Oecologia (1998) 116:565–574

Voitto Haukisalmi · Heikki Henttonen

Analysing interspecific associations in parasites: alternative methods and effects of sampling heterogeneity

Received: 20 October 1997 / Accepted: 4 May 1998

Abstract The purpose of the present study was (1) to test the ability of six alternative methods to detect random and non-random patterns of overall association in artificial presence/absence data sets, and (2) to analyse overall associations and effects of sampling heterogeneity in four empirical presence/absence data sets of helminths of the common shrew *Sorex araneus*. In the null model, the expected distribution was created by means of a randomisation procedure. Application of methods on artificial data sets indicated a generally low probability of type I statistical error. All methods were more likely to detect positive non-randomness than negative non-randomness of comparable strength, which may partly explain the predominance of positive overall associations in empirical data sets. The analyses based on artificial data sets indicated slight differences between methods in their ability to detect non-randomness of known strength (type II error). However, some of the methods failed to detect strong overall association when the artificial assemblages consisted of roughly equal numbers of positive and negative pairwise interactions. The structure of the artificial data sets always disappeared when the expected distribution was constrained to account for "sampling heterogeneity", i.e. varying prevalence of species among subsamples. The patterns of overall association in real helminth communities were variable, depending on the locality and association method used, but not usually on the simulation constraint used. Of the four empirical data sets analysed, one showed an unequivocal positive structure, in one the structure depended on the method used, and two data sets from the same locality were unequivocally unstructured (random). We discuss the applicability of various association measures, and the possible causes of positive overall associations in parasites.

Key words Helminths · Shrews · *Sorex* · Null models · Interspecific associations

V. Haukisalmi (✉)
Department of Ecology and Systematics,
Division of Population Biology, PO Box 17,
FIN-00014 University of Helsinki, Finland
e-mail: voitto.haukisalmi@helsinki.fi, Fax: +358-9-1917492

H. Henttonen
Vantaa Research Center, Finnish Forest Research Institute,
PO Box 18, FIN-01301 Vantaa, Finland

Introduction

Searching for the structure of communities is one of the key problems in ecology. An important and frequently studied aspect of community structure is the presence of non-random overall (multispecies) patterns of co-occurrence and covariance (Gotelli and Graves 1996). Because the analyses of community structure are based on observational data only, the processes responsible for the observed patterns may not be revealed (but see Lafferty et al. 1994). However, in the majority of multispecies assemblages, the recognition of pattern is practically the only available approach for elucidating the interspecific processes, and an observed non-random structure forms an obvious basis for subsequent process-oriented studies.

Despite the clear-cut problem, the diversity of methods and the lack of consensus concerning the appropriateness of alternative null models have been serious obstacles for studies of community structure. The proposed null models differ in several respects, e.g. the metric used to measure overall association, and the method of constructing the expected distribution, including the various constraints (cf. Gotelli and Graves 1996 pp. 182–185).

Association measures are usually based on information about the co-occurrence of specific pairs of species; the log-linear method of Gilpin and Diamond (1982) and the checkerboard score of Stone and Roberts (1990) are examples of such methods. There are two basically different ways of presenting the pattern of overall association: the various pairwise metrics may be condensed

into a single index (e.g. Stone and Roberts 1990), usually by averaging or summing, or presented as a frequency distribution (e.g. Gilpin and Diamond 1982). The single index or the frequency distribution of pairwise indices is then compared with an expected distribution. The use of a single index has been recommended on the grounds that when using the distribution of pairwise indices, the frequency classes are not independent of one another (Gotelli and Graves 1996). However, the methods based on a single index also have an obvious disadvantage: because positive and negative pairwise interactions cancel each other out, assemblages that include unexpectedly high proportions of both positive and negative associations may not be detected as non-random by these methods (Schluter 1984).

The null, or expected, distribution for a particular association measure may be obtained by analytical or randomisation methods. Although the analytical methods are easily applied even on highly diverse assemblages, the validity of the null distribution used is sometimes questionable. For example, the normal distribution is clearly an inappropriate null expectation for Gilpin and Diamond's (1982) method, because it yields significant results for random data (Wilson 1987), and biases the pattern towards negative structure (Lafferty et al. 1994). Furthermore, Lotz and Font (1994) have shown that the sample size and the proportion of common and rare species may affect the outcome of an association analysis. These problems may be avoided by using randomised matrices that match closely the observed matrix; such matching is achieved by constraints imposed on the random samples. The occurrence frequency of various species, the number of species on various islands and incidence effects (species occurrence frequency on different-sized islands) are the constraints used in real island systems (Harvey et al. 1983). However, randomised samples may be constrained to account for practically any kind of confounding structure in the observed data, including sampling heterogeneity (see below).

Because individual hosts are well-defined, fairly homogeneous and easily replicated spatial units, parasite assemblages are ideally suited for analysis of interspecific association and other aspects of community structure. However, parasite populations are often highly variable both spatially and temporally (e.g. Haukisalmi et al. 1988; Montgomery and Montgomery 1989). If the aim of an association analysis is to determine the existence of direct interactions between parasites, such as interspecific competition or mutualistic interactions, various sampling heterogeneities have to be controlled for. Spatial, temporal and other host-related factors (e.g. sex, reproductive status) usually exert a strong influence on infection levels of parasites (Montgomery and Montgomery 1989; Haukisalmi et al. 1995), and any direct interaction may be masked or biased by these confounding effects.

