Research

Patterns of species co-occurrence and density compensation: a test for interspecific competition in bat ectoparasite infracommunities

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Parasites constitute an ideal system with which to investigate patterns and mechanisms of community structure and dynamics. Nevertheless, despite their prevalence in natural systems, parasites have been examined less often than other organisms traditionally used for testing hypotheses of community assembly. In the present study, we investigate possible effects of competitive interactions on patterns of distribution (co-occurrence) and density among a group of streblid bat flies parasitic on short-tailed fruit bats, *Carollia perspicillata*. Using null model analyses of species co-occurrence, we did not find evidence that competition affects the distribution of bat fly species across hosts. Moreover, when non-infested hosts were included, analyses showed evidence for interspecific aggregation, rather than for the segregation predicted by competition theory. Partial Pearson correlations among bat fly species densities showed no evidence of negative covariation in two of three cases. In the species pair for which a significant negative correlation was found, a visual analysis of plotted covariation indicated a constraint line, suggesting that competition between these two species might become operational only in some infracommunities when abundances of bat flies approach a maximum set by one or more limiting resources. Moreover, when a community-wide estimation of the significance of density compensation was calculated, the result was not significant. Overall, we find no evidence that competition influences the distribution of bat flies on their hosts, and mixed support for effects of competition on the densities of species. These results are consistent with the idea that competition plays a role in structuring natural communities, but in many systems its effects are context-dependent and might not be important relative to other factors. Wider analyses across taxonomic and environmental gradients and a detailed consideration of the different hypothesized effects of competition are necessary to fully understand the importance of competition on natural communities.

Host–parasite systems offer a wealth of opportunity for understanding variation in distribution and abundance of organisms (Gotelli and Rohde 2002). Most research has focused on documenting distribution and abundance of parasites on hosts in an attempt to understand host–parasite interactions. Nonetheless, the relative frequency of interspecific interactions among parasites, how these interactions affect the structure of such communities, and how this may ultimately influence host–parasite interactions remains uncertain (Rohde 1991, Morand et al. 1999, Mouillot et al. 2005).

The nature of host–parasite relationships structures parasite communities at several hierarchical scales, ranging from host clades and species to host individuals. Parasite communities assembled at this last scale are termed infracommunities (sensu Bush et al. 1997). The infracommunity level is the most fundamental of these scales of analysis since individual hosts represent the units of distribution at which most local parasite interspecific interactions take place.

Parasite infracommunities make excellent model systems for understanding organization of natural communities.

Hosts typically provide discontinuous habitat patches that can support discrete communities of parasites (Gotelli and Rohde 2002); this is in contrast to many terrestrial habitats that often subtly intergrade, thereby making delimitation of communities difficult. Additionally, abundant hosts provide ample and convenient replication with which to conduct powerful statistical analyses to assess hypothesized processes underlying community structure (Holmes and Price 1986). Individual hosts can also vary considerably in terms of morphology, condition, fitness and life history, thereby providing natural treatment effects from which to explore causative mechanisms.

Several mechanisms have been hypothesized to determine variation in community composition and species abundance. Primary among these has been the classical hypothesis of competitively induced deterministic structure. According to this hypothesis, species in a particular community comprise a nonrandom subset of species available from the regional pool, and possess characteristics that minimize competitive interactions among them, thereby allowing coexistence (Hutchinson 1957, MacArthur and

Levins 1967). The interspecific competition hypothesis has been recently challenged from at least two different perspectives: neutral theory and non-equilibrium ecology. The latter has been supported by some studies on parasite communities (Rohde 1991, Morand et al. 2002, Mouillot et al. 2003), suggesting that in these systems population densities often remain low, resources are not limiting, and consequently communities do not become saturated. Therefore, neither resource-based competition nor zero-sum dynamics are likely to be important (Rohde 2005). However, other experimental and observational studies have provided evidence that competition might be an important force structuring parasite communities by causing competitive exclusion (mainly among snail parasites, Kuris and Lafferty 1994) or by increasing differentiation of microhabitat use within the host (mainly on platyhelmith worms, Bush and Holmes 1986). Studies on parasite community structure, particularly those addressing the effects of competition on composition and species abundance, have focused on a relatively limited set of parasitic taxa. Given the vast diversity of host–parasite systems, the generality of competitive interactions on parasite communities remains unclear.

Ectoparasitic relationships between bat flies and their chiropteran hosts provide an interesting and useful setting from which to better understand variation in community structure, as well as the mechanistic bases to such structure. Bat flies (Diptera: Streblidae) are obligate blood-feeding ectoparasites exclusively associated with bats (Dick and Patterson 2006). Streblid bat flies exhibit high host specificity (Dick 2007, Dick and Patterson 2007), suggesting tight co-evolution between hosts and parasites in this system. Bat hosts are often abundant, especially in the tropics, and easy to sample (Rui and Graciolli 2005). In particular, the host species of this study, *Carollia perspicillata*, is widely distributed in South and Central America and is considered one of the most abundant species in the Neotropics (Cloutier and Thomas 1992). On a particular host, bat flies can be numerous and are readily identifiable (Dick and Gettinger 2005). Hosts are often parasitized by multiple fly species; in a survey in Venezuela, 63% of infested bats hosted simultaneously 2–4 fly species (Wenzel 1976). Moreover, all bat flies consume a common resource (blood) and live in relatively small patches of habitat (hosts). How these ectosymbionts co-exist, given seemingly limiting resources, remains a question.

In the present study, we test the hypothesis that interspecific competition is a major force structuring infracommunities of streblid flies that parasitize *C. perspicillata*. We analyze the distribution and densities of these parasite species to evaluate two predicted effects of competition on the structure of natural communities. If competition is an important force, it is expected that 1) competitors will be distributed across communities in a way that avoids, or at least reduces, their common occurrence (competitive exclusion, less-than-expected patterns of species co-occurrence), or that 2) in communities where competitors co-exist, abundance of one species will be inversely related to the abundance of others (density compensation, negative correlations among the abundances of competing species).

Methods

Data collection

Individuals of *Carollia perspicillata* and their bat fly ectoparasites were collected from several lowland and mountainside localities on both sides of the Andes in Ecuador. Bat sampling at a particular site typically consisted of six mist-nets set at ground level within an area <1 km^2 for up to five consecutive nights. When a *C. perspicillata* was captured, it was immediately euthanized, removed from the net and placed into a sealed zip-lock plastic bag. At the field laboratory, each bat and bag were carefully examined for streblids and other ectoparasites. This protocol minimized parasite escape and inter-host contamination, allowing for more accurate estimation of the proportion of hosts infested (prevalence) and streblid individuals per parasitized host (intensity).

Bat flies were identified, individuals were quantified and a value of abundance (sensu Bush et al. 1997) was assigned for each streblid species on each bat host examined. All bat and bat fly specimens collected were deposited in the scientific collections of the Museo de Zoología of the Pontificia Univ. Católica del Ecuador (QCAZ).

Data analysis

Sampling was conducted at 18 localities. However, because the purpose of our study was to examine patterns of fly co-occurrence and abundance under the hypothesis of competitive interactions at the infracommunity level (on host individuals), we reduced the dataset to only those hosts collected at geographic localities from which all three streblid species under study were recorded. By doing this, we intended to reduce or eliminate the effect of mechanisms acting at scales larger than the infracommunity, such as forces shaping geographic distribution or locality occupancy of the bat fly species. We acknowledge that one of these forces could be competition, and that competitive interactions could have different effects at other scales of analyses (e.g. at the level of geographic locality or even region), which we are not addressing in this paper. The restriction of localities to those containing all bat fly species analyzed, and the fact that sampling at each locality was homogeneous in time and space, maximized the probability that every host could be colonized by every bat fly species. This process of data filtering yielded information on the occurrence of 376 streblids on 92 hosts collected at seven localities; four of these localities were located west and three east of the Andes. The mean linear distance between localities was 230 km.

