

Endemicity biases nestedness metrics: a demonstration, explanation and solution

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Nestedness is frequently investigated to understand complex patterns of species occurrences. Temperature (T) is commonly used for comparisons of nestedness of different-sized datasets. However, the assumptions made for the standardization of this metric have not been fully explored, particularly the effects of endemicity. Here we show that T incorrectly indicates an increase in nestedness with the addition of non-nested endemics to matrices that are not perfectly nested – a consequence of standardizing matrix size by the product of species and sites. This problem is common both to test matrices and to real matrices that are typically subjected to nestedness analyses. The latter are often characterized by substantial numbers of endemics and by variation in the numbers of endemics in different taxa. Standardizing by occupancy resolves this problem, which is demonstrated using a derivative of discrepancy, d_1 . A small modification to T, such that it standardizes matrices by occupancy, would resolve the current problems with this nestedness metric.

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The distribution of species across a landscape can be envisaged as a species by site matrix with varying levels of occupancy. How species ranges overlap to produce the pattern of matrix fill is an essential component of understanding assemblage structure and the mechanisms underlying it (Diamond 1975, Cutler 1998, Ovaskainen 2002, Bell 2003), and can be investigated from the perspectives of variation in range size, beta-diversity, and matrix nestedness (Gaston and Blackburn 2000). All of these approaches have enjoyed increasing attention over the last decade (Brown 1995, Wright et al. 1998, Gaston 2003, Koleff et al. 2003). Nestedness analyses are thought to be useful because they apparently expand ecologists' abilities to deal with complex patterns of occurrence, and provide insight into the mechanisms underlying these patterns, such as the relative importance of colonization, extinction and nested physiologi-

cal tolerances in generating assemblage-level patterns (Cutler 1991, Atmar and Patterson 1993, Lomolino 1996, Cutler 1998, Mac Nally et al. 2002). Indeed, nestedness analyses are now widely used to investigate assemblage patterns and, because of their apparent utility, are also being incorporated into assessments of the effects of human disturbance on assemblages, and into conservation planning (Boecklen 1997, Fernández-Juricic 2002, Fleishman et al. 2002, Wethered and Lawes 2005).

Two recurrent themes in the nestedness literature are which metric should be used to calculate nestedness (there are various ways of doing so) and the randomization procedure that should be applied to test for significance of the metric (for review see Cutler 1998, Wright et al. 1998, Gaston and Blackburn 2000). In the former case, several major issues have been of concern,

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including the use of both unexpected presences and absences to calculate a metric, the likelihood of unexpected presences and absences given differential patterns of species occupancy, and standardization for matrix size to facilitate matrix comparisons (Patterson and Atmar 1986, Cutler 1991, Wright and Reeves 1992, Atmar and Patterson 1993, Cutler 1998, Cook and Quinn 1998, Wright et al. 1998, Jonsson 2001). Owing to their ability to account for these issues, the metric *C*, which counts the number of times that a species' presence at a site correctly predicts its presence at a richer site (quantified by N_c), standardized for matrix size (Wright and Reeves 1992), and the metric *T*, or a measure of the disorder of a matrix (Atmar and Patterson 1993), have been used extensively (Patterson et al. 1996, Boecklen 1997, Sfenthourakis et al. 1999, 2004, Hadly and Maurer 2001, Fernández-Juricic 2002, Fleishman et al. 2002, Mac Nally et al. 2002, Bruun and Moen 2003).

Because nestedness metrics and the null models used to assess their significance have received much attention, and the use of *T* (and *C*) is now widespread, it might seem obvious that investigations of nestedness can now move beyond the stage of pattern documentation to a more explicit understanding of underlying mechanisms. Indeed, this is not only being done more commonly, but the adoption of such an approach is widely advocated (Lomolino 1996, Cutler 1998, Fleishman et al. 2002, Bruun and Moen 2003). In both cases the implicit assumption made is that the behaviour of these metrics, and the associated randomization procedures, are reasonably well understood – at least sufficiently well to allow any potential artefacts to be readily comprehended (reviewed in Wright et al. 1998). However, like other ecological indices, nestedness metrics have the potential to hide as much as they reveal. Therefore, they require ongoing, careful scrutiny, such as that given to beta-diversity measures (Koleff et al. 2003).

Recently, whilst comparing *T* values for different higher taxa that occur on the Southern Ocean islands, it became clear that *T* behaved in counter-intuitive ways, especially when its values for different taxa were compared across the same sets of islands (Greve et al. 2005). To further explore the likely origin of the counter-intuitive results, we assessed the behaviour of *T* and *C* by comparing these metrics for a series of presence–absence matrices that we constructed specifically for this purpose. Here, we present the results of these analyses and demonstrate that the way in which *T* corrects for matrix size is problematic. We show that as the numbers of non-nested single occurrences (hereafter singletons) in a matrix, which is not perfectly nested to begin with, increase, and nestedness thus declines, *T* (Atmar and Patterson 1995), and to a lesser extent *C* (Wright and Reeves 1992), incorrectly indicate an increase in nestedness (or at best no decline in nestedness). Given that

most sets of islands or habitat patches are characterized to varying degrees by single species occurrences (or endemism) (Wilson 1959, Paulay 1985, Ricklefs and Bermingham 2002) (see also below), this characteristic of *T* especially could prohibit a clear understanding of the mechanisms underlying nestedness, especially in those archipelagos or sets of habitat patches where large numbers of endemic species are involved (Hawaii, Wagner and Funk 1995, Grant 1998). Moreover, the potential for obscuring understanding is particularly large when levels of nestedness are compared amongst archipelagos or amongst taxa. By examining three methods of standardizing Brualdi and Sanderson's (1999) metric, *d*, for matrix size, we demonstrate the reason why *T* behaves in the way it does. We also show that one of the derivatives of *d*, *d*₁, resolves the problems caused by non-nested singletons, and argue that *T* can be corrected in a similar fashion.