This study covers two main problems. First, we apply six association methods on artificial assemblages to determine their ability to detect non-randomness of known strength and structure, and to test whether the apparent non-randomness due to the heterogeneity of subsamples can be controlled by a modified randomisation approach. We concentrate on methods previously used for parasites; however, all of these methods are very general and may be applied equally well on any patchily distributed assemblage. Second, we describe the patterns of spatial and temporal occurrence and overall interspecific association in helminths of the common shrew *Sorex araneus*. The common shrew is a ubiquitous and abundant insectivorous mammal which has a fairly rich helminth fauna dominated by cestodes and nematodes (Haukisalmi 1989; Haukisalmi and Henttonen 1994).

Materials and methods

Association measures

Method 1: species density distribution

The frequency distribution of the number of species per host (Fig. 1a,b), called here the species density distribution (Janovy et al.

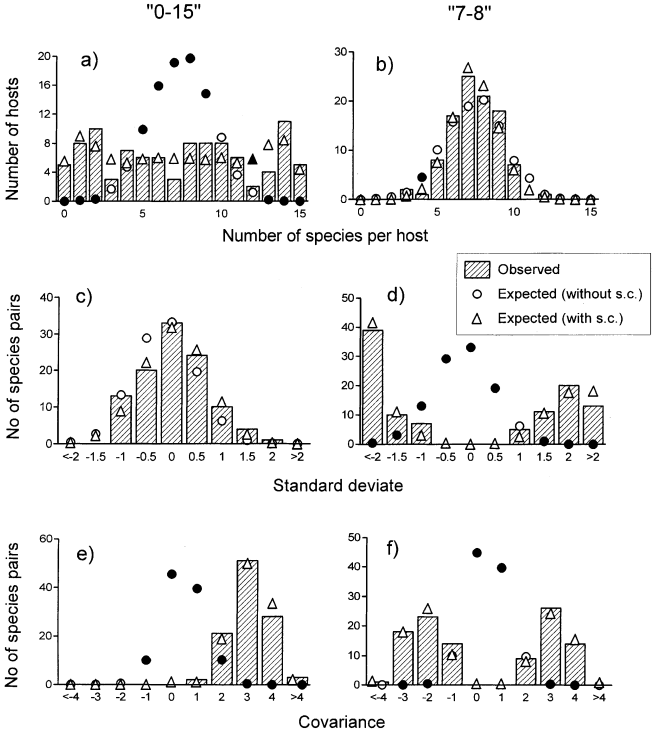

Fig. 1 Examples of overall associations for two artificial, non-random matrices by three association methods: species density distribution (**a, b**), log-linear method (**c, d**) and covariance distribution (**e, f**). In the matrix "0–15", all 15 species show parallel subsample variation, creating a positive overall association. In the matrix "7–8", there are two sets of species with parallel subsample variation within sets, but reversed variation between sets; this results in roughly equal numbers of positive and negative pairwise interactions (cf. Table 1). The expected distributions were simulated with and without the "subsample constraint" (*s.c.*). The expected values indicated by *closed symbols* differ significantly from the observed values

1995), has often been used to establish the structure of parasite communities (e.g. Goater et al. 1987; Dobson 1990; Pence 1990; Forbes et al. 1994). The observed distribution has conventionally been tested against the Poisson distribution which, however, is an improper null expectation (Lotz and Font 1991; Janovy et al. 1995). More appropriate null distributions are obtained by alternative analytical methods (Janovy et al. 1995; Poulin 1996) or by randomisation (Lotz and Font 1991). The species density distribution differs from the other methods used here by utilising data on species numbers per host, instead of data on specific pairs of species.

Method 2: log-linear method

In Gilpin and Diamond's (1982) log-linear method (Fig. 1c,d), the probability of species i occurring on island j (P_{ij}) is estimated as $P_{ij} = R_i C_j / T$, where R_i and C_j are the marginal totals of the species-island matrix, and $T = \sum R_i = \sum C_j$. The expected number of islands shared by species i and k (E_{ik}) is then obtained as $E_{ik} = \sum P_{ij} \sum P_{kj}$. The next step is to calculate the standard deviates (d_{ik}) for the differences between the observed (O_{ik}) and expected co-occurrence frequencies for all species pairs: $d_{ik} = (O_{ik} - E_{ik})/SD_{ik}$, where $SD_{ik} = \sqrt{\Sigma\, P_{ij}\, P_{kj}(1 - P_{ij}P_{kj})}$. Finally, the frequency distribution of standard deviates is compared with a normal distribution with a mean of 0 and standard deviation of 1; significant deviation from the normal distribution indicates non-random community structure.

Because of the inappropriateness of the original null model (Wilson 1987), we use a null distribution created by a randomisation procedure, instead of applying the standard normal distribution. P_{ij}s exceeding unity, a characteristic problem of this method, were handled by truncating them at 1. Lafferty et al. (1994) have used the log-linear method, applying the standard normal distribution, to analyse the structure of trematode assemblages in snails.

Method 3: checkerboard score

The checkerboard score of Stone and Roberts (1990) is the mean number of "checkerboard units" formed by all pairs of species in an assemblage. A basic checkerboard unit consists of two species showing an exclusive distribution on a pair of islands. In practice, the number of checkerboard units formed by species i and k is calculated as $C_{ik} = (r_i - O_{ik})(r_k - O_{ik})$, where O_{ik} is the number of co-occurrences and r_i and r_k are the numbers of occurrences (row sums) for the two species. The checkerboard score is then obtained as $C = \Sigma\Sigma C_{ik}/N$, where N is the total number of species pairs. This method has not been previously applied on parasite assemblages.