Null model analyses of co-occurrence
Given that resources are limiting, a superior competitor could drive other competitors locally extinct (competitive exclusion, Gause 1934). Competitively induced local extinction distributed across a landscape or a series of habitat patches would create a checker-board occupancy pattern among competitors. Thus, competition theory predicts less-than-expected patterns of species co-occurrence

(Diamond 1975). To test this prediction, null-models of species co-occurrence were carried out.

Null models are statistical tools based on the randomization of empirical or theoretical data that allow comparison between observed patterns and those expected under a particular null hypothesis (Gotelli and Graves 1996). In the case of null models of species co-occurrence, analyses are often based on a presence–absence matrix. Columns represent the units at which occurrences are measured (e.g. geographic localities, host individuals) and rows correspond to species. Each entry can be either 1 (presence), or 0 (absence). From this empirical matrix, a single co-occurrence index is extracted, representing the degree of common occurrences of species throughout the communities. Then, 1s and 0s are reshuffled in the matrix with each occurrence being relocated independently of any other, but according to the constraints of a particular randomization algorithm. After a new random matrix has been created, the co-occurrence index is recalculated. This process is repeated a number of times, yielding a distribution of the index under the null hypothesis of independence among occurrences. The empirical index can then be compared to the randomly generated distribution and a value of its probability of outcome by chance can be obtained.

Delimitation of the empirical matrix is critical (Gotelli and Graves 1996). Thus, matrices must be logically structured and justified; but at the same time, they provide a flexible means to modify the scenario in which hypotheses are tested and to further explore data. The decision to include or exclude empty sites is one that can have substantive effects on the outcome of randomization analyses. Some decisions are obvious, such as the exclusion of sites that fall outside of the dispersal capabilities of focal taxa (Reddingius 1983). By limiting our samples to geographic locations where all species of ectoparasites were known to occur, we eliminated hosts that were unsuitable in terms of isolation. Non-parasitized hosts within the dispersal range of focal taxa present a quite different scenario. Hosts could be empty because they are not suitable habitats for ectoparasites, because by chance they have not been colonized (stochasticity of assembly) (Gotelli and Rohde 2002), or because they represent spatial dynamics of parasite populations. Because of these different interpretations, we analyzed our data including and excluding non-parasitized bats. We agree with Gotelli and Rohde (2002) that complementary analyses with and without empty sites can provide important insights into the assembly of communities.

After our data were constrained to geographic localities where all bat fly species were recorded, the remaining data were used to build two matrices. The first consisted of 92 columns, representing the total 92 hosts for which data on streblid infracommunity structure were gathered. The second matrix was the portion of the first that remained after removing all hosts that did not harbor any bat fly species; this process yielded a matrix with 75 columns.

Several indices have been proposed to quantify patterns of species co-occurrences, but not all are equally suitable for a particular dataset. In this study, the C-score was used. This index has the advantage that it does not require species to have perfect mutually exclusive distributions (Gotelli

2000), so it can be used in "noisy" datasets, where species tend to competitively exclude each other, but still co-exist in certain communities. Gotelli (2000) showed that this "noise", caused by imperfect divergent distributions of species, can reach high values (around 50%) with C-scores being still sufficiently powerful to detect less-than-expected co-occurrences. Additionally, when coupled with appropriate algorithms, it generates null models with good statistical behavior (Gotelli 2000). High values of this index indicate that the overall pattern is infrequent co-occurrence of species, while low C-score values mean that species frequently occur together.

The final step in null model analyses of species co-occurrence is to select suitable randomization algorithms. Algorithms define constraints imposed on the process by which random matrices are generated. Gotelli (2000) demonstrated that algorithms maintaining the empirical number of occurrences per species in the reshuffled matrices (fixed row algorithms) generally have good type I error properties (average false null hypothesis rejection $<15\%$; Gotelli 2000). Thus, the null models used in this study were based on four fixed-row algorithms, but with the following constraints imposed on the host (column) totals:

1. Fixed rows, equiprobable columns. All hosts have the same probability of obtaining a parasite species during the randomization process.
2. Fixed rows, column probabilities given by host body weight. Considerable variation in host weight was observed (14.9–29.8 g). Host weight may be used as a surrogate for resource availability and habitat patch size, which may in turn influence parasite species richness per bat. Also, if body size is indicative of host age, it can be associated to species richness by parasite accumulation (Pulkkinen and Valtonen 1999). These potential effects were controlled using this algorithm, whereby probability of obtaining a parasite species is proportional to the host's body weight.
3. Fixed rows, fixed columns. The total number of species observed on each host is also maintained, and only their identity is randomized.
4. Fixed rows, proportional columns. The probability of a host acquiring a parasite species is proportional, but not constrained to be identical, to the number of parasite species found on that host in the empirical matrix.

The fixed-equiprobable algorithm differs from the others in that it considers all hosts to be strictly homogeneous in the likelihood of being colonized and the number of parasite species they can sustain. The other algorithms account for host-to-host variation that could influence their suitability for parasite colonization or coexistence. This host-to-host heterogeneity can be known (body mass) or unknown, but assumed to be reflected in the number of parasite species observed in the empirical data (in the case of fixed-fixed and fixed-proportional algorithms). Results stemming from the different null model algorithms can indicate the degree that host-to-host heterogeneity influences patterns of parasite co-occurrence.

Only algorithms 1 and 2 were used in analyses that included empty hosts because when occurrences are

reshuffled under constraints of fixed or proportional columns (algorithms 3 and 4), the probability that an empirical empty host will receive a parasite species in any of the null matrices equals zero. On the other hand, all four algorithms were used in the scenario that excluded non-infested hosts. All null models were based on 10 000 iterations and degenerate matrices were retained in all cases. Analyses were carried out using Ecosim 7.72 (Gotelli and Entsminger 2001).

Kuris and Lafferty (1994) have criticized the use of observed values of occupancy to parameterize co-occurrence null models. They argue that if competition causes exclusion, then the empirical values of parasite occupancy are themselves already affected by competition. Consequently, their use could lead to detection of false random distributions, hiding real less-than-expected patterns of co-occurrence. This issue has the potential to influence our results since all algorithms constrained the null matrices to match the observed number of occurrences per species (fixed row algorithms). To address this concern, we re-analyzed our data following a solution advocated by Kuris and Lafferty (1994). Accordingly, occupancy values for model parameterization must be calculated for each species using only the fraction of the host sample that does not also contain a superior competitor. Although it is not possible to a priori define superior–inferior competitor hierarchies among our parasite species, we calculated values of occupancy for each bat fly species using only those hosts that did not simultaneously harbor another parasite species. We then used the highest prevalence value for each bat fly species to parameterize our algorithms, and the null models were re-run. These results showed no more indication of competitive exclusion than the original results, suggesting that our analyses are not biased. Thus, we present results only from the fixed row algorithms.

Pooling hosts from all localities into a single analysis may obscure effects of spatial heterogeneity among geographic localities, thereby confounding our results. This possibility was suggested also by Kuris and Lafferty (1994) in their study of larval trematode infracommunities. To account for the effects of pooling sites, we re-analyzed our data by locality and interpreted the overall trends by using a meta-analysis. The results and conclusions from these analyses (Supplementary material: Appendix 1) match closely those obtained from pooled data analyses, for which we develop below.

Bi-variate correlations of species densities
Competitive exclusion, and the resulting less-than-expected co-occurrence pattern, is a fundamental prediction made by competition theory. However, species may also respond to competition in other ways. For example, there can be changes in abundance of a species depending on presence or abundance of competitors, observed primarily as negative density covariation (Houlahan et al. 2007); this response is termed density compensation (Stevens and Willig 2000). The observation of density compensation in natural systems suggests that competition for a common limiting resource is determining abundances of species within the community. Thus, although co-occurrence patterns may themselves not

point to competition, patterns of abundance may still be indicative of competitive interactions.