Methods

Test matrices

Three matrices with 20 islands and 20 species each were produced: Perfectly Nested matrices (Fig. 1a), which Atmar and Patterson (1993) define as matrices where each smaller island contains only a proper subset of the species found on all larger islands, RandomA matrices (this matrix has several outliers and holes, Fig. 1b), and RandomB matrices (more outliers and holes than RandomA – Fig. 1c). The RandomA matrix was specifically produced such that its nestedness, measured with *T*, was within the range of significant nestedness values of the real assemblages provided by Atmar and Patterson (1995), and thus a realistic model of assemblages occupying either islands or habitat

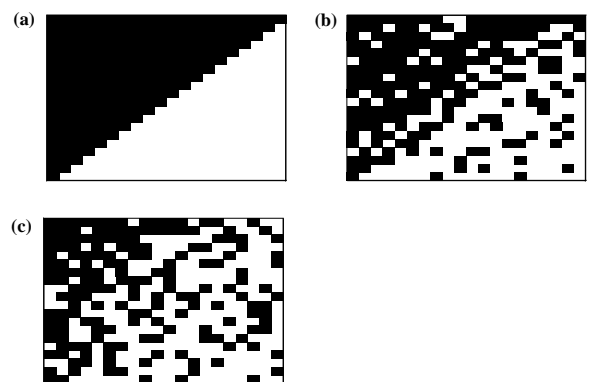


Fig. 1. Three matrices which were constructed and subsequently altered to test the effect of singletons on nestedness values. a) Perfectly Nested matrix. b) RandomA matrix. c) RandomB matrix. By convention, sites are displayed in rows and species are displayed in columns. Filled squares indicate species presences.

patches. RandomB has a nested value that is not typical of many assemblages (though by no means wholly unusual), but was specifically included for illustrative purposes. The behaviour of T , C , and derivatives of d were then assessed by varying the levels of endemism (5–19 endemics, and multiples of 19 endemics), and the positions of the endemics, in the original matrices (Fig. 2). For assemblages such as birds or insects on tropical islands these levels of endemism are not unusual (Diamond 1975, Adler and Dudley 1994, Wagner and Funk 1995, Ricklefs and Bermingham 2002). However, the $4 \times$ diagonal matrices are somewhat

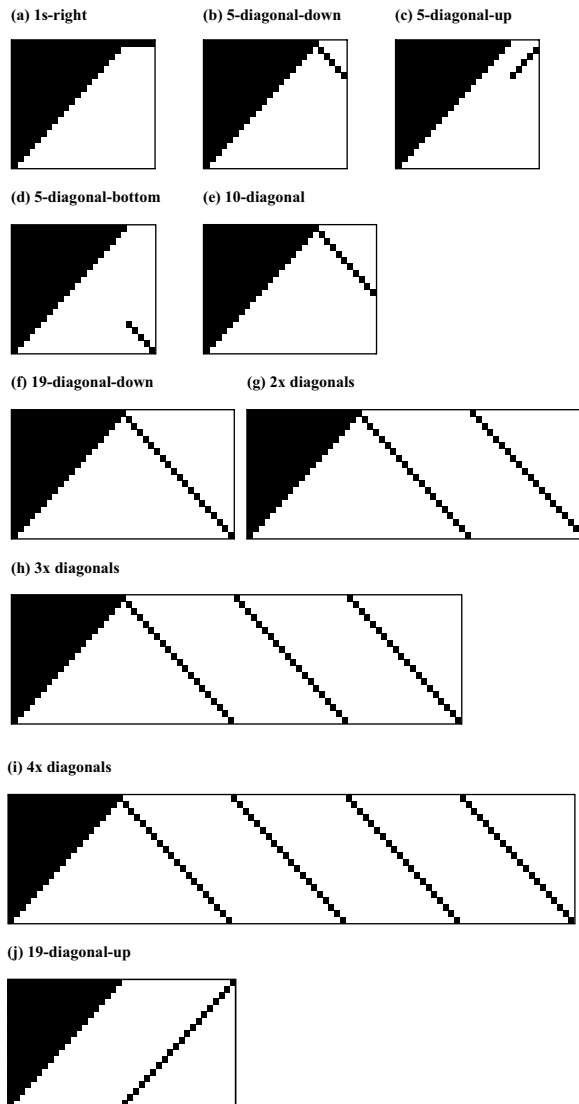


Fig. 2. Schematic representation of the twelve ways in which the original matrices were altered in order to examine the effect of single occurrences on measures of nestedness. In this example, the original matrix from which the above matrices are derived is the Perfectly Nested matrix (see Fig. 3a). By convention, sites are displayed in rows and species are displayed in columns.

biologically unrealistic (though see Gillespie 2004). The diagonal addition of endemics (or singletons) was non-nested (i.e. singletons were always added to different islands). Although endemism is typically not spread equally across all islands in a matrix, this is the only non-nested manner in which singletons (endemics) can be added to test the effect of singletons on matrices. Assemblages are identical whether singletons are presented in a diagonal or a random manner in a matrix: the order in which singletons are added to patches should not affect the measure of nestedness obtained, because it is merely a factor of the arrangement of islands and species in the matrix. The null expectation is that nestedness should decline with the addition of non-nested endemics to matrices that are not perfectly nested. Nestedness was determined using T (Atmar and Patterson 1993) calculated with Atmar and Patterson's (1995) temperature calculator, C (Wright and Reeves 1992), and three derivatives of d (Brualdi and Sanderson 1999).