Method 4: summed covariance

An association method frequently used for presence/absence data is the variance ratio, i.e. the ratio of the variance of the total number of species to the sum of variances of the occurrence frequency of individual species. However, this method is also applicable for abundance data (Schluter 1984). The null hypothesis of the Schluter test is that the sum of pairwise covariances equals zero. Thus, an alternative way of calculating the association measure is to sum all pairwise covariances; this procedure, called the summed covariance, is applied here. Previous studies applying Schluter's test on parasite assemblages include Lotz and Font (1991, 1994), Dobson and Pacala (1992), Haukisalmi and Henttonen (1993) and Forbes et al. (1994).

Method 5: proportion of positive covariances

Lotz and Font (1991, 1994) have used the proportion of positive covariances of all pairwise covariances as an index of community structure in helminth assemblages; the direction and significance of the observed index were assessed by randomisation.

Method 6: covariance distribution

Although the methods 3–5 have the attractive property of describing the overall co-occurrence pattern as a single index, it is possible that they fail to detect non-randomness in assemblages which include both positive and negative significant covariances (Schluter 1984). Therefore, we also present an alternative method, the frequency distribution of pairwise covariances (Fig. 1e,f).

Null model

For the reasons outlined in the Introduction, we create the expected distributions for all association measures by means of a randomisation procedure. In each analysis, the particular association measure is calculated from the observed data matrix and from 1000 randomised matrices in which the number of species (rows) and hosts (columns) equal those in the observed data. The association measures calculated from randomised matrices form the expected distribution which is used to determine the direction and statistical significance of the association. If the observed association measure is among the 25 most extreme values at either end of the simulated distribution (i.e. a two-tailed test with 5% significance level), the association is significantly non-random.

When the association is described as a frequency distribution of several (pairwise) measures (methods 1, 2 and 6), the observed frequencies and mean simulated frequencies of various frequency classes are compared with a standard goodness-of-fit test (log-likelihood). In addition, the significance of differences between the observed and expected frequencies is determined separately for each frequency class. The former test is used as the primary criterion for determining whether the particular sample is structured or unstructured.

Our randomisation approach constrains the total number of occurrences of each species (row sums), but not the total number of species per host (column sums). Under these constraints, 0s and 1s are randomly shuffled among hosts. We do not constrain the column sums because some of the tests we use measure the variability of the number of species among hosts, and the column sum constraint would imply zero variability. Also, the variation in prevalence among parasite species (row sums) is typically much more pronounced than variation in the number of parasite species per host (column sums).

To control for the effects of sampling heterogeneity on interspecific associations, we use an additional constraint, i.e. the occurrence frequency of parasites in the subsamples ("subsample constraint"). This procedure is related to the method of pooled within-site variation used by Wilson and Roxburgh (1994) to study assembly rules in plant communities. When this constraint is imposed, shuffling is performed separately within each subsample according to the species' observed prevalence in that subsample. The association measures are then calculated for the whole simulated sample, and the significance is determined as described above.

Artificial data sets

Power analysis

The ability of the association measures to detect positive and negative non-randomness is analysed by the procedure of Gotelli et al. (1997). We first created two artificial presence/absence matrices, one with a maximum positive overall association and another with a maximum negative association among species. Both matrices had 12 species and 200 hosts, and the occurrence frequencies of species were fixed between 1 and 34. In the positive matrix, species had a completely nested (overlapping) occurrence

pattern, whereas in the negative matrix, the occurrence of species was completely non-overlapping (no co-occurrences). Completely non-overlapping occurrence is achieved only when all species are relatively scarce; therefore the species occurrence frequencies in artificial matrices do not correspond to those in empirical data sets.

The structure of these two matrices was gradually broken down by randomly redistributing an increasing proportion (at 10% intervals) of all species occurrences; the situation where all occurrences are redistributed corresponds to a completely randomised matrix. All six association metrics were then applied to the resulting 11 matrices with a decreasing negative structure and 11 matrices with a decreasing positive structure. The power of the various association measures was determined by their ability to detect the known structure in these two series of artificial matrices (Fig. 2).

Proportion of positive and negative pairwise interactions

Another set of artificial presence/absence matrices was created to examine (1) how the proportion of negative and positive *pairwise* interactions affect the outcome of overall association analyses, and (2) whether the confounding spatial or temporal variability in helminth occurrence can be controlled by applying the "subsample constraint". We created eight matrices with 15 species and 100 hosts. In each matrix, the host "population" was split into four subpopulations (I–IV), e.g. seasonal samples, each having 25 individuals. The infection probability of each species either increased (0.15, 0.30, 0.60, 0.85) or decreased (0.85, 0.60, 0.35, 0.15) systematically from subsample I to IV. Species were then allowed to "infect" hosts randomly, i.e. independently of other species, according to their infection probability in each subsample. The overall prevalence of all species approximated 50%.

In the first matrix ("0–15"; Table 1), the infection probability of all 15 species increases from subsample I to IV, i.e. all species show parallel "seasonal" variation. This matrix is therefore positively structured when pooled across subsamples, but unstructured (random) within subsamples. The seven remaining matrices consist of two sets of species showing parallel "seasonal" variation within sets, but reversed variation between sets. In other words, all species in the first set have an increasing infection probability and all species in the second set have a decreasing infection probability in the four subsamples. The numbers of species in the two sets varied from 1 versus 14 to 7 versus 8.

This procedure is intended to create assemblages with variable numbers of strong positive and negative pairwise interactions. When pooled across subsamples, these eight matrices are extremely non-random, because all species are involved in pairwise interactions, and because most of the pairwise interactions are (highly) significant. Therefore, all these data sets should be detected as non-random by an appropriate method. On the other hand, when controlled for the "seasonal" variation, all matrices should appear as random.