Other factors, such as spatial heterogeneity of environmental variables, could also influence species abundances and create spurious correlations. Thus, when testing for density compensation we controlled for environmental heterogeneity by using partial Pearson correlations in which variation in locality elevation, host body weight and host sex (categorical dummy variable) was accounted for. Density correlations were performed for each species pair using only those hosts that harbored one, the other, or both species whose densities were being considered (i.e. double-zero records were eliminated from all pair-wise correlations). Since we were exclusively interested in possible negative correlations, we used one-tailed probability values. All possible pair-wise combinations of species yielded three different correlation analyses, and sequential Bonferroni corrections (Rice 1989) maintained alpha at 5%.

To obtain a community-wide estimation of the importance of density compensation in our study system, we used Fisher's method to combine the probabilities of all three independent correlations in a meta-analysis (Sokal and Rolf 1995). This method consists of summing the natural logarithms of the one-tail probabilities for each correlation, multiplying it by minus two, and comparing this value to a χ^2-distribution with 2k degrees of freedom (where k is the number of individual tests; Sokal and Rolf 1995).

Results

Four streblid bat fly species were recovered from *Carollia perspicillata*: *Trichobius joblingi* (Fig. 1A), *Strebla guajiro* (Fig. 1B), *Speiseria ambigua* (Fig. 1C) and *S. peytonae*. *S. peytonae* was rare, represented by only two individuals in the total sample (<0.5% of the total number of bat flies captured). Also, this streblid species is known to be primarily associated with a different, but closely related, host species: *C. brevicauda* (Wenzel 1976). Because of this extremely low occurrence and the possibility that these records represent sampling contamination (Dick 2007), *S. peytonae* was omitted from all analyses. *Trichobius joblingi* was the most common species (prevalence = 0.7, mean abundance = 3.11), followed by *Strebla guajiro* (prevalence = 0.35, mean abundance = 0.50) and *Speiseria ambigua* (prevalence = 0.27, mean abundance = 0.48).

All null model algorithms exhibited qualitatively similar results, indicating that host heterogeneity in body size and parasite species richness do not play important roles in determining co-occurrence patterns. Nevertheless, differences were observed between scenarios that excluded and included empty hosts. The analyses excluding non-infested hosts showed that the observed co-occurrence pattern could be expected by simple random assembly (Fig. 2). However, the two null models that included empty hosts showed a strong positive non-random pattern of species co-occurrence (Fig. 3), indicating that species co-occur more often than expected by chance (Supplementary material: Appendix 1).

Of the three pair-wise correlations between species densities, two showed no significant relationships (Fig. 4A–B). In contrast, *Speiseria ambigua* and *Strebla guajiro*
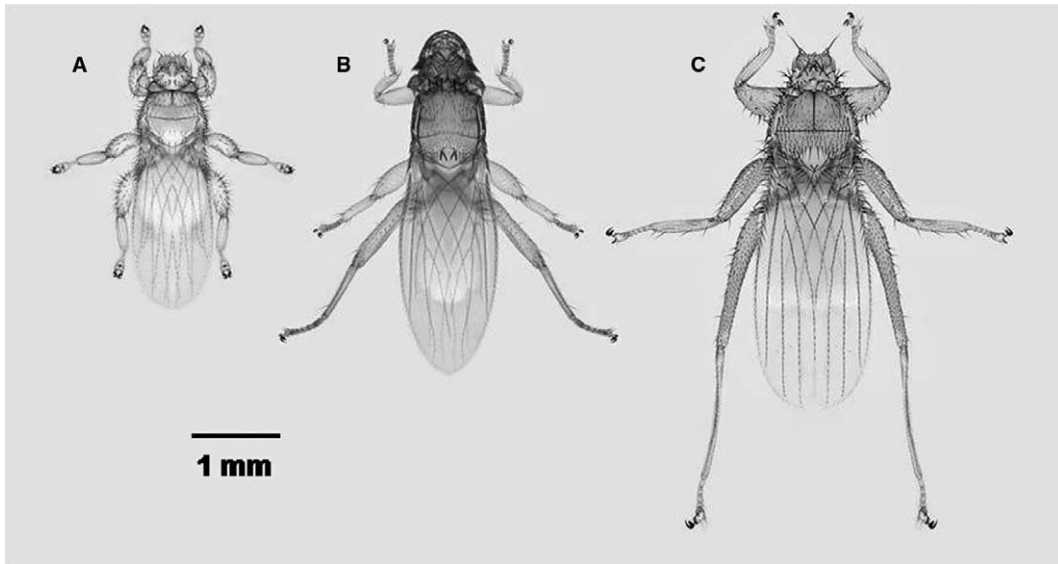
Fig. 1. Relative body size and morphology of the three bat fly species that commonly co-occur on *C. perspicillata*: (A) *Trichobius joblingi*, (B) *Strebla guajiro* and (C) *Speiseria ambigua*.

exhibited a significant negative correlation (Fig. 4C). However, the pattern of density variation in these two species does not simply fall around a negative tendency line, but indicates a constraint. This constraint is graphically observed as the triangular shape of the distribution of variation and the absence of points in the upper right corner of the bi-variate plot (Fig. 4C). Finally, after combining probabilities from all three correlations, the overall significance of negative covariations among bat fly species was not statistically significant ($\chi^2 = 10.15$, DF = 6, p = 0.118).

Discussion

The role of interspecific interactions in shaping patterns of distribution, assembly and abundance of species has been broadly investigated and remains a focus of ongoing debate. Much work has been conducted on free-living organisms, with less attention given to patterns of organization of parasite communities and the mechanistic bases for their structure. In the present study, we investigated the role of interspecific competition in structuring simple bat ectoparasite assemblages by testing two of its expected outcomes: competitive exclusion and density compensation.

Bat fly abundances and density compensation

Several studies that have investigated interspecific interactions through analysis of density correlation in parasites have reported both negative (Komeno and Linhares 1999, Alves and Luque 2001) and positive (Luque et al. 2003) relationships. However, lack of association also seems to be common (Dean and Ricklefs 1979, Haukisalmi and Henttonen 1993, Behnke et al. 2005). In our examination of density compensation, Fisher's test for combining probabilities indicated overall non-significant negative covariation, suggesting that competition may be relatively unimportant in determining community-wide abundance

patterns of these species. Nevertheless, one species pair (*Strebla guajiro* and *Speiseria ambigua*) exhibited evidence of density compensation, as expected by the effects of negative interspecific interactions. Species of the genera *Strebla* and *Speiseria* have been reported to share similar microhabitat preferences for furred body surfaces, while species in the genus *Trichobius* tend to be located more often on interfemoral and wing membranes (ter Hofstede et al. 2004). These microhabitat preferences could set the stage for the observed stronger interactions between *Strebla guajiro* and *Speiseria ambigua*.

Interestingly, visual inspection of the density covariation between these two species (Fig. 4C) reveals that points do not simply fall around a line of negative slope, but occur within an apparent upper constraint. It seems that when density of one species is low, density of the other species is free to vary from zero to high abundance; but, when density of one species is high, density of the other is constrained to be low. This observation suggests that there are some instances in which species densities become so high that one or more resources turn into limiting factors and competition becomes operational, setting a maximum to the number of individuals and species that can co-exist given the available resources. However, much variation in species abundances can occur below this constraint, and this variation may be attributable to other causes, such as dispersal limitation, disturbance or chance. In some situations, mechanisms that prevent infracommunities from becoming saturated may operate so frequently that species never become abundant enough as to reach carrying capacity and thus exhaust resources; in such cases, these constraints and competitive effects may never become apparent (Rohde 2005).

