ANALYSIS OF LARVAL TREMATODE COMMUNITIES[1]

K. D. Lafferty, D. T. Sammond, and A. M. Kuris
Department of Biological Sciences, University of California, Santa Barbara, California 93106 USA

Abstract. We can compare natural communities with null models of communities to indicate how they differ from random assemblages of species (i.e., how much structure is present). However, because null models draw on observed values of species' prevalences, whatever structure already exists in natural communities affects the composition of a null model and weakens its comparative power. To address this, we developed formulae to estimate "pre-interactive" species prevalences permitting a more sensitive quantification of community structure. Nonetheless, if a null model deviates from the community that we base it on, it is difficult to separate the effects of heterogeneity in recruitment from competition. We have developed a method to test for each independently. Applying our analytical techniques to a well-studied guild of larval trematodes in the salt marsh snail *Cerithidea californica* revealed that competitive interactions among species were the most significant structuring force. Interestingly, spatial heterogeneity acted to significantly intensify species co-occurrences. This differs from previous studies, which argued that the isolating effects of spatial heterogeneity, not competition, structure these communities by reducing co-occurrences.

Key words: Cerithidea californica; community; Digenea; double infections; larval trematode; null model; prevalence; random model; recruitment.

INTRODUCTION

Considerable debate centers around whether communities are more than just random assemblages of species (Strong et al. 1984, Sih et al. 1985, Sale 1991). Although disturbance, physical stress, recruitment dynamics, predation, and competition all might alter the distribution and composition of species in a community, the role of interspecific competition is at the center of the community structure debate (e.g., Diamond 1975, Connor and Simberloff 1979, Connell 1980, 1983, Schoener 1983, Gurevitch et al. 1992). To resolve this issue, we must explicitly and consistently define "community structure," find tractable systems for study, and use analytical approaches that can detect nonrandom structure and distinguish the relative importance of potential structuring forces. For this, we and others have chosen a model system to address questions of community structure: guilds of larval trematodes in their first intermediate host snails. We will describe methods specifically developed for this system, use them, and then discuss how our approach applies to the study of other communities. Our goals are to specify the major hypotheses concerning what structures larval trematode guilds, refine testable predictions that stem from these hypotheses, and provide tests to determine whether our observations are consistent with predictions. To do the latter, we applied our approaches to data on larval trematodes that parasitize the horn snail, *Cerithidea californica*.

We see "community structure" as the pattern of distribution and abundance of species in a community. Following May (1984), we will term a group of species lacking statistical association to be an "unstructured" community. Hence, the more a community differs from a random association of species, the more "structured" it is. Likewise, forces may "structure" a community if they cause the association of species to depart from a null model of species abundance and distribution. We have found it useful to separate structuring forces that affect patterns of recruitment from those forces that occur after recruitment. It is of particular interest to examine how patterns in recruitment can affect the importance of postrecruitment structuring forces. A truly null model would spatially and temporally homogenize intraspecific recruitment rates, allow interspecific coexistence, and not allow species to partition the habitat. In reality, recruitment of a species will usually exhibit spatial and temporal variation, and species may interact or vary in their habitat preferences. These forces can add structure to an assemblage.

Communities of parasites are a valuable model for the analysis of such questions because hosts represent well-defined and replicated habitats (Esch et al. 1990). Although most studies of parasite communities have focused on helminths in the guts of vertebrates or the gills of fishes, ecological parasitologists have recently begun to investigate the structure of larval trematode guilds (e.g., Kuris 1990, Sousa 1990, 1993, Fernandez and Esch 1991a, b). Guild, sensu Root (1973), is the term that best describes an assemblage of such trematodes (Kuris 1990). Snails serve as first intermediate

hosts for trematodes that, as adults, are parasites of vertebrates. Larval trematodes typically castrate their snail hosts; asexual reproduction of larvae from even a single infection substantially increases the worm population in an infected snail such that trematode tissue largely replaces the gonads and digestive glands of the snail. Thus, each snail represents a limited resource for a trematode infection and competition is, therefore, a potentially important structuring force operating within trematode guilds (Kuris 1990, Sousa 1992). Consistent with this logic are well-documented observations of interference competition between trematode species (reviewed by Lim and Heyneman 1972). Competition between two species of trematodes within individual snails, herein termed the "infracommunity" (Holmes and Price 1986), often results in losses of subordinate species (reviewed in Lie et al. 1973, Combes 1982, Sousa 1992). It is not clear, however, that interactions are frequent enough to significantly affect attributes of community structure at the level of the trematode community within a host population (the "component community" sensu Holmes and Price 1986) (Holmes 1990, Sousa 1990, 1992, 1993, Fernandez and Esch 1991b). Here, we introduce methods to determine whether competition at the infracommunity level significantly affects the proportion of interacting trematodes in the component community. In addition, we are able to estimate the proportion of individuals excluded from the metapopulation of each species. Our approach is more sensitive than alternative approaches that compare diversity indices to assess the importance of competition (Sousa 1990) as these are only likely to be useful in the limiting case where competition leads to the exclusion of some species (Kuris 1990, Kuris and Lafferty 1994).

Determining structure with a null model

To determine if a given community is structured, it is necessary to test whether it is significantly different from a random assemblage of species. The degree to which a community departs from a null model represents a quantitative measure of community structure (Grant and Schluter 1984). For example, certain species combinations may occur more or less frequently than expected by chance. A null model that uses a species combination approach (analysis of species co-occurrences) can serve to construct "null communities" for comparison with observed communities. This approach is particularly appropriate for parasite studies because the opportunity to sample large numbers of hosts provides sufficient statistical power to detect structure (Simberloff 1990). Several authors have proposed methods for constructing and testing null models of species combinations (see reviews in Harvey et al. 1983, Jackson et al. 1992). Because hosts are much less variable than other habitat units such as islands or lakes, constraints on column totals (the number of trematode species in each snail individual) used to ac-

count for habitat heterogeneity (see Gilpin and Diamond 1984) are often unnecessary. For a null model used in past studies (e.g., Cort et al. 1937, Bourns 1963, Vernberg et al. 1969, Werding 1969, Combescot-Lang 1976, Vaes 1979, Rohde 1981, Kuris 1990, Fernandez and Esch 1991a), the expected number of double infections of trematode species i and j is E_{ij}: $E_{ij} = Np_ip_j$, where N is the number of hosts (infected and uninfected) and p_i and p_j represent the prevalences (where prevalence is the proportion of hosts infected, Margolis et al. 1982) of species i and j. Summing E_{ij} over all species pairs gives the expected number of double infections in a sample (ΣE_{ij}). The difference between the expected (ΣE_{ij}) and observed (ΣO_{ij}) number of double infections indicates the degree of structure.