Field data

The material consists of 465 common shrews (*S. araneus*), originating from three localities, Pallasjärvi ($n = 278$) in western Finnish Lapland (68°03′ N, 24°09′ E), Kainuu region ($n = 51$) in eastern mid-Finland (ca 64° N, 27°30′ E), and the Heinävesi-Enonkoski region ($n = 136$) in south-eastern Finland (ca 62° N, 29° E). Shrews were caught with live-traps or pitfalls. All shrews were immature young-of-the-year (237 males and 228 females).

The Pallasjärvi material, which was collected specifically for a study of spatial and temporal patterns in shrew helminths, represents 2 years (1992 and 1994), 3 months (July, August and September), and seven study grids. The habitat of all study grids was similar: old forests characterised by a thick moss layer and dominance of spruce (*Picea abies*) and blueberry (*Vaccinium myrtillus*). With one exception (4.5 ha), the size of the grids was 1.8 ha, and the average distance between adjacent grids was about 400 m. The sample size (number of shrews) for each month per year varied from 41 to 51.

The Kainuu and Heinävesi-Enonkoski shrews were both collected from four widely separated forest sites (average distance between sites was ca 40 km in Kainuu and 34 km in Heinävesi-Enonkoski) during a week in September (Kainuu in 1987 and Heinävesi-Enonkoski in 1995). The sample size varied between 3–29 in Kainuu and 24–44 in Heinävesi-Enonkoski. Compared to the Pallasjärvi material, these samples do not allow a proper analysis of sample heterogeneity on helminth occurrence. However, these data were included because their helminth assemblage and diversity differed from those at Pallasjärvi. Comparison between these three localities may elucidate the role of helminth diversity and species composition on patterns of interspecific association.

The significance of spatial and temporal variability for the occurrence frequency of the eight common helminth species (Pallasjärvi data set) was assessed by log-linear models, i.e. generalised linear models for contingency tables using Poisson error distribution and log link function. The idea of generalised linear modelling is to find the minimal adequate model, i.e. the simplest model that fits satisfactorily ($P > 0.05$) the observed data (Crawley 1993). In the log-linear analyses, we used simultaneously the study grid, year, month and presence/absence of a particular helminth species as classifying variables (one of the seven grids was excluded because of an incomplete data set). Since the prevalence of the two *Longistriata* species was very high (97–100%), we classified hosts in two groups

Table 1 Significance (*P*-values) of overall associations by alternative association measures for artificial, structured data sets (15 species and 100 hosts in each) with variable proportions of significant positive and negative pairwise interactions. In the matrix "0–15", all 15 species show parallel subsample variation, resulting in positive pairwise interaction among all species in the pooled sample. In other matrices, species are divided into two sets which show parallel interspecific variation within sets, but reversed variation between sets. The labels "1–14"…"7–8" denote the numbers of species in the two sets (see text for more details). The lower part of the table gives the number of positive and negative pairwise covariances in each artificial matrix [+ significant positive association (observed index higher than expected), − significant negative association (observed index smaller than expected), *ns* not significant ($P > 0.05$)]

	0–15	1–14	2–13	3–12	4–11	5–10	6–9	7–8
(1) Species density distribution	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	ns	ns
(2) Log-linear method	0.020	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
(3) Checkerboard score	0.000^{+}	0.000^{+}	0.000^{+}	ns	0.000^{-}	0.000^{-}	0.000^{-}	0.000^{-}
(4) Summed covariance	0.010^{+}	0.000^{+}	0.000^{+}	0.000^{+}	0.000^{+}	0.020^{+}	ns	0.000^{-}
(5) Positive covariances	0.000^{+}	0.000^{+}	0.000^{+}	0.000^{+}	0.000^{+}	ns	ns	ns
(6) Covariance distribution	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Positive pairwise covariances	105	91	79	69	61	55	51	49
Significant	91	80	71	63	55	48	44	44
Negative pairwise covariances	0	14	26	36	44	50	54	56
Significant	0	13	20	30	37	43	48	50

according to the median intensity of infection (number of parasites in a host individual); the intensity classes were ≤23 and >23 for *Longistriata depressa*, and ≤12 and >12 for *L. pseudodidas*.

We applied the six alternative association methods with and without the "subsample constraint" on the empirical data. In addition to the overall associations, we report the numbers of pairwise covariances among helminth species in each data set. All association analyses in the empirical assemblages are based on presence/absence data (0s and 1s).

Results

Power analysis

The main pattern emerging from the power analysis is that all methods are more likely to detect positive non-randomness than negative non-randomness of corresponding strength (Fig. 2). For example, the statistical significance of the overall association based on covariance distribution disappears when 40% of occurrences are redistributed in the negatively structured matrix. In the positively structured matrix, the significance of the test does not disappear until 80% of occurrences are redistributed [Fig. 2.(6)]. This pattern suggests that positive associations are more likely to be detected than negative ones irrespective of the test statistic used.