Parasite species distributions on host populations

Research on other groups of parasitic animals indicates that negative, random, or positive associations in the
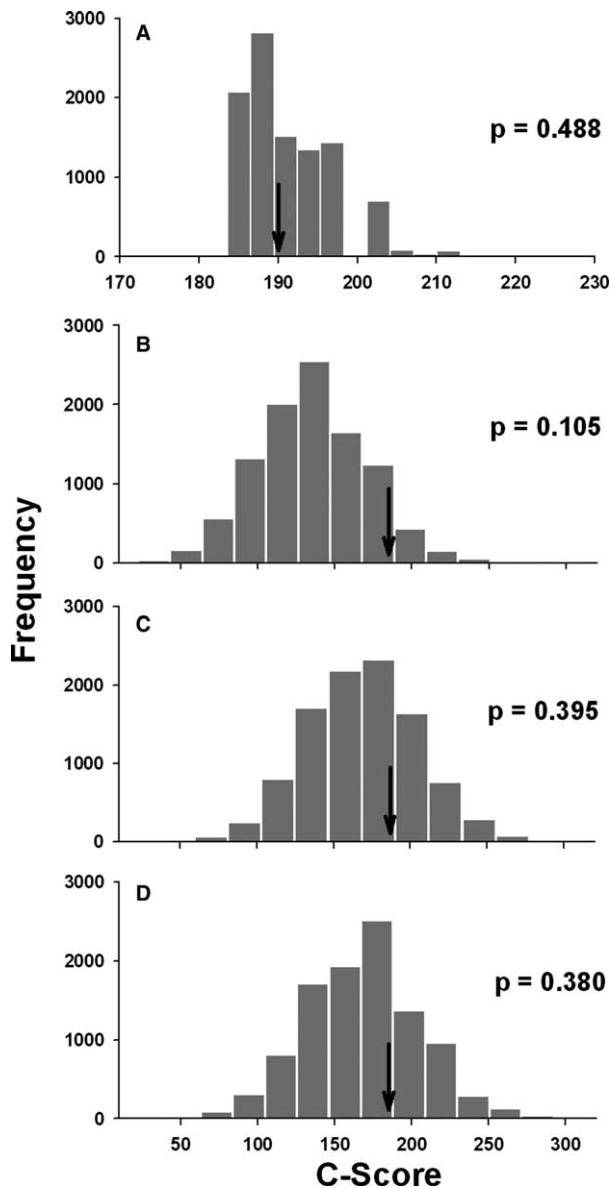
Fig. 2. Results of null model analyses based on the matrix excluding empty hosts. The figure shows the position of the empirical C-score (arrow) in a frequency distribution of values generated randomly based on four different algorithms: (A) fixed rows–fixed columns, (B) fixed rows–proportional columns, (C) fixed rows–equiprobable columns, and (D) fixed rows–column probabilities given by host body weight. Note that box (A) does not share the scaling in the abscissa with (B), (C) and (D).
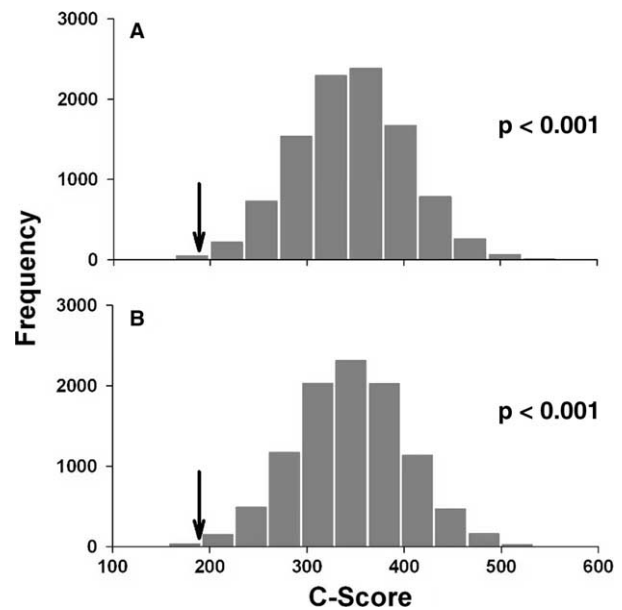

Fig. 3. Results of null model analyses based on the matrix including empty hosts. The figure shows the position of the empirical C-score (arrow) in a frequency distribution of values generated randomly based on two different algorithms: (A) fixed rows–equiprobable columns and (B) fixed rows–column probabilities given by host body weight.

distribution of coexisting species are all possible. Much evidence for competitive exclusion among parasites comes from studies on larval trematodes on snail hosts (Kuris and Lafferty 1994). However, a diverse set of study systems shows random occurrence or interspecific aggregation, thereby bringing into question the importance of competition in determining the assembly of parasites communities (e.g. helminths in voles, Haukisalmi and Henttonen 1993; Copepoda, Monogenea, Monopisthocotylea, Trematoda, Cestoda, Isopoda and Branchiura on marine fish, Gotelli and Rohde 2002; nematodes, cestodes and trematodes in antelopes, Fellis et al. 2003; nematodes, cestodes and

digeneans on mice, Behnke et al. 2005; fleas on rodents, Krasnov et al. 2006).

Our results support this line of evidence. When all hosts were incorporated into the analyses, the results indicate that bat fly species are aggregated: they occur together more often than expected. This pattern is the opposite of that predicted by competition; thus, it represents clear evidence against the idea that negative interspecific interactions drive the pattern of distribution of parasite species across host populations in our study system. However, such aggregation can be caused by forces (below) that do not necessarily involve positive species interactions. Consequently, there is still the possibility that competition is important at a smaller scale of analyses, namely when only the part of the distribution that represents reachable and suitable habitat is considered (parasitized hosts). In fact, it has been suggested that parasite aggregation could enhance conditions for interspecific competition on the parasitized part of the host population (Poulin 1998). Contrary to this idea, our results show that when empty hosts were excluded from the co-occurrence analyses, the emerging pattern was of random assembly. Such a result is inconsistent with the competition hypothesis, and strongly suggests that negative interspecific interactions are not the main force affecting the distribution of parasites across habitat patches (hosts) in our study system. It also suggests that other forces may limit the distribution of all species in a similar way, thereby causing interspecific aggregation within host populations; but, this mechanism(s) may not operate (or be strong enough) to also generate aggregation within the part of the host population that is parasitized.

When empty hosts were included in analyses, the fixed-equiprobable and the fixed-body weight algorithms did not provide different results. This suggests that host differences

in body mass do not explain the observed aggregation. This is further supported by a lack of correlation between host body weight and total parasite abundance, or parasite species richness in our system ($r = -0.044$, $p = 0.706$ and $r = -0.001$, $p = 0.992$ respectively; unpubl.). Similarly, within the part of the host population that is parasitized, the similarity among results from the null model algorithms suggests that the lack of structure is not associated with host-to-host heterogeneity in body mass or probability of colonization.

Mechanisms contributing to aggregation

The observed frequent co-occurrence when all hosts were considered can result from positive species interaction, host-to-host (patch) heterogeneity in rates of dispersal and extinction, variability in host suitability for bat fly colonization or survival, or other evolutionary or ecological processes shaping distribution of the parasite species in similar ways. First, positive indirect interaction among parasite species have been suggested in which the presence of a parasite species may reduce the effort of the host to avoid or eliminate individuals from other species. Facilitation of this nature would increase the chance for local coexistence of various parasite species (Krasnov et al. 2005). This mechanism can be considered analogous to predator-mediated positive interactions (e.g. due to predator switching) proposed to explain the coexistence of prey species in free-ranging organisms (Holt and Lawton 1994), where the presence of a prey species enhances existence of another by reducing the net predatory effect. Predator-mediated coexistence can be reached only if the effect of the predator is dependent on its own density, but its density is limited by other mechanisms besides prey exhaustion (Abrams and Matsuda 1996). In the case of parasite infracommunities, intensity of host anti-parasitic response (e.g. intensity of grooming behavior or immunological reaction, which is analogous to predator density) must reach a limit set primarily by constraints on the amount of energy or time that can be invested in parasite avoidance or removal (Giorgi et al. 2001, Krasnov et al. 2005). In this way, the anti-parasitic response would increase relative to the number of parasites, until the threshold set by energetic constrains is reached. Individuals or species numbers beyond this point would be facilitated by a dilution of the anti-parasitic host effects.