Because d increases with the size of a dataset (Brualdi and Sanderson 1999) it cannot be used for the comparison of different-sized matrices. No metrics have been derived from d which standardize the size of the datasets analyzed. Hence, d_0 and d_1 were created here, while d_2 was adapted from Wright et al.'s (1998) PN. The equations for these three metrics are:

$$d_0 = d_{\text{obs}}/d_{\text{check}} \quad (1)$$

$$d_1 = d_{\text{obs}}/f \quad (2)$$

$$d_2 = (d_{\text{obs}} - d_{\text{exp}})/(d_{\text{perf}} - d_{\text{exp}}) \quad (3)$$

where d_{obs} is the nestedness of the original matrix, d_{check} is the discrepancy of a checkerboard matrix with the same number of columns and rows as the original matrix, f is the sum of the number of presences in the matrix, and d_{exp} is expected discrepancy, which equals the mean discrepancy of, in this study, 10 000 randomly generated matrices. The value of d_{perf} (the discrepancy of a perfectly nested matrix) is always zero and is only shown here for completeness. While d_0 and d_1 decline, d_2 increases with increasing nestedness.

These three derivatives of d standardize for matrix size in different ways. Therefore, they provide a means to understand the effects of different standardization approaches on the estimate of nestedness. Initial examination of the behaviour of T suggested that the standardization approach might be responsible for problems with estimates of nestedness when singletons (or endemics) are present. Matrix size in T is compensated for by dividing the measure of disorder by the product of rows and columns, i.e. habitat patches and species, of the matrix. To obtain C , N_c is not standardized by the product of the number of habitat patches and species, but only by a term containing the latter. The value of d_{check} is the product of the number of rows and

the number of columns of the original matrix divided by four. Like T , d_0 is thus a function of the number of sites (rows) and the number of species (columns) in the dataset, i.e. the absolute matrix size, regardless of how many species occur at each site or of species occupancy. d_1 , on the other hand, is related to the number of occurrences in the dataset only. It therefore assumes that discrepancy increases as the number of species presences, rather than the number of sites \times species, increases. The size of d_2 is a function of the mean d of the random matrices, which, in turn, is informative of the null model used to generate the random matrices. In this particular study, the null expectation was that some species are more ubiquitous than others and therefore more likely to be present than other species (Fischer and Lindenmayer 2002). The software used for calculating d and its derivatives, and C , as well as their significance was custom-written and is available from the authors on request.

Real assemblages

As we demonstrate below, we found that T indicates an increase in nestedness even with the addition of non-nested singletons. A potential criticism of the matrices we created to investigate the behaviour of the metrics is that the incidence of singletons (and doubletons, or species occurring twice, or tripletons, etc.) we used does not reflect the natural situation. To obtain an idea of how relevant these findings are to patterns in nature, the incidence of singletons, doubletons and tripletons was recorded in each of 173 presence–absence matrices that are available with the temperature calculator (Atmar and Patterson 1995). The same matrices that Boecklen (1997) selected were used here, except that the files *aflrm*, *brazpfb*, *namlagc* and *bajapo* were not available or contained incomplete matrices, and that *adfsh*, *afrmtbrd*, *amazfish*, *coloaqh*, *gbmam93*, *kadmon*, *madbird*, *manuinf*, *parasite*, *punukb*, *saweevil* and *texaqh* were included. All the matrices used here are independent, i.e. contain assemblages from different regions or of different taxonomic groups. Type III generalized linear models with a Poisson distribution and log link function were used to explore the relationships between each of the three combinations of singletons, doubletons and tripletons (i.e. singletons vs doubletons, singletons vs tripletons, and doubletons vs tripletons). A t -test was used to examine the null hypothesis that the slopes of the singletons:doubletons, singletons:tripletons and doubletons:tripletons relationships are equal to one, i.e. that assemblages always have similar numbers of singletons and doubletons. If they have equal numbers of endemics, doubletons or tripletons, then comparisons of nestedness using T would not be problematic. However, as soon as they have different numbers of these “rare” species, then

comparisons among them would be confounded because of problems associated with T .

To further determine whether the problems we discovered with T are likely to be encountered in analyses of real data (as opposed to those of the test matrices), nestedness values for published datasets were re-analysed. Sfenthourakis et al. (1999, 2004) used T to calculate nestedness of terrestrial snails and isopods on the Aegean islands. In their first paper, Sfenthourakis et al. (1999) calculated nestedness of all isopods and snails, the taxa excluding singletons, and the taxa without those species endemic to the archipelago as a whole. In their second paper they measured nestedness of isopods at different spatial scales – between sites on islands, between sites of each of two island groupings (Eastern and Kyklades), and between sites on all islands (Sfenthourakis et al. 2004). In addition, they measured the nestedness of entire island assemblages of the Eastern and Kyklades groups, and of all islands. The published T -values of the matrices were compared to d_1 and to C . The second analysis was of data from Greve et al. (2005), who compared nestedness of seabird, land bird, insect and vascular plant assemblages across Southern Ocean Islands using d_1 . Here we re-analysed the data of all species across all islands, also using T and C .