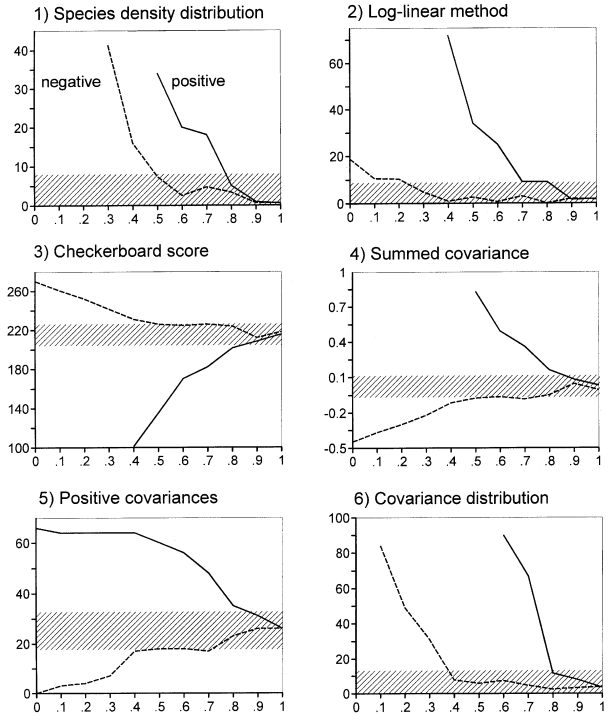

Fig. 2 Ability to detect non-randomness of known strength by alternative association measures (power analysis). The analysis is based on two artificial, non-random matrices, one with a maximum positive structure (*continuous line*) and another with a maximum negative structure (*dashed line*). The structure of these matrices was broken down by redistributing an increasing proportion of species occurrences. The labels on the *x*-axis denote how large a proportion of occurrences was redistributed: *1* is for a completely randomized matrix. The *y*-axis gives either the χ^2-value (the fit between the observed and expected frequency distributions: methods 1, 2 and 6) or the observed test statistic (methods 3–5). Values in the *shaded region* are non-significant ($P > 0.05$). In some cases, the part of the curve showing highly significant differences has been omitted

The ability to detect positive and negative non-randomness did not show clear differences between various association measures. The significant patterns disappeared when 30–50% and 70–90% of occurrences in the negative and positive matrices, respectively, were redistributed. However, the three measures based on frequency distributions (methods 1, 2 and 6) tended to have less power (i.e. a higher probability of type II error) than the measures based on a single index (methods 3–5) (Fig. 2). The log-linear method seemed to be particularly insensitive to negative overall associations.

The completely randomised matrices were detected as non-random by all methods (Fig. 2), indicating a generally low level of type I error.

Proportion of positive and negative pairwise interactions

The log-linear method and covariance distribution were the only null models detecting as non-random all the eight artificial, structured data sets with variable proportions of strong positive and negative pairwise interactions (Table 1, Fig. 1). The four remaining methods detected non-randomness in assemblages dominated by positive pairwise interactions, but not necessarily in other kinds of data sets. Notice that the three methods based on a single index (methods 3–5) gave different results for assemblages with roughly similar numbers of positive and negative pairwise interactions. For example, the matrix "5–10", with 55 positive and 50 negative pairwise covariances, appeared to be negatively structured by the checkerboard score, positively structured by the summed covariance, and unstructured (random) by the method based on the proportion of positive covariances.

The apparent structure of the eighth matrices always disappeared when the subsample constraint was applied (Fig. 1).

Helminth assemblages

The helminth material consists of 21 species representing trematodes (3 species), cestodes (13 species) and nematodes (5 species) (Table 2). All these species, with the exception of *Dilepis undula* (a cestode parasite of passerine birds) and larval spirurids (nematode parasites of larger mammals), are specialists in shrews. Despite the equal number of helminth species, there were pronounced differences in species composition and infection levels of helminths between the three localities, e.g. three cestodes that were common in Kainuu and Heinävesi-Enonkoski were totally missing at Pallasjärvi. Furthermore, the average number of species per host was markedly higher in Kainuu ($x = 6.5$, SD = 1.9) and Heinävesi-Enonkoski ($x = 5.6$, SD = 2.1) than at Pallasjärvi ($x = 3.7$, SD = 1.3).

Table 2 Prevalence (% infected) and microhabitats of helminths in the common shrew *Sorex araneus* (*s* stomach, *i* intestine, *b* bladder, *n* number of hosts)

Helminth species	Pallasjärvi 1992 $n=143$	Pallasjärvi 1994 $n=135$	Kainuu $n=51$	Heinävesi-Enonkoski $n=136$
Trematoda				
Brachylaemidae				
Brachylaemus fulvus (s)	1	1	18	18
Pseudoleucochloridium soricis (i)	1	0	0	0
Omphalometridae				
Neoglyphe locellus (i)	4	3	0	0
Cestoda				
Dilepididae				
Molluscotaenia crassiscolex (i)	32	30	28	46
Dilepis undula (i)	7	10	6	6
Hymenolepididae				
Neoskrjabinolepis schaldybini (i)	33	15	29	30
N. singularis (i)	4	4	12	1
Lineolepis scutigera (i)	34	30	16	19
Pseudobotrialepis globosoides (i)	6	6	0	0
Staphylocystis furcata (i)	1	2	41	10
Vigisolepis spinulosa (i)	0	0	45	28
Soricinia infirma (i)	0	0	18	5
Ditestolepis diaphana (i)	0	0	92	80
D. tripartita (i)	0	0	45	22
Ditestolepis sp. A (i)	28	37	0	0
Ditestolepis sp. B (i)	0	0	18	0
Nematoda				
Heligmosomidae				
Longistriata depressa (i)	99	100	100	97
L. pseudodidas (i)	97	99	89	91
Capillariidae				
Capillaria kutori (s)	18	18	20	27
Liniscus incrassatus (b)	15	21	–	18
Strongyloididae				
Parastrongyloides winchesi (i)	1	0	71	46
Spiruridae spp. (i)	1	0	0	13

[a] Bladder not examined
[b] Larvae probably belonging to the genera *Ascarops* and *Physocephalus*

Spatial and temporal patterns of helminth parasitism (Pallasjärvi)

In the Pallasjärvi material, the most commonly observed interaction was that between month and helminth occurrence (MH) (Table 3), i.e., the prevalence of most helminth species differed significantly between the 3 months. The effect of year (YH) was included for three species, and the effect of study grid (GH) for one species (*Ditestolepis* sp. A) only. The selected models thus indicate that temporal variability, and especially the month of collection, affect the occurrence of helminths more than spatial variability.