Another possible explanation for interspecific aggregation may relate to environmental and/or host characteristics needed by parasites to survive. This explanation requires that all parasite species have similar habitat preferences. This resemblance could be the case if there is a high degree of conservatism in the evolution of niche characteristics among species (Weins and Graham 2005). However, the three bat fly species belong to different genera, which present substantial morphological differentiation (Fig. 1). In turn, this phenotypic dissimilarity is a sign of niche evolution, divergence in niche characteristics and possibly also a lack of correspondence in host preference among bat fly species. On the other hand, this apparent morphological segregation could also suggest within-host microhabitat specialization. Microhabitat partitioning has been found in many other parasite systems (especially among Platyhel-minthes; Bush and Holmes 1986, Cilso and Caira 1993), and could be an important way in which parasite species share resources (Combes 2001), allowing their coexistence, or even allowing their aggregation (but see Rohde 1994 for an interpretation of parasite niche specialization unrelated to competition).

The observed aggregation could also be related to dispersal limitation. The role and importance of dispersal in patterns of community assembly and species abundance has been studied and is now well supported (Leibold et al. 2004). Since movement of individuals is strongly dependent on the spatial relationships among sites, dispersal could be especially important for parasites because their habitat patches (hosts) are often not spatially static, but move and change in position relative to each other. Thus, depending on the host's spatial interactions (e.g. movement and grouping associated with social behavior), there can be heterogeneous rates of dispersal among them. Consequently, parasites may aggregate on those hosts with the highest probabilities of transmission. This is supported by the observation that hosts with higher chances of physical encounter also have elevated rates of infestation (Arneberg et al. 1998, Patterson et al. 2007). Particularly, in *Carollia perspicillata*, sex-biased rates of contact among hosts can help explain the observed aggregation. The host species has a polygynous mating system, where females form harems controlled by one dominant male; single males can form bachelor groups, but these are usually smaller and less stable (Cloutier and Thomas 1992). Consequently, female hosts would tend to accumulate more parasites. This possible explanation is supported by evidence suggesting that female *C. perspicillata* have higher loads of parasitism by bat flies (Fritz 1983, Tello and Jarrín unpubl.), which seems not to be the result of a sex biased choice by the parasites (Dick and Dick 2006).

Finally, disturbance is also an important force shaping community structure (Sousa 1984). Parasite infracommunities are likely subjected to near-continuous episodes of disturbance, because in most cases their habitat patches (hosts) are actively trying to get rid of them. Common manifestations of disturbance in parasite infracommunities can be host grooming behavior and host immunological responses, which remove individuals from the system. Local extinction caused by the host could also exacerbate the effects of dispersal limitation; if species are removed from a site, and there is a long lapse of time before dispersal restores the species to that site, then species will tend to aggregate on the sites where local extinction due to disturbance is low or where re-colonization by dispersal is high. In vertebrates, heterogeneous rates of grooming and immunocompetence can result from social behavior, sex, body size, age, etc. (Hart et al. 1992, Mooring et al. 1996, Roulin et al. 2003).

Concluding comments

Overall, we find no evidence of competition in patterns of distribution and mixed effects on the patterns of covariation in density among bat fly species. These results support the idea that, although there might be a signature in co-occurrence patterns of free-ranging species that is consistent
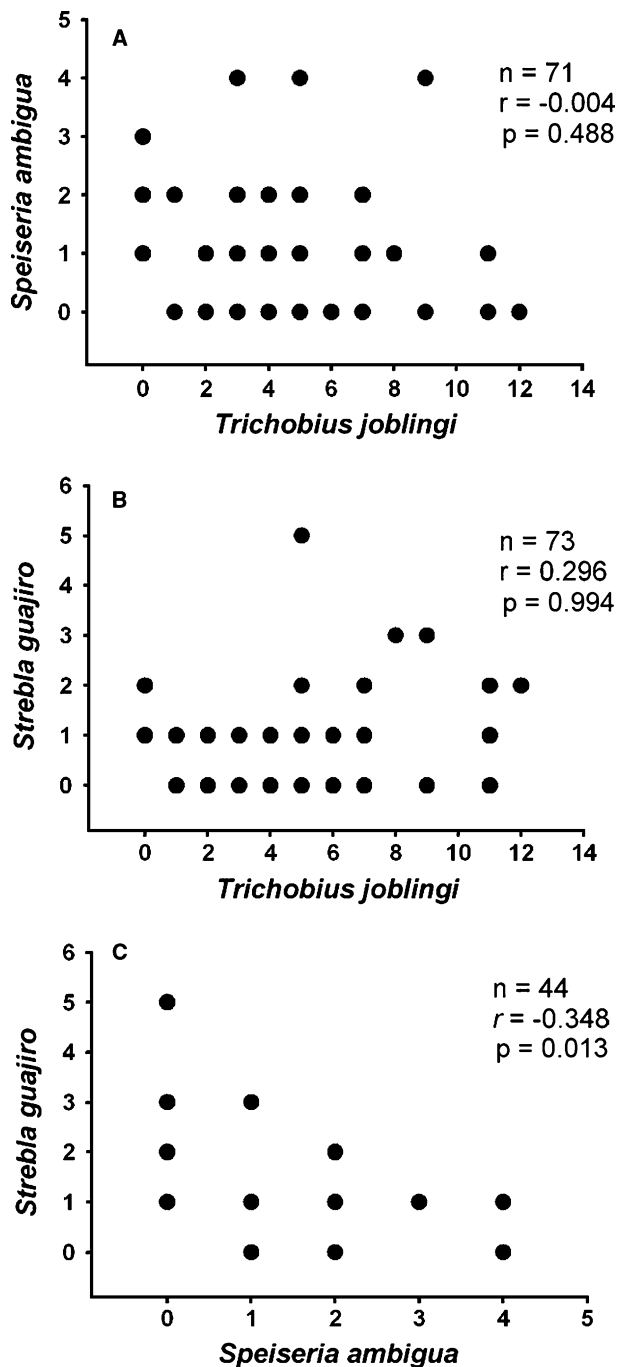
Fig. 4. Scatterplots of density for each of the three species pairs and partial correlation results. Sampling size for density correlations are different since for each pair-wise correlation only hosts with at least one of the two bat fly species being correlated were included. See text for further explanation of the quantitative analyses.

analyses suggest that competition is present but not prevalent, being restricted to one pair of species. Moreover, the effects of competition might be important when abundances of these species are high; but when a competitor is rare, the other species does not necessarily respond by increasing in abundance. It is possible that in such low abundance situations other factors become more important determinants of species abundance. This is also consistent with research on parasitic and free-ranging organisms that shows that competition interacts with others mechanisms and is context dependent, being important in some situations but unimportant in others (Combes 2001, Houlahan et al. 2007, Kaplan and Denno 2007).

Recent research on parasite community ecology has provided important insights into how parasite assemblages are structured and how they might differ from more traditional model organisms, but clear general trends have not emerged. These studies have also indicated that it is necessary to investigate how different hypothesized mechanisms shape communities in a wider array of systems in order to evaluate their effects across taxonomic and environmental gradients. For example, some evidence suggests that the strength with which competition might shape patterns of co-occurrence and abundance could vary depending on characteristics of the taxon being analyzed (e.g. poikilotherms vs homeotherms, Gotelli and MacCabe 2002, or core vs satellite species, Ulrich and Zalewski 2006). These differences in species and environmental characteristics might create communities that form a continuum ranging from random to deterministically structured (Rohde 2005, Fig. 11.1).