Unfortunately, it is often difficult to distinguish a null model from a hypothetically structured assemblage (Colwell and Winkler 1984). Therefore, it is prudent to opt for sensitive statistical analyses. It is common to compare expected frequencies of double infections with observed frequencies of double infections using a chi-square or G test, separately testing each species pair in a 2×2 table (Kuris 1990, Fernandez and Esch 1991a). Each test, however, is not strictly independent, violating the assumptions of the heterogeneity test (Schluter 1984). The likelihood of obtaining a false positive (Type 1 error: rejecting the null hypothesis when it is true) increases with the number of statistical tests conducted (Pielou 1974, Sousa 1993). Type 2 error is also a potentially serious problem because the small sample size for each comparison of a pair of species makes it difficult to statistically detect a biologically significant effect (e.g., Fernandez and Esch 1991a). An alternative approach is to conduct a single test for the entire guild (e.g., Sousa 1990). Although such an approach loses species-specific information (Harvey et al. 1983), it has considerably more power than pairwise comparisons and is much less likely to produce a false significant result.

Null parameters

Because observed species abundances and distributions used to parameterize null models may have been shaped by interspecific interactions, the expected values generated are not truly what we would expect if species did not interact or vary in their ecological requirements (Grant and Abbott 1980, Gilpin and Diamond 1984). The following is an example for two species, dominant a and subordinate b. Assuming that the probability of recruitment to a host is independent of whether that host is infected, we can try to determine how many b individuals species a eliminated. Out of a population of 100 snails that have all shared the same risk of infection, imagine that a recruits to 80 snails and b to 50 snails. By independent assortment, we would expect 40 snails were infected at some point with both a and b, 40 by a only, and 10 by b only (leaving 10 snails uninfected). We might expect that a

has displaced some of the b in the 40 double infections. Let's say we find 70 snails with a only, 10 with a and b, and 10 with b only (30 individuals of b lost to competition). Here, the observed overall prevalence of a is 0.8 and the overall prevalence of b is 0.2. Using the general random model, Np_ip_j, we would expect $100 \times 0.8 \times 0.2 = 16$ double infections. Since we observed 10 double infections, this suggests that we have lost six individuals of b to competition (a gross underestimate compared with the 30 individuals actually lost to competition), and competition does not seem very important. This null model nearly always underestimates expected co-occurrences. Gilpin and Diamond (1984) argue that this obstacle is the most intractable difficulty of using "null" models. To avoid this requires parameterizing the null model with the prevalence that each species would have obtained if no interactions occurred. We can calculate these "null parameters" (in this case, prevalence) if we know certain aspects of the biology of the interacting species. In the Appendix, we derive formulae that estimate the null prevalence of a species from observed values. These formulae vary depending on assumptions about how species interact.

It is possible to estimate the prevalence at which a species had recruited by using its present observed prevalence. For our previous example, we assume that, since a is dominant, it should not have lost any members to competition. Therefore, its null prevalence is the same as its postinteractive prevalence (0.8). By the same reasoning, we also know that single infections of species b were never doubly infected with species a. To find the null prevalence of b, we return to the rules for independent assortment. Twenty uninfected snails were available to b for single infections (a infected the other 80). Species b only infected 10 of these; this is a 50% infection rate. As we assume that b infects parasitized snails with the same probability as uninfected snails, we estimate that the null prevalence of species b was 50%. Now, using the null prevalence to estimate the initial overlap between a and b, $100 \times 0.8 \times 0.5 = 40$ expected double infections. This leaves $40 - 10 = 30$ individuals of b lost to competition with a (the correct value). The difference between this approach and using observed prevalences in the null model can be substantial for cases where competitively dominant species are common.

Interpretation of structure

Often, many fewer double infections occur than are expected from a null model (reviewed by Sousa 1992). Two general mechanisms could reduce the number of double infections and structure the guild. First, the opportunity for co-occurrences may be less frequent than expected because of spatial or temporal heterogeneity in recruitment or the differential distribution of parasite species among host phenotypes. Second, co-occurring trematodes might not survive because (1) hosts with multiple-species infections suffer higher mortality rates than do hosts with single infections or (2) interspecific competition among trematodes could reduce the number of multiple-species associations that persist. None, some, or all of these factors might influence an assemblage.

In previous analyses of expected infections, investigators often pooled temporal (e.g., Kuris 1990) or spatial (e.g., Fernandez and Esch 1991a) collections, making it impossible to interpret the potential effects of heterogeneity in recruitment. Other studies have assumed (without testing specifically) that spatial (or temporal) heterogeneity in recruitment explains the occurrence of fewer double infections than expected (e.g., Fernandez and Esch 1991a). However, although spatial (or temporal) variation in recruitment can hypothetically reduce interspecific interactions (Cort et al. 1937, Kuris 1990, Sousa 1990, 1992, Fernandez and Esch 1991a), it can also intensify their frequency (Robson and Williams 1970, Kuris 1990, this paper). For heterogeneity to affect community structure, there must be intraspecific variation in recruitment. If this variation is negatively correlated among species, there will be fewer co-occurrences than expected and species will be isolated. On the contrary, a positive association among species will intensify interactions. For example, if trematode species a recruits mostly to site 1 while trematode species b recruits mostly to site 2, they will rarely co-occur. Conversely, if the prevalence of both species is disproportionately high at site 1, there will be more co-occurrences between the two species than if there was no intraspecific variation in recruitment among sites.

The analysis of heterogeneity in recruitment requires obtaining several samples of hosts that each have experienced uniform risks of infection. That is, each sample should consist of individuals of similar age, collected at the same time from an area in which they are likely to mingle. Host mobility increases the likelihood that a sample from a given area will represent a homogeneous unit (Fernandez and Esch 1991a). It is also important to sample from a narrow host age class as older hosts will have a greater cumulative risk than young hosts. Although trematode species might isolate themselves if they specialized among various host phenotypes, or more readily infected unparasitized than parasitized snails, there is no evidence that this occurs in our host–parasite system with the possible exception of size selectivity (Sousa 1990, 1993). In experimental studies of other snail–trematode systems, if miracidia (searching stages hatched from trematode eggs) were selective, they were more likely to penetrate snails infected with other trematode species than uninfected snails (Heyneman et al. 1972, Kuris 1973, Lie et al. 1973, 1976, Boss 1977, Jourdane 1980, Lie 1982). The propensity of trematode miracidia to penetrate infected hosts would increase the incidence of parasite co-occurrences and make our analysis of structure conservative. If the probability of infecting a host is strongly
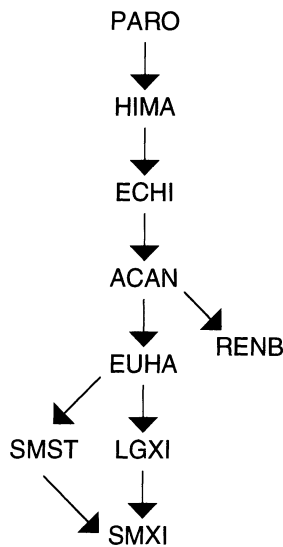
POSTULATED DOMINANCE HIERARCHY

FIG. 1. Dominance Hierarchy as per Kuris (1990). Species codes are as follows: ACAN = *Acanthoparyphium spinulosum*, ECHI = *Echinoparyphium* sp., EUHA = *Euhaplorchis californiensis*, HIMA = *Himasthla rhigedana*, LGXI = large xiphidiocercaria, PARO = *Parorchis acanthus*, RENB = *Renicola buchanani*, SMXI = small xiphidiocercaria, SMST = small strigeid (actually a cyathocotylid).

dependent on whether the host is already parasitized, both the null model and the method for determining null prevalence will require modification.