Table 3 The best log-linear models and their fit to the observed data for interactions between the year (*Y*), month (*M*), study grid (*G*) and occurrence of helminth species (*H*) in the common shrew *S. araneus* (Pallasjärvi data). A high *P*-value indicates a good fit between the model and observed data

Species	Model	χ^2	*df*	*P*
M. crassiscolex	YMH	67.8	60	0.23
N. schaldybini	YH	51.5	68	0.93
L. scutigera	MH	62.7	66	0.59
Ditestolepis sp. A	YH, MH, GH	47.1	54	0.74
L. depressa	MH	77.5	66	0.16
L. pseudodidas	YH, MH	71.0	64	0.25
C. kutori	MH	59.3	66	0.71
L. incrassatus	MH	52.5	66	0.89

Overall associations among shrew helminths

The association analyses included 14 species in the Pallasjärvi data sets (to make these data sets comparable, one rare species that was absent in 1994 was excluded), and 16 species in the Kainuu and Heinävesi-Enonkoski data sets. In the Pallasjärvi data sets, we analysed the possible effects of temporal heterogeneity (three monthly samples) on interspecific associations. In Kainuu and Heinävesi-Enonkoski, where the shrews were collected during a short period, sampling heterogeneity is due to the variation of infection levels among the four study sites

Overall, the results suggest that the three helminth assemblages differed markedly with respect to the nature of overall interspecific associations. The two Pallasjärvi data sets showed a neutral (random) structure and the data set of Heinävesi-Enonkoski showed a consistent

Table 4 Significance (*P*-values) of overall associations in empirical helminth assemblages of the common shrew *S. araneus* by six alternative methods. The column labels *A* and *P* indicate whether the null model controlled for sample heterogeneity by means of a "subsample constraint" (*A*, absent, *P*, present). The lower part of the table gives the numbers of species pairs and pairwise covariances in each data set, applying the subsample constraint [+ significant positive association, *ns* not significant ($P < 0.05$)]

Method	Pallasjärvi 1992		Pallasjärvi 1994		Kainuu		Heinävesi-Enonkoski	
	A	P	A	P	A	P	A	P
(1) Species density distribution	ns	ns	0.002	0.008	ns	ns	0.005	0.041
(2) Log-linear method	ns	ns	ns	ns	ns	ns	< 0.001	0.003
(3) Checkerboard score	ns	ns	0.024^+	ns	0.044^+	0.024^+	0.000^+	0.000^+
(4) Summed covariance	ns	ns	ns	ns	0.007^+	0.024^+	0.000^+	0.000^+
(5) Positive covariances	ns	ns	ns	ns	ns	ns	0.000^+	0.000^+
(6) Covariance distribution	ns	ns	ns	ns	0.022	ns	< 0.001	0.001
Number of species pairs		9		91		120		120
Positive pairwise covariances		40		40		53		84
Significant		1		0		3		10
Negative pairwise covariances		5		38		52		36
Significant		0		0		0		1

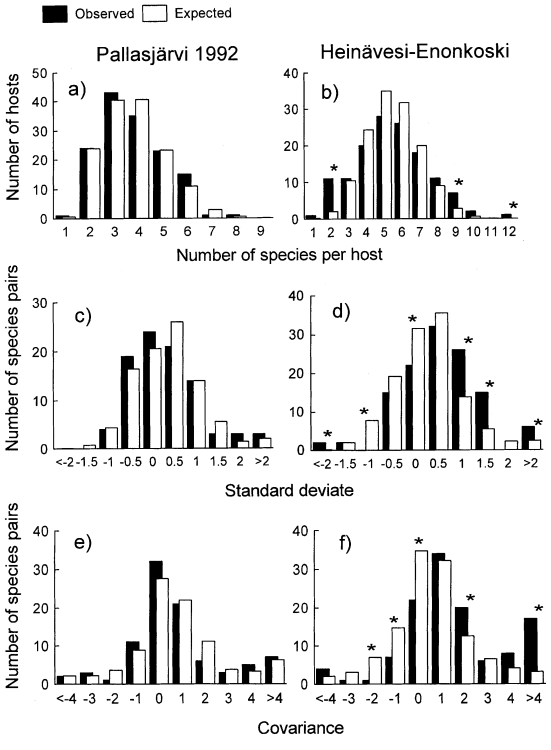

Fig. 3 Overall interspecific associations for two helminth assemblages of the common shrew *Sorex araneus*: the unstructured (random) assemblage of Pallasjärvi 1992, and the positively structured assemblage of Heinävesi-Enonkoski. The frequency distributions are based on three association methods: species density distribution (**a, b**), log-linear method (**c, d**), and covariance distribution (**e, f**). The expected distributions were simulated using the subsample constraint (see text). An *asterisk* above the columns indicates frequency classes in which the observed frequency differs significantly ($P < 0.05$) from the mean expected frequency. See Table 4 for overall differences between the observed and expected distributions

positive structure (Table 4, Fig. 3). The Kainuu assemblage was intermediate between these two assemblages: two of the six methods indicated a significant (positive) structure and four methods indicated no structure.

The simulation constraint used did not usually affect the outcome of association analyses. However, in a few cases, the level of significance decreased when the assemblage was analysed with the subsample constraint (Table 4).

Discussion

Applicability of association methods

The testing of null models on artificial data sets showed that rejecting a true null hypothesis of no overall structure (type I statistical error) is not a problem with the methods applied here. This seems to suggest that, as long as the expected distribution is created by randomisation, the method used is not likely to affect the outcome of association analysis. However, this clearly was not the case, because the results for the structured data sets depended on the method used.