Results

Test matrices

T and *C*

Any additions of singletons to the Perfectly Nested matrix resulted in an increase in T , implying a decline in nestedness (Table 1). Even the 1s-right matrix derived from the Perfectly Nested matrix was less nested than the original matrix, although it is perfectly nested (Fig. 2a). In addition, T increased progressively as more singletons were added to the Perfectly Nested matrix (Fig. 3a). However, T always declined with singleton additions to the RandomA and RandomB matrices (Table 1). Although the sequential additions of singletons actually enhances the departure from nestedness, T of the RandomA and RandomB matrices declined with the first two (5-diagonal and 10-diagonal) sequential addition of singletons, and only increased again from the 10-diagonal to the 19-diagonal-down matrix (Fig. 3b, c). Similarly, the metric C correctly indicated a decrease in nestedness as 5, 10 and 19 singletons were sequentially added to the nested matrix, but incorrectly suggested that nestedness increased in the RandomA and RandomB matrices (Table 1).

The 19-diagonal-down, 2 \times diagonals, 3 \times diagonals and 4 \times diagonals matrices are a series of matrices of successive additions of complete rows of diagonals

Table 1. Temperature (T), C and discrepancy (d) values, and percentage fill of the Perfectly Nested, RandomA and RandomB matrices and their derivatives. A decline in T, d, d0 and d1 indicate increasing nestedness, whilst an increase in d2 and C indicate increasing nestedness.

Matrix	T	C	d	d0	d1	d2	% Fill
Perfectly Nested							
Original	0	1	0	0.000	0.000	1.000	52.5
1s-right	0.26	1	0	0.000	0.000	1.000	43
5-diagonal-down	2.61	0.969	5	0.040	0.023	0.898	43
5-diagonal-up	2.61	0.969	5	0.040	0.023	0.898	43
5-diagonal-bottom	9.61	0.845	5	0.040	0.023	0.898	43
10-diagonal	4.66	0.913	10	0.067	0.046	0.807	36.6
19-diagonal-down	13.38	0.786	19	0.097	0.083	0.666	29.3
2 × diagonals	18.99	0.687	38	0.129	0.153	0.450	21.1
3 × diagonals	22.74	0.611	58	0.147	0.216	0.296	17
4 × diagonals	25.03	0.551	77	0.156	0.266	0.203	14.5
19-diagonal-up	13.53	0.786	19	0.097	0.083	0.666	29.3
RandomA							
Original	37.59	0.134	52	0.520	0.252	0.237	51.5
1s-right	23.99	0.339	52	0.416	0.246	0.267	42.2
5-diagonal-down	27.66	0.330	54	0.432	0.256	0.240	42.2
5-diagonal-up	27.47	0.330	54	0.432	0.256	0.238	42.2
5-diagonal-bottom	33.20	0.299	57	0.456	0.270	0.195	42.2
10-diagonal	26.85	0.399	57	0.380	0.264	0.226	36
19-diagonal-down	30.32	0.415	66	0.338	0.293	0.160	28.8
2 × diagonals	31.89	0.414	78	0.264	0.318	0.133	20.7
3 × diagonals	33.23	0.388	94	0.238	0.354	0.077	16.7
4 × diagonals	31.3	0.359	107	0.216	0.375	0.061	14.3
19-diagonal-up	30.24	0.415	66	0.338	0.293	0.159	28.8
RandomB							
Original	54.49	0.071	67	0.670	0.381	0.019	0.071
1s-right	36.12	0.202	70	0.560	0.387	0.018	0.202
5-diagonal-down	40.23	0.198	72	0.576	0.398	-0.010	0.198
5-diagonal-up	38.28	0.199	72	0.576	0.398	-0.010	0.198
5-diagonal-bottom	47.94	0.181	71	0.568	0.392	0.003	0.181
10-diagonal	38.09	0.253	75	0.500	0.403	-0.008	0.253
19-diagonal-down	39.1	0.278	81	0.415	0.415	-0.014	0.278
2 × diagonals	37.22	0.287	91	0.308	0.423	0.017	0.287
3 × diagonals	37.2	0.271	105	0.266	0.447	0.008	0.271
4 × diagonals	35.47	0.251	120	0.242	0.471	-0.006	0.251
19-diagonal-up	38.63	0.278	81	0.415	0.415	-0.015	0.278

(Fig. 2f–i). As more diagonal rows were added to the Perfectly Nested matrix, T increased (Fig. 4a). In the case of the RandomA and RandomB matrices, the addition of diagonals always led to a decline in T to below the original value. Successive additions of diagonal rows initially resulted in a steep decline in T, whereafter T stabilized (Fig. 4b, c). C indicated a similar behaviour in nestedness (Table 1).

Five columns were added to the original matrices to produce the 1s-right, 5-diagonal-down, 5-diagonal-up and 5-diagonal-bottom matrices. Of these, the latter always had the highest T, while the 1s-right matrices always possessed the lowest T and highest C. The 5-diagonal-up and the 5-diagonal-down matrices have identical species compositions and differ only by virtue of the fact that the positions of the singleton species in the matrix that is fed into the calculator are inverted (Fig. 2b, c). Accordingly, T of the 5-diagonal-down and 5-diagonal-up Perfectly Nested matrices was identical. However, the two 5-diagonal RandomA and RandomB matrices showed small differences in their T values. To further explore this behaviour, the 19-diagonal-up matrix, with the same species composition

as 19-diagonal-down, was created (Fig. 2f, j). None of the complementary 19-diagonal-up and 19-diagonal-down matrices possessed equal T values (Table 1). C generated identical values for the complementary inverted matrices (Table 1).

d0

In several respects, d0 behaved similarly to T. Nestedness of the Perfectly Nested matrices declined with any additions of presences (i.e. d0 increased), with the exception of the 1s-right matrix, where d0 remained zero. d0 was greatest for the original RandomA and RandomB matrices and declined with any additions to the original matrices. Furthermore, sequential additions of singletons (Fig. 2b, e, f) resulted in an increase in d0 of the Perfectly Nested matrix, and a decline in d0 of both random matrices (Table 1).

