

Research note

# Nestedness, anti-nestedness, and the relationship between prevalence and intensity in ectoparasite assemblages of marine fish: a spatial model of species coexistence

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## Abstract

Nested species subset patterns consist in a hierarchical structure of species composition in related assemblages, with the species found in depauperate assemblages representing non-random subsets of progressively richer ones. This pattern has been found at the infracommunity level in about a third of the fish ectoparasite assemblages studied to date. Here we present evidence for another non-random structural pattern in assemblages of fish ectoparasites, anti-nestedness, which corresponds to situations in which parasite species are always absent from infracommunities richer than the most depauperate one in which they occur. We show that this pattern is exactly as common as nestedness, and that anti-nested assemblages are characterised by significantly lower prevalence and mean intensities of parasites than nested assemblages. In addition, we found a positive relationship between the prevalence and the mean intensity of parasites across the different assemblages. We propose a link between the nestedness/anti-nestedness continuum and the prevalence-intensity relationship that may involve colonisation-extinction processes. The results presented here suggest that, although nestedness may not be common in parasite communities, other departures from random species assembly are possible, and that some form of structure may be present in many communities. The continuum between nestedness and anti-nestedness also has implications for recent models of species coexistence in communities. © 2000 Australian Society for Parasitology Inc. Published by Elsevier Science Ltd. All rights reserved.

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In recent years, the search for non-random structure in parasite communities has turned to the application of nestedness analyses [1–6]. This approach has been widely used in ecology to detect patterns in species assemblages of all kinds [7–9]. In parasite assemblages, nestedness analyses can serve to determine whether infracommunities (i.e. all parasites of all species on individual hosts) are random collections of locally available species, or whether they follow more rigorous assembly rules (see Ref. [10] for a review). In the most comprehensive analyses to date, Rohde et al. [6] found that nested patterns were found in less than one-third of parasite assemblages on marine fish that could be tested properly. The authors concluded that since most parasite communities are distinctly not nested, parasite assemblages in marine fish were generally unstructured, depauperate and unpredictable at least in the sense of the hierarchical species structure.

The departure from random assembly that results in a nested pattern can easily be illustrated as a progression from species-poor infracommunities to species-rich ones, with the species found in depauperate infracommunities representing non-random subsets of progressively richer ones (Fig. 1). If nestedness represents a departure from randomness in one direction, however, there must also exist possible departures in the other direction.

There are two kinds of possible departures. First, testing for the existence of nestedness involves comparing species assemblages against the hypothesis of a nested subset species pattern (see Ref. [1]). If parasite communities are structured in such a way that depauperate infracommunities consist of subsets of progressively richer ones, then the alternative hypothesis of nested hierarchy is accepted instead of the null hypothesis of random assortment. On the contrary, if the null hypothesis is accepted, species assemblages are considered as not nested, but it does not imply that parasite assemblages are not structured in some

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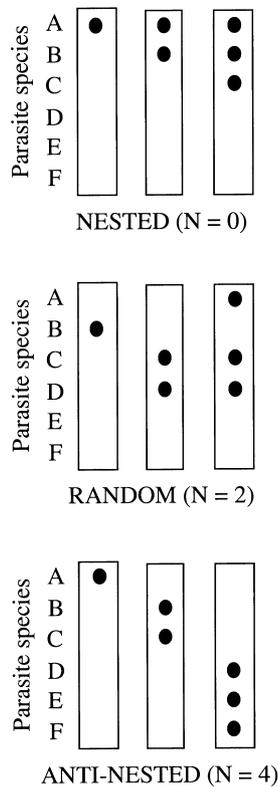


Fig. 1. Three hypothetical distributions of parasite species among infracommunities (i.e. among individual hosts). Each rectangle represents a different infracommunity, arranged from least (left) to most (right) species-rich. The mean infracommunity richness and the mean prevalence of the six parasite species are the same in the three examples. The  $N$ -values are the sum, across all parasite species, of instances where a parasite species is absent from infracommunities richer than the most species-poor one in which it occurs.

other ways (see [11,12] for criticisms of null models). The next step would be to use appropriate models in order to test other possible kinds of structuration which may, or may not, exist. Second, due to the specific nature of the nestedness pattern, an alternative departure from this model is that parasite species are always absent from infracommunities richer than the most depauperate one in which they occur. Then we have a case of anti-nestedness (Fig. 1). This pattern, although not considered in the ecological literature, represents a non-random assembly of species and may imply the action of some structuring force. Here we re-examine the data of Rohde et al. [6] to show that anti-nestedness is as common as nestedness in communities of ectoparasites on marine fish. We then investigate some of the ecological correlates of nestedness and anti-nestedness. Finally, we link these findings with the interspecific positive relationship between spatial distribution and local abundance that is well established in ecology, and we discuss the processes that could push the structure of parasite assemblages in one direction or the other.

