrence of nested subsets speaks for the value of single larger areas to protect because they necessarily contain more species than any number of smaller sites. However, Boecklen (1997) and Fischer and Lindenmayer (2005) convincingly showed that this argument is only valid for perfectly nested subsets, which are very rare in nature. Even for highly significantly (but not perfectly) nested subsets, the total species numbers from subsets of many smaller sites are often higher than the respective number of species from a single larger site of the equivalent total area.

Nestedness an species co-occurrence

Nestedness is a pattern of species co-occurrence intrinsically related to the degree of species aggregation. A perfectly nested matrix is also a matrix with a maximum number of perfect pair wise species aggregations, but the opposite does not necessarily hold. Fig. 4 relates the Z-scores for BR and NODF of 286 Atmar and Patterson data matrices to the respective Z-transforms of the widely used C-score (Stone and Roberts 1990), which measures matrix-wide species' segregation. With the equiprobable–equiprobable null model, both metrics are highly correlated ($r = 0.97$; $p < 0.0001$) indicating that they capture essentially the same pattern. In other words in these matrices a nested pattern corresponds to species aggregation and vice versa. Indeed the C-score is a normalized matrix wide count of the number of joint occurrences (Stone and Roberts 1990) and measures therefore essentially the same as NC (Wright and Reeves 1992). This result together with the strong correlations shown in Fig. 3 call for a reassessment of what has actually been measured in previous analyses of nested subsets that have used the equiprobable–equiprobable null model. We are afraid that many previous studies have quantified a pattern of matrix-wide species aggregation instead of nestedness. Further analysis is needed to determine whether nestedness is measuring something above and beyond a simple pattern of species aggregation.

As with the nestedness metrics in Fig. 3, the relationships of C-scores with BR and NODF vanished under the fixed-fixed model (Fig. 4C–D). In fact, both nestedness metrics showed a weak negative correlation with the C-score (BR: $r = -0.62$; $p < 0.001$; NODF: $r = -0.48$; $p < 0.01$). When co-occurrence and nestedness patterns are both analyzed with the fixed-fixed model, the majority of matrices remained significantly segregated (Fig. 4; Gotelli and McCabe 2002, Gotelli and Ulrich unpubl.) whereas only a small minority appeared to be significantly nested (Ulrich and Gotelli 2007a). The contrasting results in Fig. 3 and 4 again emphasize that patterns of nestedness and species segregation depend on the particular combination of metric and null model that are used. These combinations must be carefully benchmarked against artificial random data sets before they can be used to understand patterns in empirical data matrices.

The correlation between nestedness and co-occurrence metrics might be used to identify non-random species associations. An idiosyncratic species is by definition more segregated than expected in a nested pattern, and this pattern could be useful in co-occurrence analysis. The detection of non-random species segregation is central to the ecological assembly rule discussion (Diamond 1975, Weiher and Keddy 1999), and has motivated much of the work on matrix-wide measures of species segregation. However, detecting individual species pairs that are non-random has proven to be a statistical challenge (Sfenthourakis et al. 2006, Gotelli and Ulrich unpubl.). The reason is simple. Even a moderate number of species gives hundreds or even thousands of unique species pairs, of which tens or even hundreds will be significantly non-random just by chance at the 5% or 1% error benchmarks. Recent attempts to solve the problem of identifying true non-random species pairs used sequential Bonferroni corrections and Bayesian
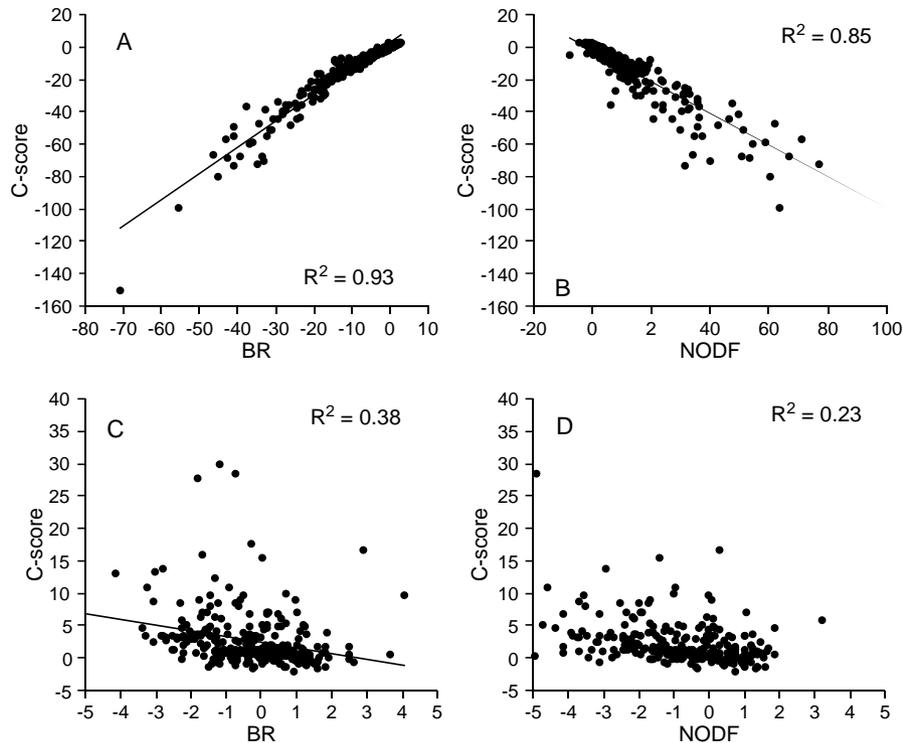
Figure 4. Correlations of Z-scores between the C-score and BR and NODF under the equiprobable (A, B) and the fixed–fixed (C, D) null models for 286 data sets provided by Atmar and Patterson (1995).