It is also important to understand and evaluate the multiplicity of effects that competition can have on natural systems. In this study, we have addressed the effects of negative species interactions based on limiting resources on the co-occurrence and covariation in abundance of species, but other predictions from competition theory should also be tested. Competition can also be related to microhabitat specialization (Reed et al. 2000, Friggens and Brown 2005) or patterns of morphology (e.g. morphological overdispersion, Stevens and Willig 1999, or character displacement, Dayan and Simberloff 2005). Habitat specialization has been suggested among co-existing bat flies (ter Hofstede et al. 2004) and phenotypic differences are also readily apparent (Fig. 1). These potential differences in niche utilization could be the reason for the lack of a stronger competition signal in the distribution and abundance of bat fly species in our study system. Evaluation of how our results match those of other bat fly species on different hosts, locations and scales, and how this possible microhabitat partitioning and morphological dissimilarities among co-occurring bat flies fit predictions made by the interspecific competition hypothesis or other possible mechanisms of community assembly remains to be investigated.

with competition theory (Gotelli and MacCabe 2002, but see Ulrich 2004), it is often the case among parasites that limiting resources are not an important force shaping the assembly of their communities (Rohde 1991, 2005, Morand et al. 2002, Fellis et al. 2003, Krasnov et al. 2005). On the other hand, our density compensation

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Appendix 1. Null model analysis of species co-occurrence by geographic locality

Pooling localities in our null model analyses could have biased our results by eliminating possible effects of spatial heterogeneity in the observed levels of co-occurrence. This has the potential to hide patterns of aggregation or segregation, which might be found if the analyses were run at the geographic locality level. To assess this possibility, we ran our null model analyses independently for each locality, and interpreted the results using a meta-analysis.

Methods

For each locality, we ran the same null model analyses as we did for the pooled data: we used four different null model algorithms and analyses were carried out in two scenarios: including or excluding non-infested hosts. However, we could not run all possible null models in all localities. One of the localities incorporated in the pooled analyses was excluded from the single locality analyses because it had only three hosts; in all other localities at least nine hosts were captured, and consequently had enough individuals to run the null models. In two localities 100% of the bats were parasitized, consequently null models including empty host could not be run. Also, the number of individuals was so small in two localities that the fixed-fixed algorithm could not randomize the observed matrix (this is caused because this algorithm sets many constraints on how the observations must be reshuffled). In the end, we were able to run a total of 29 analyses by combinations of the scenario in which the algorithms were carried out (excluding or including empty hosts), the null model algorithm (fixed-equiprobable, fixed-fixed, fixed-proportional and fixed- by host body weight), and the locality (six localities).

From each of the previously described analyses, we were able to calculate one-tailed probabilities for aggregation ($p[C_{obs} \leq C_{exp}]$) and for segregation ($p[C_{obs} \geq C_{exp}]$), as well as a measure of standardized effect size

$$SES=(C_{obs}-C_{exp})/S_{sim}$$

as used by Gotelli and Rhode 2002, where C_{obs} is the C-score calculated from the empirical matrix, C_{exp} is the mean C-score from the simulated matrices and S_{sim} is the standard deviation of the null distribution.

Exploring the effect of number of individuals on null model analysis results

All null models used a total of 10 000 randomizations. However, the number of individuals that are included in each randomization process could influence the results produced by the null models and the power of the tests. To understand these effects, we conducted linear regressions of the number of individuals included in the randomization (n) on: 1) the standard deviation of the randomized distribution, 2) the standardized effect size, 3) and 4) both one tail probability values. To control for non-independence of the observations, we modified the number of degrees of freedom in the denominator for the F-tests to be equal to the number of localities; this implied a reduction from 27 to 6 degrees of freedom. It is difficult to determine exact degrees of freedom for the 29 points used in the regressions; however, we think that this reduction is sufficient for the purpose of controlling for most non-independence.

Testing standardized effect sizes against the zero null hypothesis

To test whether the observed levels of co-occurrence differed significantly from random assembly, we compared the standardized effect size against the null hypothesis of no difference from zero. We did this by using a one-sample t-test for each combination of algorithm and scenario, and also for each algorithm irrespective of the scenario, and for each scenario irrespective of the algorithm used.

Testing for differences among algorithms and scenarios

To test if there are any trends or differences among the algorithms or between the scenarios in which the null models were ran, we conducted three independent General linear models using as dependent variables: 1) the standardized effect size, 2) the probability of aggregation, and 3) the probability of segregation. In these analyses, algorithms and scenarios were included as independent variables, while locality was included as a random factor.

Results

In Fig. A1, we observe that the number of individuals had a significant impact on the null model outcomes. A greater number of individuals increases the possibility of more configurations for the randomized matrices, and consequently increases the variability in the null distribution (Fig. A1a). Similarly, the number of individuals has a significant association with the SES and the probability values (Fig. A1b–d). Increases in the number of individuals also increase the power of the null model analysis (Fig. A1c–d), by increasing the SES (Fig. A1b).

Table A1 shows the results from the null model analyses run independently per locality, algorithm and scenario. Most single null model analyses did not show a pattern that could be distin-

guished from random assembly. As suggested by the previous correlation analyses, this could result from a lack of statistical power. However, if we look at the locality that had the largest number of individuals (locality 3), we can see that all null model algorithms that excluded empty hosts were clearly not statistically significant, while the null models that included empty hosts show evidence of interspecific aggregation, being the only statistically significant results at an alpha of 0.05. The results at this locality, where presumably we have enough statistical power, showed perfect concordance with the analyses that pooled all individuals together. On the other hand, at locality 5, three of the four null models that exclude empty hosts show marginally significant support for the idea of segregation.

Figure A2 and Table A2 show the results from the one-sample t-test of the standardized effect sizes with respect to the null expectation of zero. Only one of the groups seems to be different from zero: in the case of the null models that included empty hosts (irrespectively of the null model algorithm), the one-sample t-test showed marginally significant results.

Finally, the results of the General linear models can be found in Table A3 and Fig. A2 and A3. In general, the analyses that include and exclude empty hosts seem to provide significantly different results, suggesting that when empty hosts are included, the levels of co-occurrence are suggestive of patterns of aggregation, and when these hosts are not used in the analyses, the patterns seem to be mostly random. This is supported by the statistical analyses which show that the categories of scenario are significantly different for all three dependent variables. There also seems to be significant variation in the results among the null model algorithms and localities, indicated by the significance of the algorithm term and the interaction terms with locality. However, some of the observed interactions with locality can result from differences in the statistical power across sites which will show significant results in some, while no statistical support in others.

Conclusions

Overall, these results show support for our pooled-sites null model analyses. When the null models are run separately for each locality, there is little evidence of segregation patterns hidden in the analyses in which we pooled together hosts across localities. Moreover, when we conduct meta-analyses on the single locality results, they point to the same conclusions obtained from the pooled data in that there is a clear difference between the results of the null models when empty hosts are included versus when empty hosts are excluded. It seems that when we consider the entire host population, these parasites are markedly aggregated; conversely, the parasites seem to be distributed randomly in the part of the population that is infested. This suggests that competition is not a strong force shaping the distribution and infracommunity assembly of these parasite species.

Table A1. Results from the single-locality null model analyses. For each combination of Locality, Scenario and Algorithm, for which we ran null model analyses of co-occurrence, we report the total number of individuals used in the randomization (n), the observed C-score (C_{obs}), the expected C-score (C_{exp}), which is the mean of the C-scores of the null matrices), the standard deviation of the null distribution (S_{sim}), the one-tailed probability for aggregation (p[$C_{obs} \leq C_{exp}$]), the one-tailed probability for segregation (p[$C_{obs} \geq C_{exp}$]), and a measure of standardized size effect, as defined by Gotelli and Rhode 2002 (SES). The p-values that were statistically significant (p ≤ 0.05) and marginally significant (0.05 ≤ p ≤ 0.10) are highlighted in bold letters.