Rohde et al. [6] searched for nestedness in assemblages of metazoan ectoparasites from 50 marine fish species in which

there were at least three parasite species. Nestedness scores,  $N$ , were computed for each assemblage by calculating the sum, across all parasite species, of the instances where a parasite species is absent from infracommunities richer than the most species-poor one in which it occurs (see [1,3,5] for discussions of the technical aspects). For each assemblage, the scores were compared with the  $N$ -values of 1000 randomly generated presence-absence matrices, produced using the algorithm RANDOM1 of Patterson and Atmar [13]. In these simulations, the probability of each parasite species of being included in an infracommunity was set equal to its observed prevalence in the fish sample. Rohde et al. [6] then computed the statistical significance of the difference between the observed  $N$  and the simulated scores as the proportion of simulated  $N$ -values that were lower than or equal to the observed value. They found that 15 of the 50 assemblages had a nested structure, with a probability level of  $P < 0.05$ .

One would expect that the probabilities based on the RANDOM1 simulations would range along a continuum between 0 and 1, following a normal or skewed distribution (see [14]). In fact, the RANDOM1 probabilities for the 50 assemblages including at least three parasite species (listed in Table 1 of Rohde et al. [6]) show a strongly bimodal distribution (Fig. 2). Many communities are clumped on the left of the frequency distribution curve ( $P < 0.05$ ) and many others on the right side of the curve. Intermediate communities are also frequent, but a clear dichotomy is emerging here (Fig. 2). If  $P < 0.05$  is the statistical threshold for nestedness, then we can use  $P > 0.95$  for anti-nestedness. Surely, if the observed  $N$ -value is greater than 95% or more of the randomly-generated values from the simula-

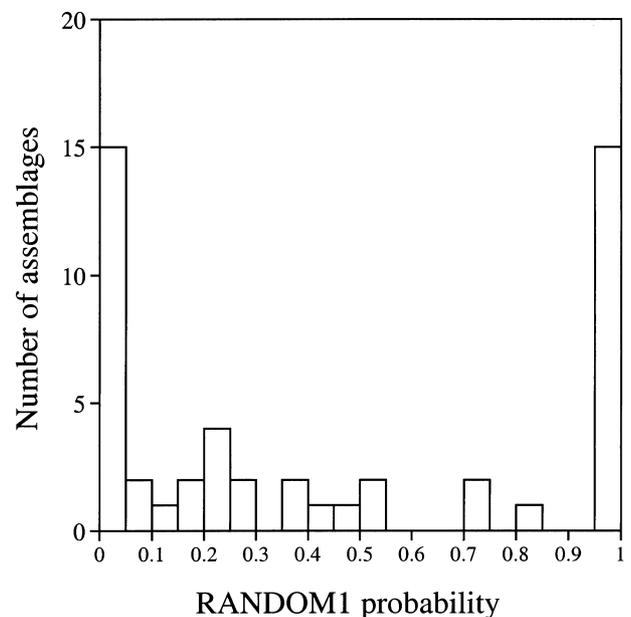


Fig. 2. Frequency distribution of RANDOM1 probability values for 50 assemblages of ectoparasites of marine fish. Data are from Table 1 in Rohde et al. [6].

tions, the assemblage is not structured randomly. Just as 15 of the 50 assemblages showed a nested pattern, another 15 display a clear anti-nested pattern: 14 of these have a  $P$ -value of 1.0 (Fig. 2). Thus anti-nestedness is just as common as nestedness among the 50 assemblages of fish ectoparasites investigated by Rohde et al. [6]. Overall, when pooling cases of nestedness and anti-nestedness, 30 out of a total of 50, (i.e. more than half) parasite assemblages of marine fish show a structured, non-random species composition.