approaches (Ulrich and Gotelli unpubl, but see Moran 2003). It would be useful to know whether the species identified by these analyses are also idiosyncratic in a nestedness analysis. Table 6 shows how such an approach might work: 13 species pairs formed by 41 Amazon gymnotiform fishes (Fernandes Cox 1995) appeared to be significantly non-random using a sequential Bonferroni test applied to significance tests for all possible pairs. Two of the 14 species in these pairs were significantly idiosyncratic. The six pairs that contain these two species are the best candidates of 14 pairs for strong non-randomness. In contrast, of the four species involved in three non-significant pairs of mammals of the Thousand Islands (Lomolino 1986), none appeared to be significantly

Table 6. Pair wise species co-occurrence analysis of Amazon Gymnotiformes (data from Fernandes Cox 1995) and the oceanic and land bridge mammals of the Thousand Island region (Lomolino (1986). Amazon Gymnotiformes: 41 species at 31 sites. Pair wise analysis of all 820 species pairs returned 13 significant pairs (sequential Bonferroni corrected after Benjamini and Yekutieli 2001). Nestedness analysis using the temperature metric points to species 3 and 4 as being significantly idiosyncratic (bold face typed). The seven pairs containing these two species have particularly low species overlap and are prime candidates of being more segregated than expected by chance. Thousand island mammals: 10 species on 18 islands. The pair wise analysis returned three significant pairs (none is expected by chance at the 5% error benchmark). However, none of the species appeared to be significantly idiosyncratic.

Species 1	Occ1	Species 2	Occ2	Joint occurrences	Corr. p
Amazon Gymnotiformes					
6	20	**4**	6	2	<0.0001
6	20	37	4	1	<0.0001
6	20	38	2	0	<0.0001
6	20	29	9	5	<0.0001
8	10	**3**	5	0	<0.0001
6	20	32	17	11	<0.001
36	9	**3**	5	0	<0.001
17	10	**4**	6	1	<0.001
41	26	6	20	16	<0.01
12	8	**3**	5	0	<0.01
25	14	**3**	5	1	<0.01
19	9	**3**	5	0	<0.01
6	20	12	8	5	<0.01
Thousand Island mammals					
2	16	5	4	2	<0.001
2	16	4	3	1	<0.0001
2	16	10	2	1	<0.0001

idiosyncratic, so perhaps these pairs are not significantly non-random, even after being selected by a sequential Bonferroni test.

In summary, the study of nestedness continues to be an active research front in ecology, and has gained new impetus with the analysis of interaction networks. As in many areas of macroecology and biogeography, it is challenging to infer biological mechanisms from the patterns of species occurrences. In this review, we have emphasized the importance of the metrics that are used to quantify patterns of nestedness, and the null models that are used as benchmarks for comparison with observed metrics. Careful attention to both of these factors will improve the quality of our inferences about pattern.

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