However, unlike T, d0 of the 5-diagonal-bottom matrices did not differ or differed marginally from d0 of the 5-diagonal-down or -up matrices, because when d is calculated, unexpected presences (or absences, for that matter) are not weighted according to their distance from the isocline of perfect nestedness. Moreover, d0

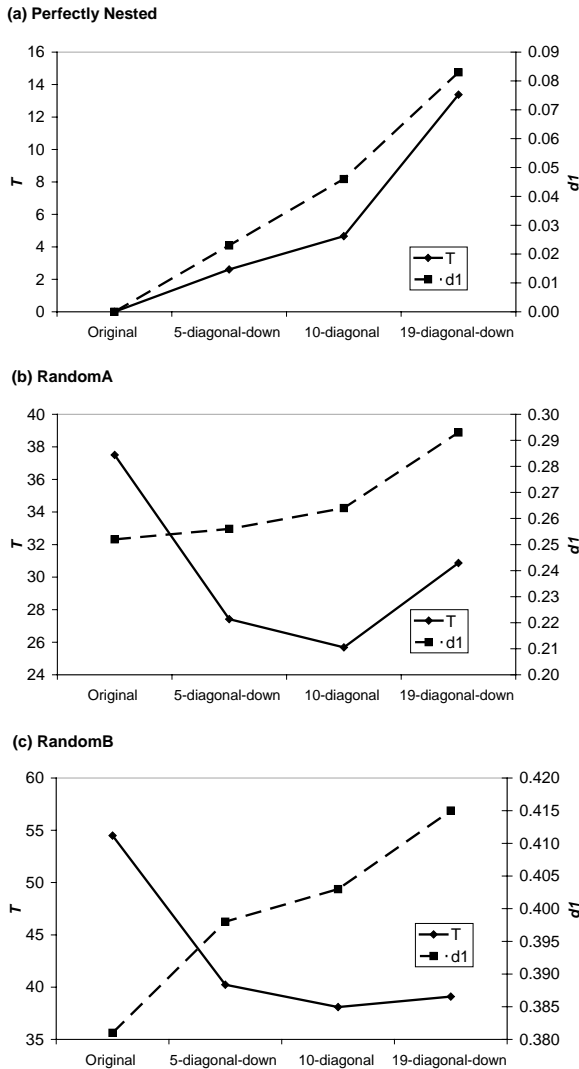


Fig. 3. The behaviour of temperature (T) and d1 with the sequential additions of non-nested singletons to the a) Perfectly Nested, b) RandomA and c) RandomB matrices.

increased continuously with the sequential addition of singletons and with the addition of diagonal rows. d_0 was identical for all complementary 5-diagonal-down and -up, and 19-diagonal-down and -up matrix pairs with identical species compositions (Table 1). This is a direct result of d being identical for these complementary matrices.

d1 and d2

When measured using d_1 and d_2 , additions of presences to the Perfectly Nested matrix resulted in a decrease in nestedness, with the exception of the 1s-right matrix, which retained a perfectly nested score (Table 1). By contrast with T and d_0 , d_1 indicated that nestedness decreased with the sequential addition of non-nested singletons (Fig. 2b, e, f) to the RandomA and RandomB

matrices (Fig. 3b, c). d_2 was more inconsistent, indicating, in the case of both random matrices, that nestedness either increased or decreased with the addition of singletons (Table 1).

The sequential addition of diagonal rows to the matrices always produced a decline in nestedness of matrices measured with d_1 (Fig. 4), although this effect was quite varied when measured with d_2 . Because the addition of singletons to the original matrices was non-nested, the results from d_1 conformed with expectations of nestedness theory (Patterson and Atmar 1986, Wright et al. 1998).

Nestedness of the 5-diagonal-down and -up matrices, and the 19-diagonal-down and -up matrices was identical when measured with d_1 , once again a consequence of the fact that d was identical in each of the pairs of matrices with the same species compositions. However, d_2 of these complementary matrix pairs differed slightly.

Real assemblages

From 173 presence-absence matrices it is clear that singletons, doubletons and tripletons do not occur in equal frequencies (Fig. 5). Singletons are more common than doubletons ($\chi^2=98.22$, Estimate=0.011, DF=171, $p<0.001$) or tripletons ($\chi^2=46.55$, Estimate=0.010, DF=171, $p<0.001$), and doubletons occur more frequently than tripletons ($\chi^2=211.13$, Estimate=0.053, DF=171, $p<0.001$). Figure 5 also clearly shows that endemics, double occurrences, and tripletons are common in matrices that are typically the subject of nestedness analyses.