Locality	Scenario	Algorithm	n	C_{obs}	C_{exp}	S_{sim}	p($C_{obs} \leq C_{exp}$)	p($C_{obs} \geq C_{exp}$)	SES ([$C_{obs} - C_{exp}$]/S_{sim})
1	Excluding empty hosts	Fixed-ByHostWeight	5	1.00	0.40	0.49	1.000	0.401	1.221
1	Excluding empty hosts	Fixed-Equiprobable	5	1.00	0.39	0.49	1.000	0.392	1.244
1	Excluding empty hosts	Fixed-Proportional	5	1.00	0.39	0.49	1.000	0.387	1.257
1	Including empty hosts	Fixed-ByHostWeight	9	1.00	3.10	1.56	0.172	0.959	-1.343
1	Including empty hosts	Fixed-Equiprobable	9	1.00	3.07	1.52	0.168	0.960	-1.365
2	Excluding empty hosts	Fixed-ByHostWeight	16	8.33	10.03	3.17	0.445	0.641	-0.537
2	Excluding empty hosts	Fixed-Equiprobable	16	8.33	10.38	3.94	0.402	0.679	-0.521
2	Excluding empty hosts	Fixed-Fixed	16	8.33	9.07	0.82	0.410	1.000	-0.902
2	Excluding empty hosts	Fixed-Proportional	16	8.33	8.13	3.88	0.645	0.446	0.053
3	Excluding empty hosts	Fixed-ByHostWeight	25	12.67	12.99	5.31	0.514	0.528	-0.061
3	Excluding empty hosts	Fixed-Equiprobable	25	12.67	13.11	5.29	0.505	0.539	-0.084
3	Excluding empty hosts	Fixed-Fixed	25	12.67	13.02	1.20	0.831	0.557	-0.298
3	Excluding empty hosts	Fixed-Proportional	25	12.67	11.13	5.06	0.657	0.389	0.304
3	Including empty hosts	Fixed-ByHostWeight	33	12.67	37.16	11.27	**0.014**	0.990	-2.173
3	Including empty hosts	Fixed-Equiprobable	33	12.67	37.51	11.41	**0.013**	0.991	-2.177
4	Excluding empty hosts	Fixed-ByHostWeight	9	1.67	2.99	1.66	0.203	0.802	-0.798
4	Excluding empty hosts	Fixed-Equiprobable	9	1.67	3.10	1.67	0.187	0.819	-0.858
4	Excluding empty hosts	Fixed-Proportional	9	1.67	2.35	1.62	0.341	0.669	-0.423
5	Excluding empty hosts	Fixed-ByHostWeight	7	5.33	2.98	1.40	0.973	**0.078**	1.680
5	Excluding empty hosts	Fixed-Equiprobable	7	5.33	3.05	1.38	0.972	**0.085**	1.646
5	Excluding empty hosts	Fixed-Fixed	7	5.33	5.35	0.39	0.756	0.630	-0.052
5	Excluding empty hosts	Fixed-Proportional	7	5.33	2.81	1.39	0.980	**0.063**	1.822
5	Including empty hosts	Fixed-ByHostWeight	8	5.33	3.54	1.52	0.919	0.177	1.178
5	Including empty hosts	Fixed-Equiprobable	8	5.33	3.70	1.51	0.908	0.203	1.076
6	Excluding empty hosts	Fixed-ByHostWeight	12	2.00	2.13	1.78	0.768	0.730	-0.073
6	Excluding empty hosts	Fixed-Equiprobable	12	2.00	2.17	1.81	0.762	0.736	-0.093
6	Excluding empty hosts	Fixed-Proportional	12	2.00	1.90	1.75	0.813	0.678	0.055
6	Including empty hosts	Fixed-ByHostWeight	14	2.00	6.40	3.17	0.134	0.919	-1.388
6	Including empty hosts	Fixed-Equiprobable	14	2.00	6.47	3.12	0.127	0.922	-1.431

Table A2. Results from one-sample t-tests to compare the values of standardized effect size to the null hypothesis of zero. See also Fig. A2. The p-values that were statistically significant (p ≤ 0.05) and marginally significant (0.05 ≤ p ≤ 0.10) are highlighted in bold letters.

Scenario	Algorithm	t_{stat}	DF	p (two-tailed)	Mean difference
Excluding empty hosts	Fixed-Equiprobable	0.545	5	0.609	0.222
Excluding empty hosts	Fixed-Fixed	−1.652	2	0.240	−0.418
Excluding empty hosts	Fixed-Proportional	1.474	5	0.200	0.511
Excluding empty hosts	Fixed-ByHostWeight	0.591	5	0.580	0.239
Including empty hosts	Fixed-Equiprobable	−1.376	3	0.262	−0.974
Including empty hosts	Fixed-ByHostWeight	−1.278	3	0.291	−0.931
All	Fixed-Equiprobable	−0.640	9	0.538	−0.256
All	Fixed-Fixed	−1.652	2	0.240	−0.418
All	Fixed-Proportional	1.474	5	0.200	0.511
All	Fixed-ByHostWeight	−0.570	9	0.583	−0.229
Excluding empty hosts	All	1.132	20	0.271	0.218
Including empty hosts	All	−2.026	7	**0.082**	−0.953

Table A3. Results from the GLM analyses for the standardized effect size (SES), the probability of aggregation (p[$C_{obs} \leq C_{exp}$]) and the probability of segregation (p[$C_{obs} \geq C_{exp}$]). See also Figs. A2 and A3. The p-values that were statistically significant (p ≤ 0.05) and marginally significant (0.05 < p ≤ 0.10) are highlighted in bold letters.

	SES ([C_{obs}–C_{exp}]/S_{sim})			p($C_{obs} \leq C_{exp}$)			p($C_{obs} \geq C_{exp}$)		
	F	DF	p	F	DF	p	F	DF	p
Scenario	13.268	1	**0.036**	9.497	1	**0.054**	9.334	1	**0.055**
Algorithm	8.659	3	**0.002**	1.235	3	0.340	11.076	3	**0.001**
Locality	3.365	5	0.151	2.261	5	0.246	4.021	5	0.102
Scenario-Algorithm interaction	2.404	1	0.219	0.358	1	0.592	0.440	1	0.555
Scenario- Locality interaction	2300.598	3	**<0.001**	8308.128	3	**<0.001**	1054.024	3	**<0.001**
Algorithm- Locality interaction	242.049	12	**<0.001**	804.206	12	**<0.001**	218.883	12	**<0.001**