When applied to artificial data sets, the methods based on a single index (methods 3–5) had slightly more power, i.e. a smaller risk of accepting a null hypothesis of no overall structure when one exists (type II error), than the methods utilizing frequency distributions (methods 1, 2 and 6). The observed difference in power may be simply due to the different way of determining statistical significance by these two types of methods. For single-index methods, the significance is determined directly from the expected distribution, whereas the fit between observed and expected frequency distributions is used to determine significance for the other three methods. When testing the fit between two frequency distributions, it is usually necessary to combine frequency classes with small expected frequencies, especially in species-poor assemblages. This inevitably leads to a loss of information and decreased statistical power.

Other drawbacks of methods utilising frequency distributions are the independence of various frequency

classes (Gotelli and Graves 1996) and possible difficulties interpreting the observed patterns. With these pitfalls in mind, the methods based on a single index appear to be better suited for overall association analysis, as suggested by Gotelli and Graves (1996). However, the single-index methods (and species density distribution) could not detect strong non-randomness in some of the artificial data sets, obviously due to the presence of roughly equal numbers of positive and negative pairwise interactions which cancel each other out (Table 1). Because natural assemblages always include variable proportions of positive and negative pairwise interactions, this drawback seems to restrict the applicability of these methods for empirical studies. Moreover, the three tested single-index methods gave contrasting results for some of the artificial data sets (e.g. the data set "5–10" in Table 1), reflecting the fact that different methods measure different things. Direct comparison of studies based on different methods is thus not meaningful.

We conclude that none of the tested methods appears to be ideal for empirical studies. The most reliable way to analyse overall associations in natural assemblages is the simultaneous use of both types of method. However, the method most commonly used for parasites, the species density distribution (method 1), appears to be least suitable for empirical studies, because it combines all the drawbacks mentioned above. In species-poor assemblages (less than ten species), the single-index methods seem to be the obvious choice.

Predominance of positive overall associations

Analyses on community structure of parasites (Lotz and Font 1991) and free-living animals (Schluter 1984) have revealed predominantly neutral and positive overall associations (but see Lafferty et al. 1994), a pattern that is supported by the present empirical data. Given the opportunities for interspecific competition in the patchy, spatially restricted environments of parasites (e.g. host intestine), the lack of observed negative overall associations is remarkable. In the following, we propose several factors that might contribute to the predominance of positive overall associations in empirical (parasite) assemblages.

First, and probably most important, the results of the power analysis suggest that detecting positive non-randomness is "inherently" much more probable than detecting negative non-randomness irrespective of the method used. A similar, but less clear-cut result was obtained by Gotelli et al. (1997). For example, in the log-linear method, the negative "signal" disappeared when only 30% of species occurrences were randomly redistributed (Fig. 2), making it very unlikely that a negative non-randomness will be detected in natural populations. Similarly, Hastings' (1987) numerical analysis showed that even strong competitive interactions among three species may remain undetected by the Schluter (1984) index, supporting the idea of generally low probability of negative non-randomness.

It could also be argued that the lack of column sum constraints in most of the null models used for parasite assemblages, including the one used by us, biases the analyses towards positive overall association (Gotelli and Graves 1996, p.168). However, there is no obvious reason why the absence of column sum constraint would increase the probability of type I error more for positive assemblages than it might do for negative assemblages. Also, the very low probability of type I error for our null model seems to refute this argument. However, it is clear that the constraints included in a null model do affect the observed patterns, and therefore the results should be evaluated and compared relative to the constraints used.

Ignorance of various sampling heterogeneities could partly explain the commonness of positive overall associations in parasites (Lotz and Font 1991). For example, ignoring the age of the host will bias the result towards positive association, because most helminths are more prevalent in adult hosts than in juveniles (Haukisalmi et al. 1994).

Finally, indirect, or apparent, interactions among species may bias the observed patterns towards positive non-randomness. In any truly non-random assemblage, there is likely to be one or more "key" species (e.g. dominant competitors) which will show negative (or positive) interactions with several other species. For example, the negative pairwise interactions among larval trematodes are asymmetric and hierarchic, i.e. the top competitors are dominant over most of the other species (Lafferty et al. 1994). If n species show independently a negative association with a dominant competitor, these pairwise interactions will produce $n(n-1)/2$ apparent *positive* associations among the n subordinate species. Thus, a predominantly negative assemblage may actually be classified as random or positively structured because of the presence of a number of indirect positive interactions. It should be noticed, however, that in shrew helminth communities, negative pairwise interactions were practically absent (Table 4), suggesting that some of these assemblages are truly structured by positive interactions.

Positive overall associations in shrew helminths

The positive overall associations observed in shrew helminths are probably not due to uncontrolled sampling heterogeneity. The shrews represented the same cohort and were collected during a short time span. Previously, we have shown that the sex of immature shrews does not affect the helminth infection level (Haukisalmi et al. 1994). Furthermore, the Pallasjärvi data showed that there are rarely significant spatial differences in helminth prevalence within a homogeneous forest habitat. The fact that pooling of various temporal or spatial subsamples did not cause clear bias to the overall associations (Table 4) suggests that the

covariation of infection levels of helminth species among subsamples was relatively weak.

If the association analysis has been properly controlled for the effects of sampling heterogeneity, positive associations among parasites may arise because of similar transmission pathways, because of direct (mutualistic) interactions, or because of indirect (host-mediated) interactions. Similarity of transmission may be responsible for positive associations among congeneric helminth species which usually have similar life cycles, but not for positive associations in general. The intermediate hosts for shrew cestodes include copro- and necrophagous beetles (*Ditestolepis diaphana*, *Staphylocystis furcata*, *Neoskrjabinolepis schaldybini*), collembolans (*Vigisolepis spinulosa*), fleas (*Lineolepis scutigera*) and snails (*Molluscotaenia crassiscolex*) (Kisielewska 1961; Prokopic 1968, 1969). Moreover, the life cycles of nematodes are usually direct (no intermediate hosts), making it more unlikely that similar transmission pathways would be responsible for the observed positive associations.