After recruitment patterns determine which species will interact, host or parasite mortality related to multiple infections may further structure the trematode guild by eliminating some double infections. Although host mortality induced by multiple infections occurs in some host–parasite systems (Anderson and May 1978), multiple infections of parasitic castrators, such as trematodes, usually do not increase host mortality rates (Kuris 1974, Sousa 1992). Instead, experiments reveal the regular occurrence of predation by dominant species (with mouthed redial larval stages) on subordinate species and that certain species with only sporocyst larval stages (mouthless) are able to indirectly suppress the development of other subordinate species in a largely linear dominance hierarchy (Lie et al. 1968, Lim and Heyneman 1972, Kuris 1973, 1990, Combes 1982, Sousa 1993) (although in some cases this can be described as asymmetric omnivorous intraguild predation [Polis et al. 1989], we will simply refer to all interactions where one species negatively affects another species as competition). Preemption, where the first species to arrive is able to stave off subsequent recruits, is rare (Lie et al. 1968, Kuris 1990, Sousa 1992). If competition structures the trematode guild, the expected number of double infections in a sample should be greater than the observed number of double

infections. Facilitation among species, on the other hand, could lead to more observed double infections than expected (Kuris 1990).

METHODS

To illustrate our method of analysis, we used the guild of larval trematodes that parasitize the marine snail, *Cerithidea californica*. This snail and its associated larval trematodes have been the subject of numerous studies (Martin 1955, 1972, Yoshino 1975, Sousa 1983, 1990, 1993, Kuris 1990, Lafferty 1991, 1993a, b). We sampled five sites (spatially separated by ≈50 m or more) at Carpinteria Salt Marsh (California, USA) over 20 d in May 1991. At each sample site, we collected snails within a 10-m stretch of tidal channel. Mark and follow experiments indicate that infected and uninfected snails mix randomly within a site over a period of 1 wk (I. Davila-Marcano, *unpublished data*). We therefore assumed the movements of individual snails effectively eliminated spatial heterogeneity within a site. Because the movement of a single snail was rarely consistent in a single direction, we do not expect that movement among sites was frequent.

We collected snails between 25 and 30 mm in length to minimize variation among sites that was not due to spatial separation and to reduce the effects of host size on infection by trematode species. Snails in this size class were abundant. Restricting the size class and spatial scale limited the intraspecific heterogeneity in recruitment within each site. From each site, we dissected snails until we found a total of 100 that were infected. We identified the trematodes found in single and double infections after Martin (1972).

For the trematodes studied here, dominants either coexist with or kill subordinates. Note that this scenario does not assume that competition occurs; rather, it assumes that if competition does occur the outcome is predictable. In this case, the null prevalence of species i, e_i, is the expected prevalence of species i without competition. This is the proportion of "competitor-free" hosts that species i occupies. In other words, $e_i = (p_i - o_{id})/(1 - p_d)$ where p_d represents the prevalence of all species (d) that are dominant to species i, and o_{id} is the prevalence of double infections observed between i and d (although we observed none, we would have counted triple infections as three observed double infections). From this, the expected number of double infections between species i and j is Ne_ie_j. Using a dominance hierarchy (Fig. 1) proposed by Kuris (1990) and Sousa (1993), we determined e_i for each species.

We used heterogeneity chi-square tests to determine whether overall prevalence was the same at each of the five sites and whether the relative prevalence of each species was the same at each site (lumping the prevalence of the six rarest species). Next, we investigated the five samples for the structuring effect of spatial heterogeneity. To do this, we compared the number of

double infections expected if there was no intraspecific variation in recruitment among sites with the number of double infections expected from the actual recruitment regime among sites. The expected number of double infections from a group of k samples is the sum of the number of expected double infections calculated separately for each sample (ΣE_{ij}). This equals $\Sigma_k \Sigma_i \Sigma_j N_k p_{ki} p_{kj}$ and can be referred to as the sum of the expected. The expected number of double infections if there was no intraspecific variation in recruitment is calculated directly from the pooled samples ($E\Sigma_i \Sigma_j$). This equals $\Sigma_i \Sigma_j (\Sigma_k N_k p_{ki})(\Sigma_k N_k p_{kj})/(\Sigma_k N_k)$ and can be referred to as the expected of the pooled. Going back to our previous example, by sampling 100 snails from an additional site where a recruited to 40% and b to 90% the sum of the expected (over both sites) would be $100 \times 0.9 \times 0.4 + 100 \times 0.5 \times 0.8 = 76$ and the expected of the pooled would be $200 \times 0.65 \times 0.65 = 84.5$ (spatial heterogeneity isolates the species). The "expected of the pooled" will equal the "sum of the expected" if heterogeneity does not structure the community. Likewise, the sum of the expected will be equal to the observed double infections if interactions do not structure the community. For each prediction, we estimated the expected frequency of double infections with observed (p_i) and null (e_i) prevalences. We compared the results from each approach in our analysis.

Since we sampled more snails from low prevalence sites, we weighted observed values according to sample size so that sites with low prevalence were not disproportionately overrepresented. The weighted frequency of a species $= F \times 170/N$, where F is the observed frequency of a species, 170 is the average number of snails sampled, and N is the number of snails in a subsample. Our analysis did not account for variation in snail density among sites.