Using the Sfenthourakis et al. (1999) data, T and C indicated that with the removal of singletons, nestedness of both snails and isopods declined (Table 2). d_1 indicated an increase in nestedness in both cases (Table 2). The differences in nestedness values were relatively small. Trends between the three measures did not differ when species endemic to the archipelago were removed. In the case of Sfenthourakis et al. (2004), when individual islands were examined little congruence was found between values generated by the three nestedness measures (Table 3). When comparing the nestedness of the island groups per sample and per island, it became clear that T indicated the opposite trend to d_1 and C. The latter both show that nestedness is greater for the islands/group matrices than for the stations/group matrices. T was lowest for the stations/group analyses, which was a result of their large matrix size, as Sfenthourakis et al. (2004) already noted, and not because of nestedness of the matrices.

For the matrices analyzed by Greve et al. (2005), T typically declined as matrix size increased, while d_1 increased or declined independently of matrix size, and in most cases nestedness using either index was

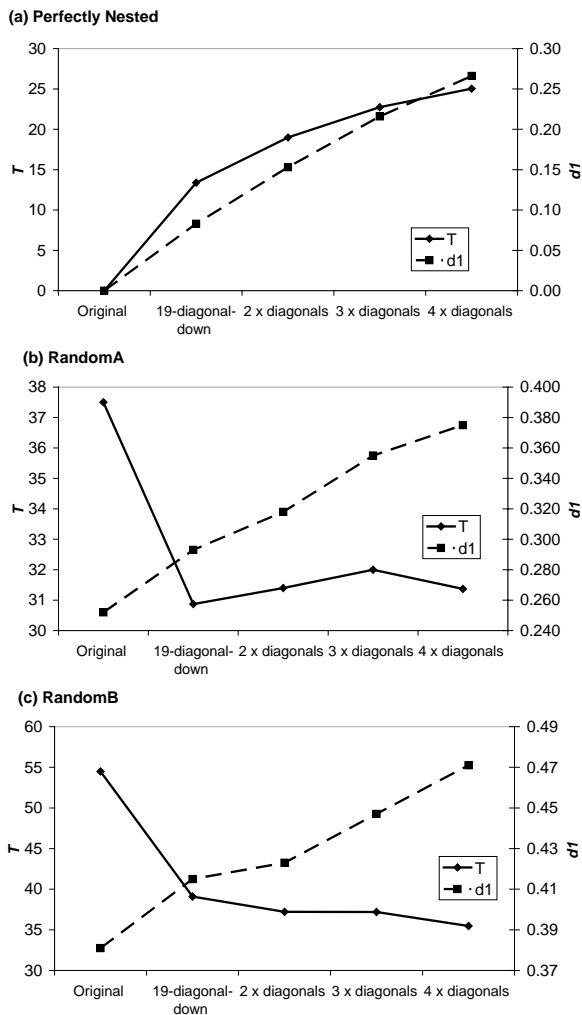


Fig. 4. The behaviour of temperature (T) and d1 with the additions diagonal rows of non-nested singletons to the a) Perfectly Nested, b) RandomA and c) RandomB matrices.

significant (Table 4). Matrices that were least nested according to T, were most nested when measured with d1. These also happened to be the smaller matrices. C behaved similarly to d1, except for the land bird matrices, which, according to C, were less nested than all other (except seabird) matrices. For example, the indigenous seabirds matrix includes 24% singleton species (total no. of endemics = 19), whilst the indigenous plant matrix is characterized by 30% singletons (total no. of endemics = 143). T for the seabirds and plants was 32.94° and 7.99°, respectively, indicating that plants, which possess large numbers of singletons, are considerably more nested than seabirds, which have considerably fewer singletons. By contrast, d1 indicates that seabird assemblages (d1 = 0.325) are more nested than those of vascular plants (d1 = 0.577), across the same set of islands (nestedness is significant in all cases).

Discussion

Additions of singletons (i.e. species that occur only at one site) to non-nested presence-absence matrices result in the inflation of nestedness estimates measured with T, even if, in reality, these additions cause a decline in the nestedness of a matrix. To a lesser extent, the problem is also true for the metric C. T and C only indicate a correct decline in nestedness with the addition of singletons when these are added to a perfectly nested matrix. This is not surprising given that T of a perfectly nested matrix cannot decrease to below the value (zero) and C cannot rise above the value (one) for a perfectly nested matrix.

The initial addition of a diagonal row of singletons had the most pronounced effect on the inflation of nestedness measured with T. Additions thereafter did not necessarily lead to further declines in T. With sequential additions of singletons to the original random matrices, nestedness measured with T also increased, except in the case of the 19-diagonal-down matrix (Fig. 3b, c). Thus, as the number of singletons in a matrix increases, so its T declines. T of the 19-diagonal-down increased to values above 10-diagonal only because the singletons in the bottom right corner of this matrix (which are absent from other matrices) are heavily weighted and contribute greatly to the temperature of the system. For this same reason T of the 5-diagonal-bottom matrices was considerably greater than that of the 5-diagonal-down or -up matrices.

An additional, relatively minor problem characterises T. Atmar and Patterson (1993) defined a perfectly nested subset as a set of islands where “each smaller island would contain only a proper subset of the species found on all larger islands”. By this definition, the 1s-right Perfectly Nested matrix is perfectly nested – it contains no unexpected species presences or absences (outliers or holes): the biotas of the habitat patches are “proper subsets” of one another. Matrices of the kind illustrated by the 1s-right Perfectly Nested matrix should thus possess a T of zero. However, this was not the case. It should also be noted that the position of the species in the unsorted matrix that is fed into the calculator has an effect on T of the matrix, as shown by T values of the complementary diagonal-down and diagonal-up (5 and 19) matrices. Thus, the position of columns of the matrix that is fed into the temperature calculator has an effect on T, although this effect is not large.