How do nested and anti-nested assemblages differ with respect to other ecological parameters? We compared the 15 nested and 15 anti-nested assemblages in terms of sample size (numbers of fish examined), prevalence (percentage of fish infected by at least one parasite species), mean intensity of infection (mean number of parasite individuals of any species per infected fish), parasite species richness (number of parasite species in an assemblage), and mean water temperature at the site of fish capture. These five variables were log-transformed prior to analyses; original data were obtained from Rohde et al. [6] or Rohde and Heap [15]. In addition, a recent interest in the regional distribution of abundance in free-living organisms has drawn the attention of researchers to a possible link between nested species patterns and the positive relationship that may exist between spatial distribution and local abundance of faunas [16]. Therefore, we checked for such a relation between prevalence and mean intensity following a recent study [17] that demonstrated that the idea of regional distribution of abundance may also apply to parasite community ecology. More interestingly, a link might exist between the positive prevalence-intensity relationship and the nestedness/anti-nestedness continuum model proposed here.

Nested and anti-nested assemblages did not differ with respect to sample size (two-tailed test:  $t = 0.051$ ,  $df = 28$ ,  $P = 0.9608$ ) or parasite species richness ( $t = 1.512$ ,  $df = 28$ ,  $P = 0.1418$ ). However, differences were found with respect to the other three variables (Fig. 3): nested assemblages have higher parasite prevalence and mean intensity of infection, and they occur in warmer waters than anti-nested assemblages. Finally, we found that sample size did not correlate with either prevalence or mean intensity (both  $P > 0.15$ ), but we found a positive relationship between prevalence and mean intensity of infection across assemblages (Fig. 4). This indicates that in assemblages characterised by a high number of parasite individuals per infracommunity, there is also a high proportion of fish harbouring infracommunities.

The above differences may or may not be real: because the different fish species harbouring the parasite assemblages are more or less related to each other, the statistical independence of each assemblage is questionable. The most widely used method to remove the potentially confounding influence of relatedness among species is the phylogenetically independent contrasts method [18,19]. We used the RANDOM1 probabilities as a continuous variable along with the other five variables examined in the above

analyses. Contrasts were computed on log-transformed data using the program CAIC version 2.0 [20]. The fish phylogeny used to derive independent contrasts is the same as in Poulin and Rohde [21]. All correlations and regressions using contrasts were forced through the origin (see [22] for justification).

A total of 32 sets of independent contrasts could be derived from the fish phylogeny. Contrasts in fish sample size did not correlate with contrasts in parasite species richness ( $P > 0.50$ ), but correlated strongly with contrasts in prevalence and contrasts in mean intensity of infection (both  $P < 0.01$ ). Therefore we regressed contrasts in both prevalence and mean intensity against contrasts in sample size, and used the residuals of these regressions as contrasts in prevalence and mean intensity corrected for sample size