We report expected and observed numbers of co-occurrences. Although it is possible to compare expected and observed double infections, we found it more biologically meaningful to compare (using 95% confidence limits) the proportion of trematodes expected to interact before and after the effects of competition. Fig. 2 illustrates the statistical power of this approach for cases with no double infections. Although this approach detects the net effects of structure, non-random structure can act in opposite directions, leading to a nonsignificant net effect. For this reason, we also suggest comparing the distribution of the standard deviate (standardized difference between observed and expected values) with a normal distribution having a mean of zero and standard deviation of one (e.g., Jackson et al. 1992). The standard deviation for the estimates of E_{ij}/N over all $n(n-1)/2$ species combinations is $SD_{ij} = [\Sigma(E_{ij}/N)(1 - E_{ij}/N)]^{1/2}$. The standard deviate between the observed and expected number of double infected hosts is d_{ij}: $d_{ij} = (O_{ij}/N - E_{ij}/N)/SD_{ij}$. We then compared the distribution of d_{ij} for all species with a standard normal distribution to determine structure
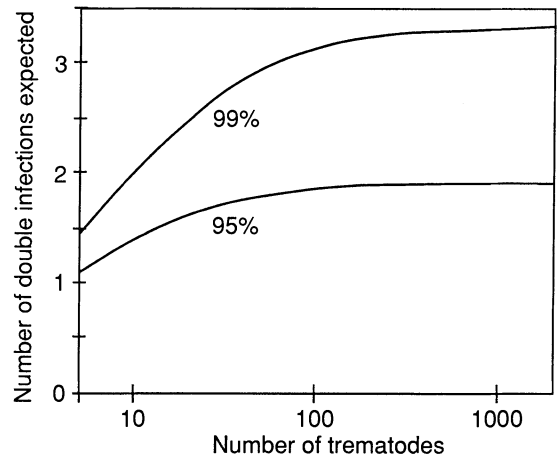

FIG. 2. Power to distinguish N expected from zero double infections. Lines indicate the 95 and 99% confidence intervals above zero double infections across a range of sample sizes. These intervals were computed for the comparison of the proportion of trematodes expected to interact (2 × expected double infections/no. trematodes).

within the observed guild. We report two-tailed probabilities for all tests.

RESULTS

Prevalence at the sites ranged from 42 to 83%. Out of 500 infected snails, we identified nine trematode species and found seven snails with double infections. This was similar to other studies that have found double infections in only 2.5% of infected snails (Sousa 1993). Table 1 shows the observed number of double infections and the numbers estimated from the null models.

Prevalence ($\chi^2 = 75.8$, df = 4, $P < 0.01$) and guild composition ($\chi^2 = 63.8$, df = 11, $P < 0.01$) varied among sites. Thus, at Carpinteria Salt Marsh, spatial heterogeneity might significantly affect community structure. The simple null model, $Np_i p_j$, indicated little difference in the expected number of double infections between the pooled sites (71) and the sum of the sites (74). This suggested no significant effect of spatial heterogeneity on the potential number of double infections (Fig. 3). There was no difference between the distributions of the standard deviates (Kolmogorov-Smirnov, $P = 0.64$). After using $Ne_i e_j$, however, there was a significant difference between the expected number of double infections for pooled sites (111) and the sum of the expected double infections calculated for each site separately (133, see Fig. 3). In this case, the distributions of the standard deviates differed significantly (Fig. 4, Kolmogorov-Smirnov, $P = 0.028$). Therefore, spatial heterogeneity caused more, not fewer, co-occurrences.

For both null models, the difference between 8.7 observed double infections and the sum of the expected double infections ($Np_i p_j = 74$, $Ne_i e_j = 133$) was con-

TABLE 1. Weighted counts of single- and double-species infections for five sites. Species are coded as in Fig. 1 and listed in order of dominance. We sampled each site for 100 infected snails between 25 and 30 mm in length. Expected double infections were calculated as Np_ip_j and Ne_ie_j for each site separately, then summed and calculated from the values of the pooled sites. The expected number of double infections from the pooled sites indicates the number of multiple-species interactions expected if intraspecific recruitment was homogeneous. The expected number of double infections summed over the individual sites indicates the increase in interactions due to intraspecific variation in recruitment. Equalities in lower right of table represent [a] the sum of observed double infections, the sum of expected double infections using [b] observed and [c] expected prevalence, and the expected double infections from pooled data using [d] observed and [e] expected prevalence.

Trematode species	Site 1	Site 2	Site 3	Site 4	Site 5		Pooled sites
None	134	33	21	60	101		349
PARO	0.7	2.6	4.2	2.1	0		9.6
HIMA	10.2	7.7	28.1	6.4	1.7		54.0
ECHI	8.0	3.8	15.5	10.6	0		37.9
ACAN	0	1.3	2.8	3.2	6.8		8.1
EUHA	53.0	106.1	75.9	80.8	80.3		396.1
RENB	0	1.3	0	0	0.8		2.1
SMST	0.7	1.3	7.0	0	0.8		9.9
LGXI	0	0	0	1.1	0		1.1
SMXI	0	3.8	7.0	2.1	0		13.0
EUHA/SMXI	0	1.3	0	0	0		1.3
EUHA/SMST	0	1.3	2.8	0	0		4.1
EUHA/ECHI	0	0	0	1.1	0		1.1
EUHA/RENB	0	0	0	0	0.8		0.8
ECHI/SMXI	0	0	1.4	0	0		1.4
Total, $\Sigma_j O_{ij}$	0	2.6	4.2	1.1	0.8	$\Sigma O_{ij} = 8.7$[a]	$\Sigma O_{ij} = 8.7$[a]
$\Sigma_i\Sigma_j Np_ip_j$	6.5	14	38	14	1.7	$\Sigma E_{ij} = 74$[b]	$E\Sigma_i\Sigma_j = 71$[d]
$\Sigma_i\Sigma_j Ne_ie_j$	8	22	83	19	1.7	$\Sigma E_{ij} = 133$[c]	$E\Sigma_i\Sigma_j = 111$[e]

siderable and highly significant (Figs. 3 and 4, Kolmogorov-Smirnov, $P < 0.001$). Thus, we infer that competition resulted in the elimination of most of the expected co-occurrences. Table 2 presents the esti-mated proportion of each species lost to competition. Over all, interspecific competition apparently resulted in an estimated 16% loss of the trematodes that recruited to 25 to 30 mm snails.

DISCUSSION

The structuring effect of heterogeneity results from the interplay of isolating and intensifying factors. The relative prevalences of each trematode species varied among subsamples, acting to isolate species. In addition, however, variation in the absolute total prevalence among subsamples intensified the likelihood of double infections. Although both factors acted significantly in our study, the net effect was to intensify interactions. This finding is contrary to assertions that variability in recruitment reduces interactions (Sousa 1990, Fernandez and Esch 1991a, Esch and Fernandez 1993, Rohde 1993). As available evidence suggests that doubly infected snails did not suffer higher mortality than did hosts with single infections, competitive exclusion apparently eliminated most of the subordinate species in these co-occurrences. Using null parameters in the null model (Ne_ie_j) gave a different indication of the magnitudes of the structure imparted by both spatial heterogeneity and competition than did standard observed parameters. The notable differences between estimates derived from these models (Fig. 3) would have been even more pronounced had dominant species been common.