One advantage of T over d and its derivatives is that T weights species absences and presences according to their distance from the matrix’s isocline of perfect nestedness (Atmar and Patterson 1993). This explains why the 5-diagonal-bottom matrices always had the lowest T of the matrices to which five columns were added. Their unexpected presences are situated far from the isocline of perfect nestedness and thus contribute more to T than unexpected presences close to the

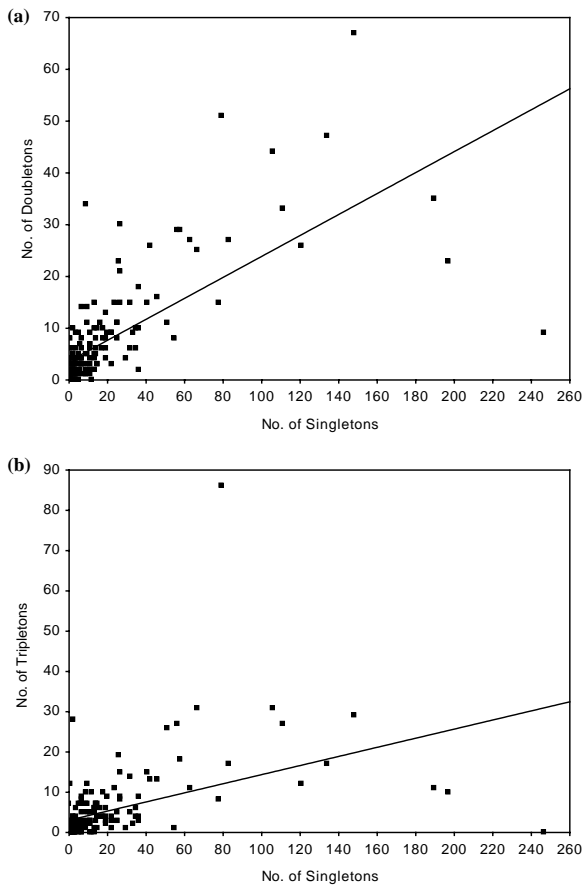


Fig. 5. The relationship between the number of (a) singletons and doubletons and (b) singletons and tripletons in 173 presence-absence matrices. Note that most assemblages have several, and commonly many endemic or rare species, and that the numbers thereof vary substantially between the assemblages.

isocline. This property of T also explains the low values calculated for 1s-right matrices (singletons were added to the matrix rows where the unexpected presences contribute least to the value of T).

The derivatives of d provide insight into the reasons for the increase in T , when in reality nestedness declines with the addition of singletons. All three metrics – d_0 , d_1 or d_2 – correctly indicate that the 1s-right Perfectly

Table 2. T , d_1 and C of presence-absence matrices from 14 Kyklades islands (Sfenthourakis et al. 1999). Here, singletons refers to species found on one island only, whilst endemics refers to species endemic to the archipelago as a whole. A decline in T and d_1 indicate increasing nestedness, whilst an increase in C indicates increasing nestedness.

Matrix	T	d_1	C
Isopods (total)	35.03	0.205	0.548
Isopods (no singletons)	36.79	0.193	0.488
Isopods (no endemics)	32.24	0.175	0.567
Snails (total)	35.19	0.281	0.42
Snails (no singletons)	39.63	0.258	0.341
Snails (no endemics)	32.68	0.271	0.425

Nested matrix is indeed perfectly nested. However, they behave very differently with the addition of non-nested singletons. When measured with d_0 , nestedness increases with the addition of singletons, even more consistently so than does T . This is because d_0 , like T , is directly related to the inverse of the product of sites and species (i.e. row and column) sums. This function was included in the metrics' equations to compensate for matrix size (Atmar and Patterson 1993). Therefore, T and d_0 effectively assume that the chances of unexpected presences or absences occurring in a matrix are directly proportional to the number of species and patches in a matrix, regardless of the species' patch occupancy or the species richness of patches. Thus, their null assumption is that all patches are likely to be occupied by all species. This is hardly the case in nature, where some species tend to be more widespread than others and some patches more species-rich than others (Gaston and Blackburn 2000, Bell 2003).

By contrast, d_1 showed theoretically appropriate behaviour by indicating that nestedness declines with the sequential addition of non-nested singletons or diagonal rows. d_2 , on the other hand, was inconsistent in indicating the effects of randomly distributed singletons on nestedness. Because the value of a matrix's d was insensitive to species ordering in a matrix, d_0 and d_1 were, unlike T , always consistent for matrices with the same species composition. The value of d_2 varied slightly for differently packed matrices with the same species composition. However, this variation declines to insignificant levels as the numbers of randomizations increase and the denominator converges on a single value.

While d_2 behaved unpredictably, the performance of d_1 conformed to nestedness theory. Unlike T and d_0 , d_1 assumes that the chance of unexpected presences or absences occurrence is related to species incidences across the patches. This is a realistic assumption given that most groups of assemblages contain a few widespread species, many narrowly distributed species, and a varying number of endemic species (depending on the type of study, e.g. archipelagos vs habitat patches, and its spatial extent) (Ricklefs and Bermingham 2002).