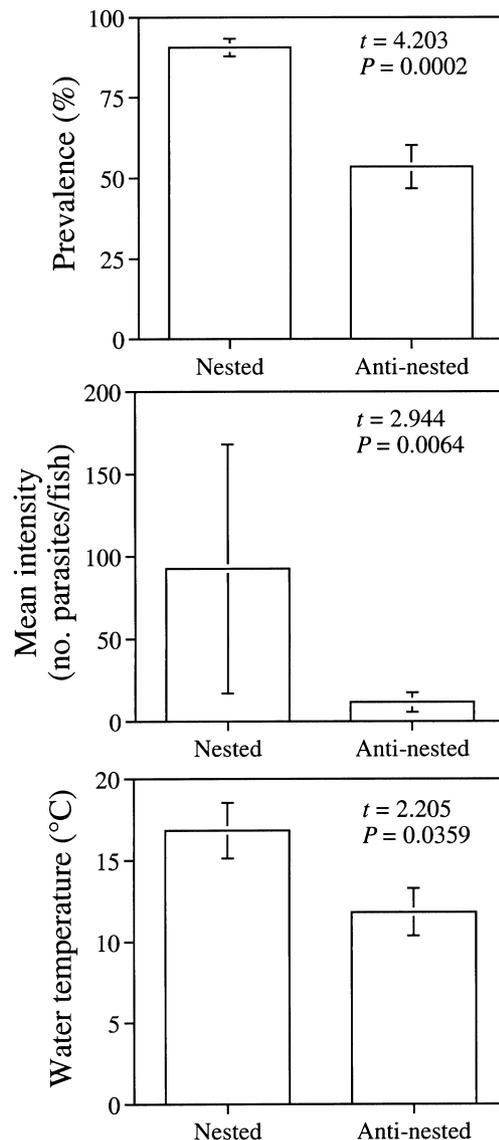


Fig. 3. Mean ( $\pm$ SE) prevalence, intensity of infection, and water temperature for 15 nested and 15 anti-nested assemblages of ectoparasites of marine fish. The results of two-tailed tests shown in the figure were obtained using log-transformed data.

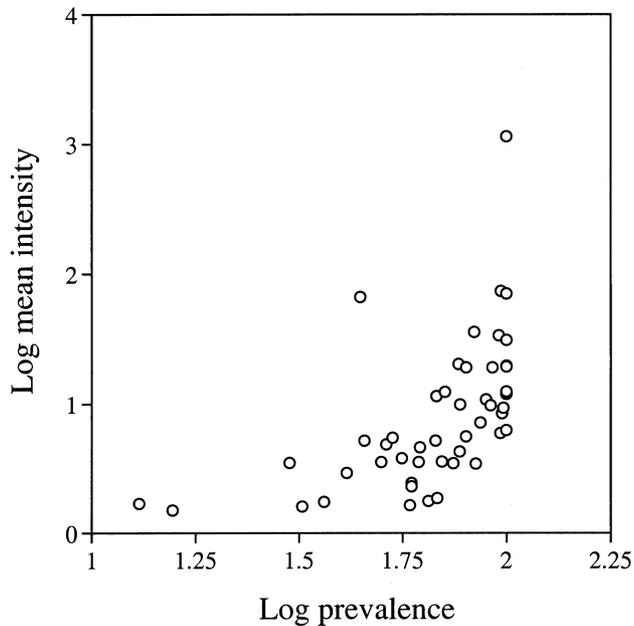


Fig. 4. Relationship between mean parasite intensity and parasite prevalence across 50 assemblages of ectoparasites from marine fish ( $r = 0.547$ ,  $P = 0.0001$ ).

in subsequent analyses. This correction for sampling effort is important. For instance, the positive relationship between prevalence and mean intensity found across assemblages (Fig. 4) remains significant when using phylogenetic contrasts ( $r = 0.493$ ,  $P < 0.01$ ), but not after the correction for sample size described above ( $r = 0.295$ ,  $P < 0.10$ ).

Contrasts in RANDOM1 probabilities did not co-vary with either contrasts in fish sample size ( $r = 0.046$ ,  $P > 0.50$ ), parasite species richness ( $r = -0.080$ ,  $P > 0.50$ ), or water temperature ( $r = -0.041$ ,  $P > 0.50$ ). However, contrasts in RANDOM1 probabilities correlated negatively with both contrasts in prevalence ( $r = -0.588$ ,  $P < 0.001$ ) and in mean intensity ( $r = -0.355$ ,  $P < 0.05$ ). Clearly, with contrasts in RANDOM1 probabilities computed to be positive, the majority of contrasts in either prevalence or mean intensity have a negative value (Fig. 5), indicating a negative association with the RANDOM1 probability, which is itself inversely related to nestedness. Thus as the prevalence or mean intensity of ectoparasites increases in a fish population, the likelihood that the parasite assemblage is nested also increases. The comparative analysis using phylogenetic contrasts reinforces our preceding analyses, although the association with water temperature is no longer apparent once phylogenetic influences are controlled. The geographic distributions of fish species from different families are not independent of water temperature, and this phenomenon produced a spurious association between temperature and levels of nestedness.

Rohde et al. [6] reported that nested patterns were more likely to be found in assemblages with high prevalence, this parameter being the best predictor of nestedness in their study. They did not discuss why assemblages with low

prevalence (and, as shown here, low mean intensity of infection) tend to display a marked departure from randomness, but in a direction opposed to nestedness. We introduce here the concept of anti-nestedness, and demonstrate its existence in ectoparasite communities of marine fish. The pattern also exists in endoparasite communities: of the nine fish endoparasite communities examined by Rohde et al. [6], two were nested and one was anti-nested (RANDOM1 probability = 1). Interestingly, this anti-nested community also had the lowest prevalence of the nine communities investigated (see Table 2 in Rohde et al. [6]).