We conclude that competition is the best explanation for the fewer than expected double infections observed within these infracommunities. Even the seven double
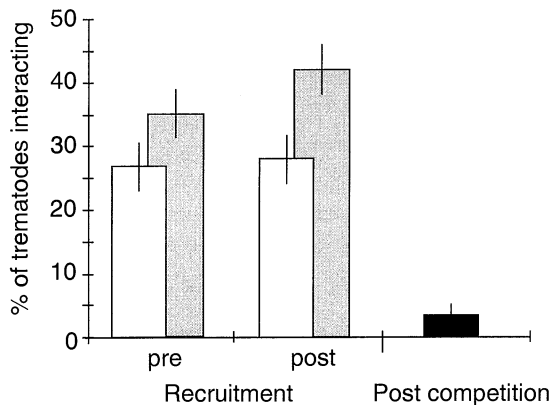

FIG. 3. Structure of the trematode community as esti-mated by two methods. The first pair of shaded and open bars is the percent of trematodes expected to interact before the effect of competition if recruitment were homogeneous among sites (expected of the pooled). The second pair of bars is the percent of trematodes expected to interact before the effect of competition with the observed heterogeneous re-cruitment among sites (sum of the expected). The solid filled bar is the observed number of double infections. Open bars were calculated using observed prevalences while shaded bars were calculated using expected prevalences. Error bars rep-resent 95% confidence limits. Spatial heterogeneity increased interactions and competition decreased the number of inter-actions that persisted.
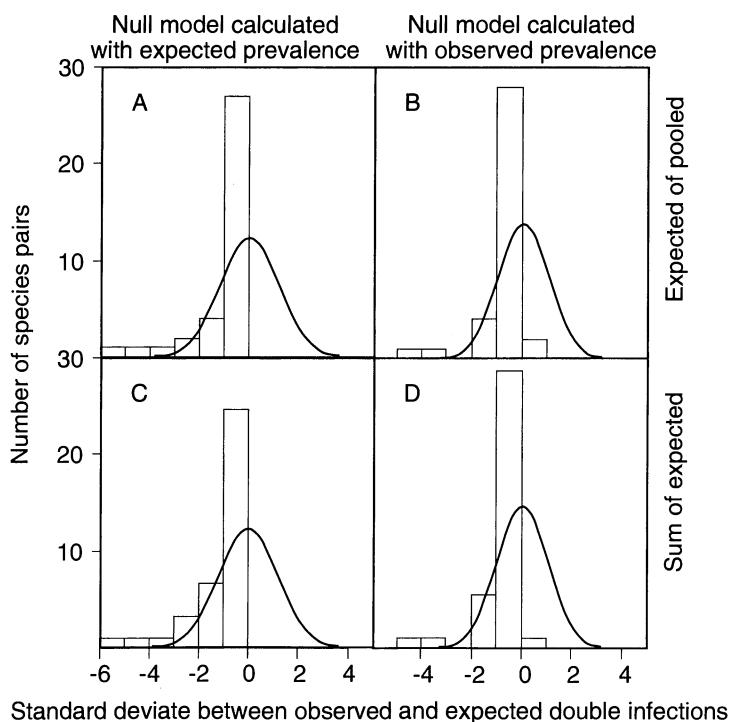
FIG. 4. Distribution of standard deviates for four comparisons between expected double infections and observed double infections. Right boxes were calculated using observed prevalences ($\Sigma N p_i p_j$) while left boxes were calculated using expected prevalences ($\Sigma N e_i e_j$). Top boxes were calculated from data pooled across sites while (homogeneous recruitment), bottom boxes were calculated from the sum of the expected values that were calculated separately for each site (heterogeneous recruitment). All distributions were significantly skewed to the left when compared with a standard normal curve (solid line), indicating that fewer double infections were observed than were expected ($P < 0.001$). Using expected prevalences in the null model resulted in significantly different distributions than using observed prevalences (A vs. B, $P = 0.03$; C vs. D, $P = 0.006$). Spatial heterogeneity was only detectable when expected prevalences were used in the null model (B vs. D, $P = 0.64$; A vs. C, $P = 0.028$).

infections that we did observe could represent competitive events in progress (Kuris 1990, Fernandez and Esch 1991a). This conclusion is consistent with the findings of Sousa (1990, 1993) and Kuris (1990) for the same host–parasite system, as well as for studies of larval trematode assemblages in other molluscan hosts where double infections were less frequent than expected (Fernandez and Esch 1991a, references in Kuris 1990 and Sousa 1992, Kuris and Lafferty 1994). We envision a three-step process determining the species composition of a guild of larval trematodes. First, the diversity and abundance of species that recruit to an area will determine the potential for co-occurrences (Kuris 1990, Sousa 1992). Then, spatial and temporal heterogeneity in recruitment will determine whether the initial number of co-occurrences is higher or lower than the potential number of co-occurrences. Finally, with time, competition, according to a hierarchy of dominance, will reduce the number of multiple-species infections that persist.

The importance of competition in structuring the trematode community is directly dependent on the proportion of hosts experiencing multiple infections. In the present case, overall prevalence was 61.6%, and competition appears to have displaced 16% of all trem-

atode individuals. However, because we restricted our analysis to a narrow size class of hosts, we have only accounted for structure in a portion of the trematode guild. In an experimental mark–recapture study where overall prevalence at the study site was only 30%, Sousa

TABLE 2. An estimation, using prevalence pooled over all sites (e_i vs. p_i), to estimate the effects of competition on the relative abundance of trematode species. Numbers represent occurrences (double and single infections) of each species. Estimated numbers before competition were rounded to the nearest whole integer. Percentage losses were calculated from the unrounded estimation.

Trematode species	Before competition	After competition	Change
			0%
PARO	9.6	9.6	−1.7%
HIMA	55.0	54.0	−11.6%
ECHI	42.9	37.9	−16.1%
ACAN	9.7	8.1	−13.1%
EUHA	455.9	396.1	−20.6%
RENB	2.7	2.1	−51.8%
SMST	20.5	9.9	−60 %
LGXI	2.7	1.1	−63.4%
SMXI	35.4	13.0	−16.2%
Total	634.3	531.8	

(1993) found that annual replacement of one trematode by another eliminated 72% of *Euhaplorchis californiensis* (by far the most common trematode in our system), 11% of *Acanthoparyphium spinulosum*, 8% of *Echinoparyphium* sp., 8% of *Himasthla rhigedana,* and 1% of *Parorchis acanthus* (the most dominant species). These results are consistent with a highly interactive system. The vast majority of replacements were by the dominant species, *P. acanthus* and *H. rhigedana* (Sousa 1993), demonstrating the importance of the competitive hierarchy.