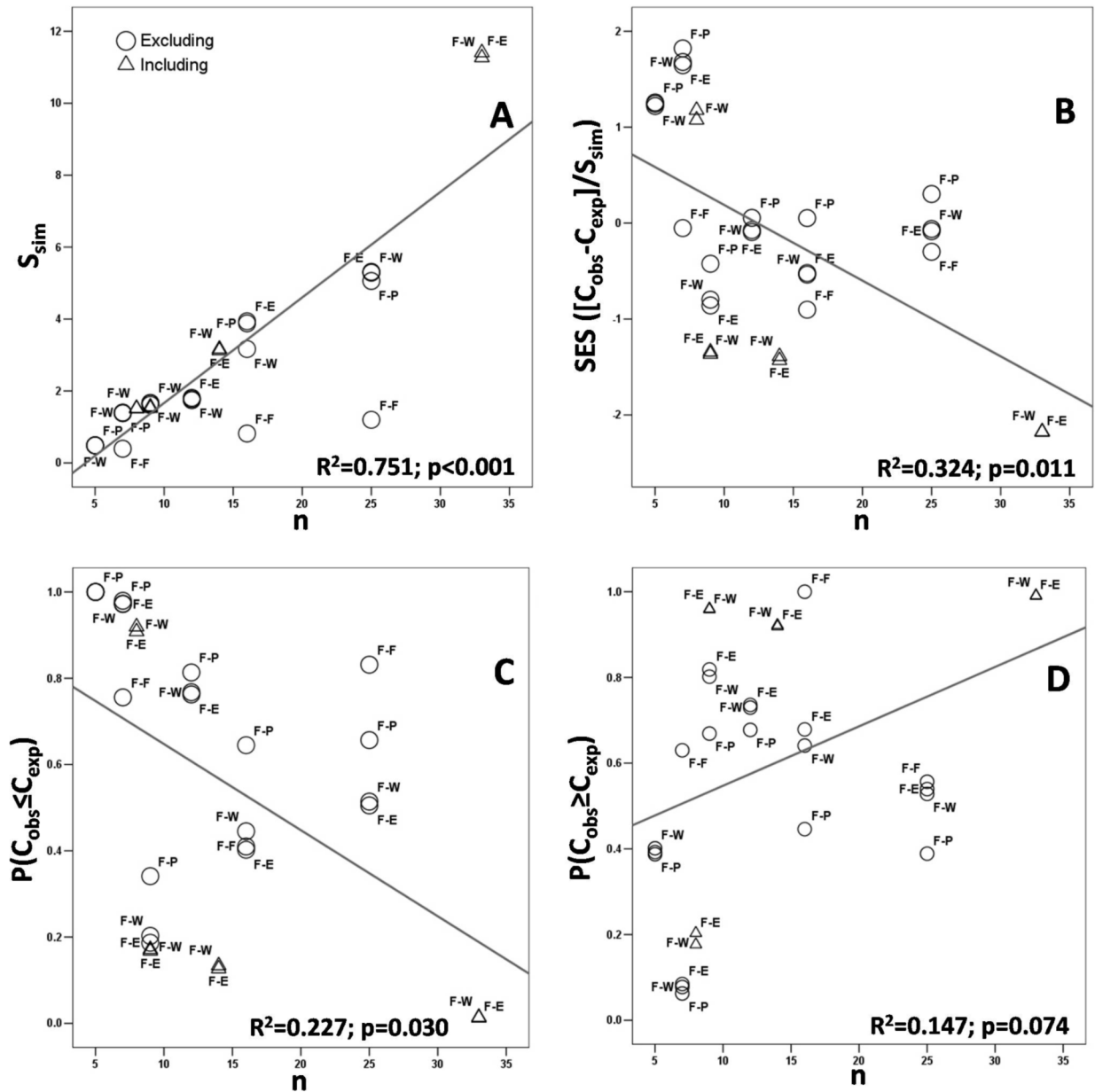Fig. A1. Relationships among the number of individuals per locality (n) and (A) the variation in the null distribution of C-scores (S_{sim}), (B) the standardized effect size (SES), (C) the probability of aggregation ($p[C_{obs} \leq C_{exp}]$), and (D) the probability of segregation ($p[C_{obs} \geq C_{exp}]$). For each relationship the p-value has been adjusted for non-independence among points (see text for details). Algorithms: F-E: fixed-equiprobable, F-F: fixed-fixed, F-P: fixed-proportional, and F-W: fixed-probability by host weight.
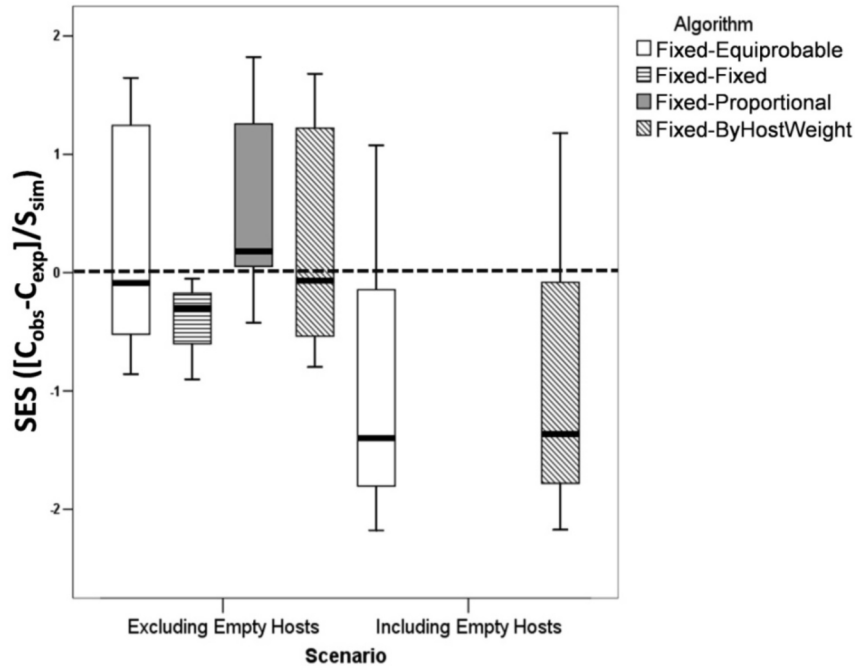
Fig. A2. Comparison of the standardized effect size values per Scenario and Algorithm categories. The dashed line across the figure represents the value expected under the null hypothesis of random assembly. For each case: the box represent the 25 to 75 percentiles of the distribution, the bold horizontal line in the box represent the median, and the top and bottom lines represent the maximum and minimum values respectively.
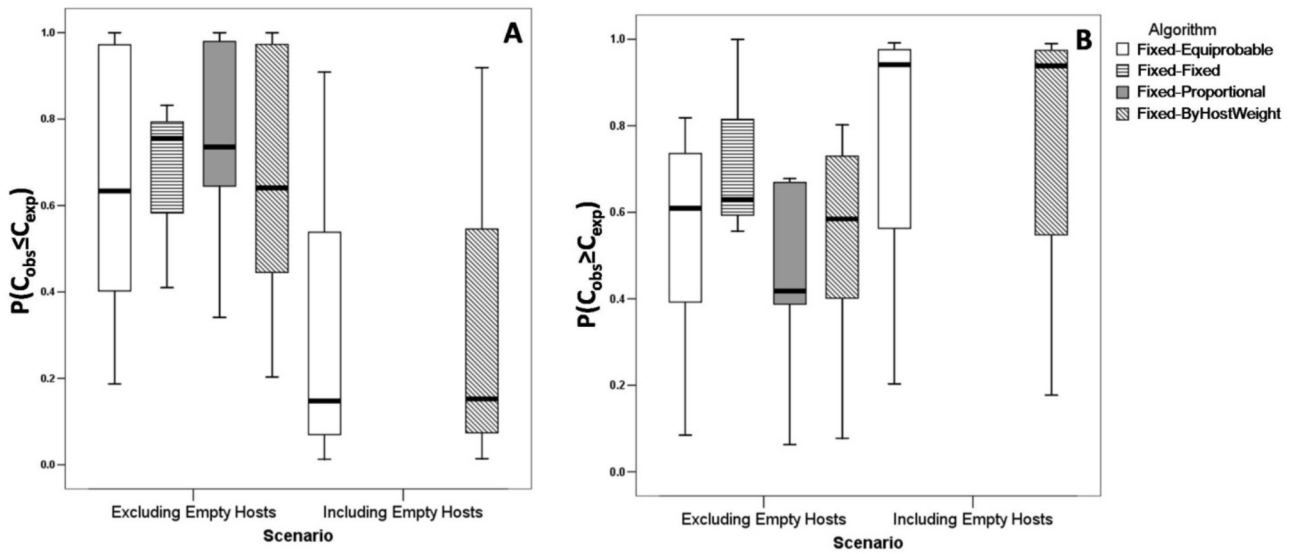

Fig. A3. Comparison of: (A) probability of aggregation, and (B) probability of segregation among the categories of Scenario and Algorithm. For each case: the box represent the 25 to 75 percentiles of the distribution, the bold horizontal line in the box represent the median, and the top and bottom lines represent the maximum and minimum values respectively.