An anti-nestedness species subset pattern, as illustrated in Fig. 1, corresponds to the case where parasite species are always absent from infracommunities richer than the most depauperate one in which they occur. Worthen [8] and Worthen and Rohde [3] stated that demonstrating that (parasite) communities are not nested provides compelling evidence that local (parasite) communities are truly unstructured, random assemblages from the local species pool. Our analysis strongly contradicts this assertion since we show that 15 parasite communities of marine fish (this study) have

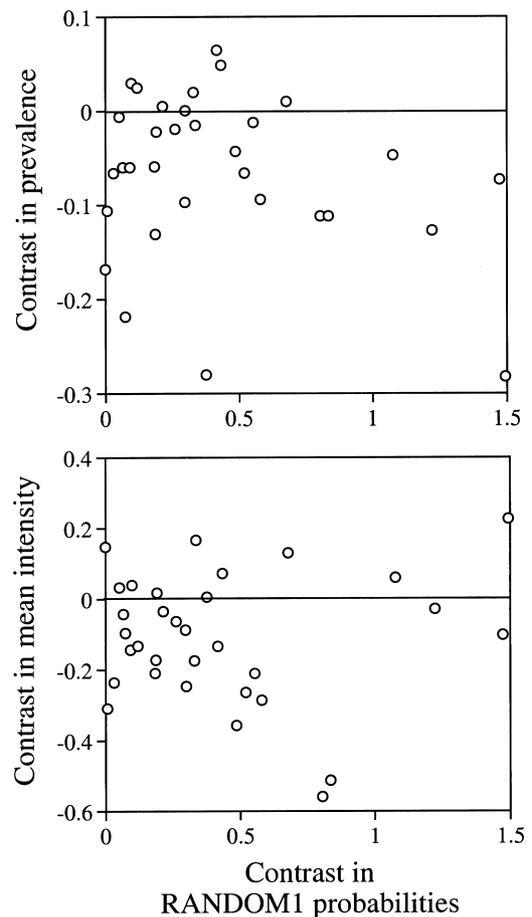


Fig. 5. Relationships between parasite prevalence and mean intensity of infection on the one hand, and the RANDOM1 probability of an assemblage showing a nested structure. Data are from 32 sets of phylogenetically independent contrasts among marine fish species; contrasts in prevalence and contrasts in intensity were corrected for fish sample size (see text).

an anti-nested structure in addition to the 15 shown to have a nested structure [6]. Overall, 30 parasite communities out of a total of 50 investigated by Rohde et al. [6] are structured, though in different ways. We recommend that two distinct tests be used when testing for the existence of a nested species hierarchy in parasite assemblages: a first test which specifically tries to detect the existence of a nested species pattern, using for instance the Patterson and Atmar [13] method, and a second test which, as proposed in this paper, checks for the existence of a bimodality in the frequency distribution of probabilities generated by the first test. In the context of species-area relationships, Guégan and Hugueny [1] have retained two of the three hypotheses generally accepted to explain nested species patterns, i.e. habitat heterogeneity and extinction-colonisation processes. The third mechanism, the passive sampling hypothesis, was rejected on the basis of Monte-Carlo simulations. Worthen and Rohde [3] suggested that differences in colonisation rates might be the cause of nestedness in parasite assemblages of marine fish, an idea also accepted by Rohde et al. [6] in their analysis. We can here argue that differences in colonisation rates might also be responsible for anti-nestedness patterns, with rare and highly-specific parasite species characterised by poor colonisation capabilities when compared to locally abundant and widespread species.