General applications

Although developed to study larval trematodes, our approach applies to the general study of communities. Granted, communities of parasites are somewhat easier to study than free-living organisms because the scale at which we determine the presence or absence of a parasite species, the host, is unambiguous and biologically meaningful (Esch et al. 1990). Analogous, though more arbitrary, spatial scales for quantifying the presence–absence of free-living organisms would be the area of a home range, territory, or, for sessile species, the area covered by an individual. To use our methodology to determine the structure of a community of two free-living species, *a* and *b,* would require determining areas used by individuals, pairs, or other logical groups of each species. Then, as we have done for host snails, one could calculate the proportion of those areas occupied by species *a, b, a* and *b,* or neither *a* nor *b.* Although simply quantifying the presence or absence of a species leads to the easiest analysis, it may be more powerful to incorporate species densities in some cases (Rahel 1990). To test for structure, it is necessary to compare the observed frequency of overlapping areas with the frequency expected if the two species were randomly distributed and did not interact. Determining the expected frequency of overlap would require choosing and developing the appropriate null model (Strong et al. 1979). To generate a truly null expected value requires using null parameters in the null model. In the Appendix, we derive null prevalences for displacement or mortality associated with dominance or preemption. Where the observed overlap is different from expected, the community is structured. In some cases, it may not be possible to predict the position of every species in a postulated hypothetical linear dominance hierarchy. The exclusion of species with questionable dominance ranks from calculations of the expected prevalence of other species is appropriate yet carries the potential price of underestimating expected prevalences.

Structure, if found, could be a result of heterogeneity or interaction. Two spatial scales for sampling these areas would allow the determination of the relative role of spatial heterogeneity. The smaller spatial scale should be one in which there is little intraspecific variation in recruitment. Replicates of these small-scale samples should then be taken over the entire community of interest. This scale could be as large as the geographic distributions of the species. Wiens (1989) recommends investigating several spatial scales to determine the effects of scaling processes on the determinants of community structure. Comparing the sum of expected values of overlap on small spatial scales with the expected overlap calculated by pooling over a single large spatial scale would indicate whether heterogeneity acted to isolate the species, intensify their overlap, or did not affect their relative distributions. Samples taken over time would generate a similar analysis for temporal heterogeneity.

As we have tried to give the most general examples possible, one might have to modify our approach to suit the biology of the community under study. Here, we have concentrated solely on methods used to analyze patterns that we can observe in nature. The use of appropriate descriptors of a community, stratified samples, appropriate null models, and powerful statistical comparisons will make it easier to determine community structure from such observations. We feel strongly that this approach can lead to valuable insight but recognize that experimental approaches, though often difficult, are necessary to further investigate these patterns.

LITERATURE CITED

Anderson, R. M., and R. M. May. 1978. Regulation and stability of host-parasite interactions. I. Regulatory processes. Journal of Animal Ecology 47:219–247.

Boss, J. M. 1977. Synergism between *Schistosoma mansoni* and *Echinostoma paraensei* in the snail *Biomphalaria glabrata.* Dissertation. University of California, Berkeley, California, USA.

Bourns, T. K. R. 1963. Larval trematodes parasitizing *Lymnaea stagnalis appressa* Say in Ontario with emphasis on multiple infections. Canadian Journal of Zoology 41:937–941.

Colwell, R. K., and D. W. Winkler. 1984. A null model for null models in biogeography. Pages 344–359 *in* D. R. Strong, D. Simberloff, L. G. Abele, and A. B. Thistle, editors. Ecological communities: conceptual issues and the evidence. Princeton University Press, Princeton, New Jersey, USA.

Combes, C. 1982. Trematodes: antagonism between species and sterilising effects on snails in biological control. Parasitology **84**:151–175.

Combescot-Lang, C. 1976. Etude des trématodes parasites de *Littorina saxatilis* (Olivi) et de leurs effets sur cet hôte. Annals de Parasitologie Humaine et Comparée **51**:27–36.

Connell, J. H. 1980. Diversity and the coevolution of competitors, or the ghost of competition past. Oikos 35:131–138.

———. 1983. On the prevalence and relative importance of interspecific competition: evidence from field experiments. American Naturalist 122:661–696.

Conner, E. F., and D. Simberloff. 1979. The assembly of species communities: chance or competition? Ecology 60:1132–1140.

Cort, W. W., D. B. McMullen, and S. Brackett. 1937. Ecological studies on the cercariae in Stagnicola emarginata angulata (Sowerby) in the Douglas Lake region, Michigan. Journal of Parasitology 23:504–532.

Diamond, J. M. 1975. Assembly of species communities. Pages 342–444 in M. L. Cody and J. M. Diamond, editors. Ecology and evolution of communities. Harvard University Press, Cambridge, Massachusetts, USA.

Esch, G. W., and J. C. Fernandez. 1993. A functional biology of parasitism: ecological and evolutionary implications. Chapman and Hall, London, England.

Esch, G. W., A. W. Shostak, D. J. Marcogliese, and T. M. Goater. 1990. Patterns and processes in helminth parasite communities: an overview. Pages 1–19 in G. W. Esch, A. O. Bush, and J. M. Aho, editors. Parasite communities: patterns and processes. Chapman and Hall, London, England.

Fernandez, J., and G. W. Esch. 1991a. Guild structure of larval trematodes in the snail Helisoma anceps: patterns and processes at the individual host level. Journal of Parasitology 77:528–539.

Fernandez, J., and G. W. Esch. 1991b. The component community structure of larval trematodes in the pulmonate snail Helisoma anceps. Journal of Parasitology 77:540–550.

Gilpin, M. E., and J. M. Diamond. 1984. Are species co-occurrences on islands non-random, and are null hypotheses useful in community ecology? Pages 297–315 in D. R. Strong, D. Simberloff, L. G. Abele, and A. B. Thistle, editors. Ecological communities: conceptual issues and the evidence. Princeton University Press, Princeton, New Jersey, USA.

Grant, P. R., and I. Abbott. 1980. Interspecific competition, island biogeography and null hypotheses. Evolution 34:332–341.

Grant, P., and D. Schluter. 1984. Interspecific competition inferred from patterns of guild structure. Pages 201–233 in D. R. Strong, D. Simberloff, L. G. Abele, and A. B. Thistle, editors. Ecological communities: conceptual issues and the evidence. Princeton University Press, Princeton, New Jersey, USA.

Gurevitch, J., L. Morrow, A. Wallace, and J. Walsh. 1992. A meta-analysis of competition in field experiments. American Naturalist 140:539–572.

Harvey, P. H., R. K. Colwell, J. W. Silverton, and R. M. May. 1983. Null models in ecology. Annual Review of Ecology and Systematics 14:189–211.

Heyneman, D., H. K. Lim, and U. Jeyarasasingam. 1972. Antagonism of Echinostoma liei (Trematoda: Echinostomatidae) against the trematodes Paryphostomum segregatum and Schistosoma mansoni. Parasitology 65:203–222.

Holmes, J. C. 1990. Helminth communities in marine fishes. Pages 101–130 in G. W. Esch, A. O. Bush, and J. M. Aho, editors. Parasite communities: patterns and processes. Chapman and Hall, London, England.