Although the significance of nestedness of individual matrices is unlikely to be affected by the singleton-related behaviour of T and d_0 , comparisons between different-sized matrices that do not possess equal proportions of single-, double-, triple, etc. -occurring species, as well as those that do not have equal species richnesses, will be confounded. As we have demonstrated, real matrices typically do not possess equal proportions of singletons, doubletons and tripletons, and they generally possess more singletons than doubletons and tripletons. In addition, comparing T with d_1 of published studies, it is apparent that T might provide a biased estimate of nestedness, which complicates biological interpretation (Sfenthourakis et al. 2004). In

Table 3. T, d1, C and matrix size (no. of rows × no. of columns) of presence–absence matrices from the Aegean Islands (Sfenthourakis et al. 2004). Nestedness was measured for patches on each of 20 islands, for all patches on all Eastern* and Kyklades**, and all 20*** islands, and for islands of the Eastern group†, Kyklades group†† and all islands†††. A decline in T and d1 indicate increasing nestedness, whilst an increase in C indicates increasing nestedness.

Matrix	T	C	d1	Matrix size
Kalymnos	20.2	0.4293	0.1857	160
Sifnos	12.6	0.6259	0.1935	231
Kythnos	31.9	0.5028	0.2184	208
Serifos	28.3	0.4766	0.2235	220
Nisyros	22.4	0.4211	0.2414	140
Patmos	39.6	0.2672	0.25	85
Tinos	37.3	0.3761	0.2609	325
Amorgos	48.9	0.3355	0.2874	253
Paros	30.2	0.3137	0.2883	270
Leros	36.4	0.104	0.3036	126
Kea	23.7	0.3921	0.3043	52.2
Astypalaia	23.5	0.3101	0.3117	220
Syros	34.2	0.3721	0.32	300
Mykonos	42.7	0.2946	0.3281	160
Andros	26.7	0.3378	0.3467	624
Ikaria	26.5	0.2938	0.3724	494
Kos	37.6	0.2456	0.391	435
Naxos	23.7	0.3457	0.3984	608
Samos	27.3	0.2958	0.424	522
Milos	26.2	0.2187	0.4643	286
Eastern†	42.6	0.4421	0.1977	368
Eastern (per station)*	19.7	0.2982	0.504	4508
Kyklades††	34.6	0.4917	0.2317	600
Kyklades (per station)**	15	0.4044	0.3984	8500
All†††	37.2	0.4561	0.2706	1180
All (per station)***	13.6	0.3545	0.4563	15812

other words, the problems we have shown to be characteristic of T, and to a lesser extent of C, are not restricted to the test matrices we used. Rather, real matrices constructed from assemblages on islands or occupying habitat patches have similar varying levels of endemism to those we used in our assessments. Moreover, we demonstrate clearly for several real matrices that assessment of nestedness using T can lead to results that are either incorrect or complicate interpretation of the actual patterns of nestedness. Indeed, the problems we have identified are not simply quantitative, but are qualitative to the extent that the different metrics often

lead to opposite conclusions. That is, wholly incorrect conclusions regarding the extent of nestedness and the way in which this differs between taxa occupying the same patches might be reached.

C does not make the assumption of equiprobable species occurrence across patches (Wright and Reeves 1992). However, the metric also displays inconsistent behaviour such that, in some cases, it decreases with additions of singletons to matrices. For that reason d1 was employed to examine the behaviour of T with addition of non-nested singletons. Despite C's inconsistency in the test matrices, nestedness between different-sized matrices measured with C and d1 were not always different. Therefore we advocate that caution be taken if C is used. One advantage of C is that it can be used to assess the effect of patch characteristics by ranking them according to variables that characterise them (Loo et al. 2002), which cannot be done using d1. Conversely, d1 can be used to measure the effect of species characteristics by ranking species according to variables differentiating them, which is not possible with C.

In conclusion, investigation of the behaviour of the three derivatives of d has shown that the way in which matrix size is standardized has a considerable effect on the response of nestedness metrics to endemism, to the extent that conclusions opposite to those actually shown by the data are reached. Thus, previous studies which compare different-sized datasets using T (Patterson et al. 1996, Wright et al. 1998, Sfenthourakis et al. 1999, Puyravaud et al. 2003), will have to be revisited. In addition, because endemism is a general property of real assemblages (Wilson 1959, Paulay 1985, Ricklefs and Bermingham 2002), its effects on certain nestedness metrics are likely to be common, and should be considered in all analyses. Nonetheless, a small modification of T, such that matrix size is standardized by occupancy, rather than by species × sites, would correct for the problems we have demonstrated here. T would then correctly take into account richness of the assemblages, endemism, and the distances of unexpected presences and unexpected absences from the isocline of

Table 4. T, d1, C and matrix size (no. of rows × no. of columns) of presence–absence matrices across 26 Southern Ocean islands (Greve et al. 2005). A decline in T and d1 indicate increasing nestedness, whilst an increase in C indicates increasing nestedness. Significantly nested if *p < 0.05, ***p < 0.001.

Matrix	T	d1	C	Matrix size
Indigenous land birds	13.86***	0.341***	0.026	1625
Indigenous seabirds	32.94***	0.325***	0.366***	2028
Indigenous insects	8.63***	0.705***	−0.007	20 150
Indigenous vascular plants	7.99***	0.577***	0.157	10 833
All indigenous taxa	10.47***	0.603***	0.167	36 114
Alien land birds	32.7	0.333*	0.030	135
Alien insects	7.56***	0.576***	0.120***	3784
Alien vascular plants	5.43***	0.434***	0.360***	8096
All alien taxa	7.41***	0.532***	0.221	12 936

perfect nestedness. Doing so is of considerable significance, especially because nestedness metrics are being increasingly used to comment on conservation questions, and especially on the suitability of particular sets of patches for different taxa.

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