Interestingly, we also demonstrate the existence of a positive relationship between parasite prevalence and mean local parasite intensities across the 50 ectoparasite assemblages. The relationship weakens when phylogenetic influences and sampling effort are taken into account, but this may be due to the fewer degrees of freedom available in the comparative analysis. This trend is similar to the interspecific relationship between local abundance and spatial occurrence that has been well documented in free-living organisms [16,23,24]. The adequacy of this interspecific relationship to parasitology has seldom been considered. Poulin [25] has demonstrated, using a comparative approach that controlled for study effort and phylogenetic influences, that it may not apply to parasites of Canadian freshwater fish. In fact, he observed a negative trend supporting, on the contrary, the existence of a trade-off between how many host species a parasite can exploit and how well it does on average in those hosts. Such negative relationships have been previously reported [26]. More recently, Poulin [27] studied parasite communities of birds and found a positive interspecific relationship that he interpreted within the context of the niche breadth hypothesis [28]. Morand and Guégan [17] also found a positive interspecific relationship between the mean abundance and the spatial distribution of nematode worms in small mammals and addressed the exact mechanisms behind this observed pattern.

Although here we report an inter-assemblage relationship and not an interspecific one, it is worth considering the ecological processes that can generate these related trends. From the eight mechanisms responsible for the positive

interspecific abundance-spatial distribution relationship in free-living organisms [16], only three have been tested on parasitic assemblages, i.e. sampling artefact [17], niche breadth hypothesis [17,25–27], and metapopulation dynamics within the context of demographic, (i.e. epidemiology) and environmental, (i.e. host fragmentation) stochasticity [17]. All three mechanisms have been shown to act on the relationship between distribution and abundance but in different ways. Morand and Guégan [17] have shown that the positive relationship might result both from sampling biases or from regional dynamics of local parasite abundance within the framework of epidemiological models. They discussed whether sampling models are a product of pure sampling or whether they reflect the action of underlying mechanisms, i.e. extinction-colonisation processes and environmental heterogeneity.

We here show for the first time that there may exist a link between the positive mean intensity-prevalence relationship and the nestedness/anti-nestedness continuum. At the moment, it is difficult to determine which pattern, i.e. the positive relationship between intensity and prevalence or the nested/anti-nested continuum, is the cause and which is the consequence of the other. We can simply point out that the nested/anti-nested pattern which emerges from the present study may be explained in terms of a positive inter-assemblage relationship between local parasite abundance and prevalence, in which the local proportion of hosts harbouring infracommunities and actual numbers of parasites tend to co-vary. One important question now is whether the positive spatial distribution-abundance relationships observed in many groups of free-living organisms are also associated with nested/anti-nested structure continuum as shown here for ectoparasite assemblages of marine fish.

If the coupling between the nested/anti-nested continuum and the positive relationships between local abundance and spatial distribution is verified, a body of common mechanisms might be at work. We strongly recommend that future community studies test for both the existence of a nested/anti-nested continuum and positive relationships between spatial distribution and local abundance in a similar vein as we did in the present work. Interestingly, one of the eight hypotheses retained to explain the positive relation between spatial distribution and abundance, and not yet tested, is that of the vital rates of organisms [29] which, to paraphrase its authors, might represent a ‘back to basics’ approach. According to this hypothesis, (parasite) species would persist solely at those sites, i.e. hosts, where their intrinsic population growth rate (which equals birth rate minus death rate) exceeds zero. That is exactly the proposition made by epidemiological models in which parasite populations increase when  $R_0$  is greater than one. Previous nestedness studies [1,3,5,6] could all be right in proposing that colonisation-extinction processes across parasite species in patchy environments could be the main determinant of parasite occurrence, prevalence and abundance in hosts.

In addition, nestedness/anti-nestedness continuum patterns should roughly parallel the aggregation model of coexistence [30] with parasite species found in nested hierarchy having levels of interspecific aggregation reduced relative to intraspecific aggregation and thus facilitating species coexistence. On the contrary, parasite species occurring in anti-nested assemblages would tend to show a strong interspecific aggregation yielding species exclusion. In fact, the nested/anti-nested continuum emerging in this study might illustrate a kind of spatial organisation of parasite species from species coexistence to species competitive exclusion.

To conclude, further studies should turn toward determining the primary causes of the two patterns emerging from this work. It is logical to think that one pattern could be responsible for the other, or vice versa, or that a third influential variable may constrain the two patterns in the same way. Parasite communities are certainly interesting models to test such ideas, and we strongly encourage parasitologists to analyse their data in order to address pertinent questions regarding the relative roles of exact mechanisms at work in structuring, or not, parasite assemblages at different spatial scales.

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