Holmes, J. C., and P. W. Price. 1986. Communities of parasites. Pages 187–213 in D. J. Anderson and J. Kikkawa, editors. Community ecology: patterns and processes. Blackwell Scientific, Oxford, England.

Jackson, D. A., K. M. Somers, and H. H. Harvey. 1992. Null models and fish communities: evidence of nonrandom patterns. American Naturalist 139:930–951.

Jourdane, J. 1980. Interference by Schistosoma mansoni with the natural resistance to Echinostoma togoensis in Biomphalaria glabrata. Proceedings of the Third European Multicolloquium in Parasitology. Cambridge University Press, Cambridge, England.

Kuris, A. M. 1973. Biological control: implications of the analogy between the trophic interactions of insect pest-parasitoid and snail-trematode systems. Experimental Parasitology 33:365–379.

———. 1974. Trophic interactions: similarity of parasitic castrators to parasitoids. Quarterly Review of Biology 49:129–148.

———. 1990. Guild structure of larval trematodes in molluscan hosts: prevalence, dominance and significance of competition. Pages 69–100 in G. W. Esch, A. O. Bush, and J. M. Aho, editors. Parasite communities: patterns and processes. Chapman and Hall, London, England.

Kuris, A. M., and K. D. Lafferty. 1994. Community structure: larval trematodes in snail hosts. Annual Review of Ecology and Systematics 25:189–217.

Lafferty, K. D. 1991. Effects of parasitic castration on the salt marsh snail, Cerithidea californica. Dissertation. University of California, Santa Barbara, California, USA.

———. 1993a. Effects of parasitic castration on growth, reproduction and population dynamics of the marine snail Cerithidea californica. Marine Ecology Progress Series 96:229–237.

———. 1993b. The marine snail, Cerithidea californica, matures at smaller sizes where parasitism is high. Oikos 68:3–11.

Lie, K. J. 1982. Survival of Schistosoma mansoni and other trematode larvae in the snail Biomphalaria glabrata: a discussion of the interference theory. Tropical and Geographic Medicine 34:111–122.

Lie, K. J., P. F. Basch, D. Heyneman, A. J. Beck, and J. R. Audy. 1968. Implications for trematode control of interspecific larval antagonism within snail hosts. Transactions of the Royal Society in Tropical Medicine and Hygiene 62:299–319.

Lie, K. J., D. Heyneman, and K. H. Jeong. 1976. Studies on resistance in snails. 7. Evidence of interference with the defense reaction in Biomphalaria glabrata by trematode larvae. Journal of Parasitology 62:608–615.

Lie, K. J., H. K. Lim, and C. K. Ow-Yang. 1973. Synergism and antagonism between two trematode species in the snail Lymnaea rubiginosa. International Journal of Parasitology 3:719–733.

Lim, H. K., and D. Heyneman. 1972. Intramolluscan inter-trematode antagonism: a review of factors influencing the host-parasite system and its possible role in biological control. Advances in Parasitology 10:191–268.

Martin, W. E. 1955. Seasonal infections of the snail, Cerithidea californica Haldeman, with larval trematodes. Pages 203–210 in Essays in natural science in honor of Captain Alan Hancock on the occasion of his birthday, July 26 1955. University of Southern California Press, Los Angeles, California, USA.

———. 1972. An annotated key to the cercariae that develop in the snail Cerithidea californica. Bulletin of the Southern California Academy of Science 71:39–43.

Margolis, L., G. W. Esch, J. C. Holmes, A. M. Kuris, and G. M. Schad. 1982. The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). Journal of Parasitology 68:131–133.

May, R. M. 1984. An overview: real and apparent patterns in community structure. Pages 3–18 in D. R. Strong, D. Simberloff, L. G. Abele, and A. B. Thistle, editors. Ecological communities: conceptual issues and the evidence. Princeton University Press, Princeton, New Jersey, USA.

Pielou, E. C. 1974. Population and community ecology. Gordon and Breach, New York, New York, USA.

Polis, G. A., C. A. Meyers, and R. D. Holt. 1989. The

ecology and evolution of intraguild predation: potential competitors that eat each other. Annual Review of Ecology and Systematics **20**:297–330.

Rahel, F. J. 1990. The hierarchical nature of community persistence: a problem of scale. American Naturalist **136**: 328–344.

Robson, E. M., and I. C. Williams. 1970. Relationships of some species of Digenea with the marine prosobranch *Littorina littorea* (L.) I. The occurrence of larval Digenea in *L. littorea* on the North Yorkshire Coast. Journal of Helminthology **44**:153–168.

Rohde, K. 1981. Population dynamics of the two snail species, *Planaxis sulcatus* and *Cerithium moniliferum*, and their trematode species at Heron Island, Great Barrier Reef. Oecologia **49**:344–352.

———. 1993. Ecology of marine parasites. Second edition. Commonwealth Agricultural Bureau International, Wallingford, England.

Root, R. B. 1973. Organization of a plant-arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). Ecological Monographs **43**:95–124.

Sale, P. F. 1991. Reef fish communities: open non-equilibrial systems. Pages 564–598 *in* P. F. Sale, editor. The ecology of fishes on coral reefs. Academic Press, San Diego, California, USA.

Schluter, D. 1984. A variance test for detecting species associations, with some applications. Ecology **65**:998–1005.

Schoener, T. W. 1983. Field experiments in interspecific competition. American Naturalist **122**:240–285.

Sih, A., P. Crowley, M. McPeek, J. Petranka, and K. Strohmeier. 1985. Predation, competition and prey communities: a review of field experiments. Annual Review of Ecology and Systematics **16**:269–311.

Simberloff, D. 1990. Free-living communities and alimentary tract helminths: hypotheses and pattern analysis. Pages 289–319 *in* G. W. Esch, A. O. Bush, and J. M. Aho, editors. Parasite communities: patterns and processes. Chapman and Hall, London, England.

Sousa, W. P. 1983. Host life history and the effect of parasitic castration on growth: a field study of *Cerithidea californica* Haldeman (Gastropoda: Prosobranchia) and its trematode parasites. Journal of Experimental Marine Biology and Ecology **73**:273–296.

———. 1990. Spatial scale and the processes structuring a guild of larval trematode parasites. Pages 41–67 *in* G. W. Esch, A. O. Bush, and J. M. Aho, editors. Parasite communities: patterns and processes. Chapman and Hall, London, England.

———. 1992. Interspecific interactions among larval trematode parasites of freshwater and marine snails. American Zoologist **32**:583–592.

———. 1993. Interspecific antagonism and species coexistence in a diverse guild of larval trematode parasites. Ecological Monographs **63**:103–128.

Strong, D. R., D. Simberloff, L. G. Abele, and A. B. Thistle. 1984. Ecological communities: conceptual issues and the evidence. Princeton University Press, Princeton, New Jersey, USA.

Strong, D. R., L. A. Szyska, and D. Simberloff. 1979. Tests of community-wide character displacement against null hypotheses. Evolution **33**:897–913.

Vaes, M. 1979. Multiple infection of *Hydrobia stagnorum* with larval trematodes. Annals de Parasitologie Humaine et Comparée **54**:303–312.

Vernberg, B., F. Vernberg, and F. Beckerdite, Jr. 1969. Larval trematodes: double infections in the common mud-flat snail. Science **164**:1287–1288.

Werding, B. 1969. Morphologie, Entwicklung and Okologie digener Trematoden-Larven der Strandschnecke, *Littorina littorea*. Marine Biology **3**:306–333.

Wiens, J. A. 1989. Spatial scaling in ecology. Functional Ecology **3**:385–397.

Yoshino, T. P. 1975. A seasonal and historical study of larval Digenea infecting *Cerithidea californica* (Gastropoda: Prosobranchia) from Goleta Sough, Santa Barbara County, California. Veliger **18**:156–161.

APPENDIX

Here, we derive the number of individuals of a species, i, expected to have recruited to a sample of territories (for parasites, these territories are hosts). For five biological scenarios, we describe an equation for the observed frequency of a species as a function of its pre-interactive frequency, solve for the pre-interactive frequency, and then calculate the pre-interactive frequency using hypothetical observed values. These equations are the equivalent of performing a series of binomial trials on each territory. A subordinate (according to dominance or preemption) that recruits to the same territory as a dominant could coexist, be killed, move to a conspecific territory, move to an empty territory, or move to a nonheterospecific territory. Scenario 1 (below) fits what we expect most often happens for the larval trematodes in our system. Kuris (1990) used a similar approach to calculate the pre-interactive prevalence of trematode species by incorporating information from a proposed dominance hierarchy.

Scenario 1.—Dominants either coexist with or kill subordinates, e.g., parasitic castrators or parasitoids (or the subordinate species moves to a conspecific territory). The observed prevalence of a species i will be equal to its initial prevalence minus the initial prevalence of double occurrences with dominant species plus the fraction of those double occurrences observed at the time of sampling. This is expressed as $p_i = e_i - p_d e_i + o_{id}$, where p_i is the observed prevalence of species i, e_i is the prevalence of species i that recruit and would in isolation survive to the sampling date, p_d is the combined prevalence of all species that are dominant to i, and o_{id} is the

proportion of double occurrences observed between i and d. Solving for e_i yields $e_i = (p_i - o_{id})/(1 - p_d)$. In this case, the prevalence of species i expected at recruitment is the portion of available hosts times the proportion of "competitor-free" hosts that species i occupies. Using hypothetical values of $p_i = 0.2$, $p_d = 0.8$, and $o_{id} = 0.1$, $e_i = 0.5$.

Scenario 2.—Subordinates coexist with dominants or move to an empty territory, e.g., nesting song birds, hermit crabs, pontonean shrimps in penn shells. The observed prevalence of a species will be equal to the initial prevalence minus the initial prevalence of double occurrences with dominant species plus the prevalence of those double occurrences observed at the time of sampling plus the prevalence of double occurrences that move. This is expressed as $p_i = e_i - p_d e_i + o_{id} + (p_d e_i - o_{id})$. Solving for e_i yields $e_i = p_i$. For the same hypothetical observed values, $e_i = 0.2$.

Scenario 3.—Subordinates coexist with dominants or move to a territory that does not contain a dominant, e.g., anemone fishes, ungulates. The prevalence of individuals of a species will be equal to the prevalence of initial individuals minus the proportion that moves to a conspecific territory. Assuming that two subordinates do not move to the same open territory, this is expressed as $p_i = e_i - (p_d e_i - o_{id})(e_i - p_d e_i)/(1 - p_d)$ or $p_i = e_i(1 - p_d e_i + o_{id})$. Solving for e_i yields $e_i = (1 + o_{id} - [(o_{id} + 1)^2 - 4p_d p_i]^{1/2})/2p_d$. For the same hypothetical observed values, $e_i = 0.22$.

Scenario 4 (preemption).—The first species to arrive coexists with or kills the second species to arrive, e.g., sessile,

filter-feeding invertebrates (or the second species to arrive moves to a conspecific territory, e.g., barnacles with selective, gregarious settlement). Assuming each species has a 50% chance of being the first to arrive, the observed number of individuals of a species will be equal to the total number of initial individuals minus half the initial amount of double occurrences with other species plus half the number of those double occurrences observed at the time of sampling. This is expressed as $p_h = e_i - e_ie_h/2 + o_{ih}/2$, where e_h is the fraction of heterospecific individuals expected at recruitment. The observed number of heterospecific individuals, p_h, will be equal to the total number of initial heterospecific individuals minus half the initial amount of double occurrences with species i plus half the number of those double occurrences observed at the time of sampling. This is expressed as $p_h = e_h - e_ie_h/2 + o_{ih}/2$. Using both equations to solve for e_i yields $e_i = 1 + \{p_i - p_h - ([p_h - p_i - 2]^2 - 4[2p_i - o_{ih}])^{1/2}\}/2$. For the same hypothetical observed values, and for $p_h = 0.8$, $e_i = 0.26$.

Scenario 5 (preemption). — The second species to arrive coexists with the first species to arrive or moves to an empty territory, e.g., mantis shrimps. The observed number of individuals of a species will be equal to the total number of initial individuals minus the initial amount of double occurrences plus the number of those double occurrences observed at the time of sampling plus the number of double occurrences that move. This is expressed as $p_i = e_i - e_ie_h + o_{ih} + (e_ie_h - $

$o_{ih})$. Solving for e_i yields $e_i = p_i$. For the same hypothetical observed values, $e_i = 0.2$.

It is not possible to explicitly calculate e_i for the scenario where the second species to arrive coexists with the first species or moves to a nonheterospecific territory. This may not be a likely pattern of interaction for preemption because if recruitment order determines dominance, early recruiting individuals should be able to exclude members of their own species.

The goal of these formulae is to provide values for null models that are free from the effects of competition. Although the scenarios incorporate assumptions about interactions, they do not assume that competition occurs; they only assume that if competition does occur its outcome is predictable. Only by determining the number of each species that recruit, is it possible to estimate the number of territories with co-occurring species that would be expected were structuring forces such as competition or variation in habitat selection not operating. For two species, the expected null frequency of co-occurrences equals Ne_ie_j. This may vary strongly from the more generally used null model Np_ip_j in scenarios where species kill or move to conspecific territories, especially if dominants are common. For situations where subordinate species may move to another habitat, it may be justifiable to modify our equations to account for certain percent loss of those individuals that move.