We regard host-mediated interactions as the most probable explanation for the positive overall associations in shrew helminths. Experimental work has shown that positive (synergistic) associations among mammalian helminths often arise because of immunosuppressive effects of certain species (see Christensen et al. 1987 for a review). The immunosuppressive effect induced by a helminth species may be non-specific, i.e. it impairs the host's ability to resist both homologous (species responsible for the effect) and heterologous (other species) infections, including totally unrelated helminths (Alghali et al. 1985). Thus, even a single key species might be able to create an overall positive structure, if several other species are affected by its immunosuppressive capacity. Such pairwise associations would also create a number of indirect positive interactions which would reinforce the overall positive association.

Acknowledgements Our ongoing research on the ecology of parasites in small mammals has been funded primarily by the Maj and Tor Nessling Foundation, the Finnish Forest Research Institute and the Research Council for Natural Resources in Finland. Sirpa Kaksonen, Pia Vikman and Taina Mikkonen have helped in processing the shrew intestines, and Tapio Juutilainen and Petri Kuortti helped in trapping the shrews. John C. Holmes, Nicholas J. Gotelli and J.B. Wilson are acknowledged for critical comments on various versions of the manuscript.

References

Alghali STO, Hagan P, Robinson M (1985) *Hymenolepis citelli* (Cestoda) and *Nematospiroides dubius* (Nematoda): interspecific interactions in mice. Exp Parasitol 60: 364–370

Christensen NO, Nansen P, Fagbemi BO, Monrad J (1987) Heterologous antagonistic and synergistic interactions between helminths and between helminths and protozoans in concurrent experimental infection of mammalian hosts. Parasitol Res 73: 387–410

Crawley MJ (1993) GLIM for ecologists. Blackwell. Oxford

Dobson AP (1990) Models for multi-species parasite-host communities. In: Esch GW, Bush AO, Aho JM (eds) Parasite communities: patterns and processes. Chapman & Hall, New York

Dobson AP, Pacala SV (1992) The parasites of *Anolis* lizards in the northern Lesser Antilles. II. The structure of the parasite community. Oecologia 91: 118–125

Forbes M, Weatherhead PJ, Bennett GF (1994) Blood parasites of blue grouse – variation in prevalence and patterns of interspecific association. Oecologia 97: 520–525

Gilpin ME, Diamond JM (1982) Factors contributing to nonrandomness in species co-occurrences on islands. Oecologia 52: 75–84

Goater TM, Esch GW, Bush AO (1987) Helminth parasites of sympatric salamanders: ecological concepts at infracommunity, component and compound community levels. Am Midl Nat 118: 289–300

Gotelli NJ, Graves GR (1996) Null models in ecology. Smithsonian Institution Press, Washington, DC

Gotelli NJ, Buckley NJ, Wiens JA (1997) Co-occurrence of Australian land birds: Diamond's assembly rules revisited. Oikos 80: 311–324

Harvey PH, Colwell RK, Silvertown JW, May RM (1983) Null models in ecology. Annu Rev Ecol Syst 14: 189–211

Hastings A (1987) Can competition be detected using species co-occurrence data? Ecology 68: 117–123

Haukisalmi V (1989) Intestinal helminth communities of Sorex shrews in Finland. Ann Zool Fenn 26: 401–409

Haukisalmi V, Henttonen H (1993) Coexistence in helminths of the bank vole *Clethrionomys glareolus*. I. Patterns of co-occurrence. J Anim Ecol 62: 221–229

Haukisalmi V, Henttonen H (1994) Distribution patterns and microhabitat segregation in gastrointestinal helminths of *Sorex* shrews. Oecologia 97: 236–242

Haukisalmi V, Henttonen H, Tenora F (1988) Population dynamics of common and rare helminths in cyclic vole populations. J Anim Ecol 57: 807–825

Haukisalmi V, Henttonen H, Mikkonen T (1994) Parasitism by gastrointestinal helminths in the shrews *Sorex araneus* and *S. caecutiens*. In: Merrit JF, Kirkland GL, Rose RK (eds) Advances in the biology of shrews. Special publication of Carnegie Museum of Natural History, vol 18. Carnegie Institute, Pittsburgh

Haukisalmi V, Henttonen H, Batzli GO (1995) Helminth parasitism in the voles *Microtus oeconomus* and *M. miurus* on the North Slope of Alaska: host specificity and the effects of host sex, age and breeding status. Ann Zool Fenn 32: 193–201

Janovy J Jr, Clopton RE, Clopton DA, Snyder SD, Efting A, Krebs L (1995) Species density distributions as null models for ecologically significant interactions of parasite species in an assemblage. Ecol Model 77: 189–196

Kisielewska K (1961) Circulation of tapeworms of *Sorex araneus araneus* L. in biocenosis of Bialowieza National Park. Acta Parasitol Pol 9: 331–367

Lafferty KD, Sammond DT, Kuris AM (1994) Analysis of larval trematode communities. Ecology 75: 2275–2285

Lotz JM, Font WF (1991) The role of positive and negative interspecific associations in the organization of communities of intestinal helminths of bats. Parasitology 103: 127–138

Lotz JM, Font WF (1994) Excess positive associations in communities of intestinal helminths of bats: a refined null hypothesis and a test of the facilitation hypothesis. J Parasitol 80: 398–413

Montgomery SSJ, Montgomery WI (1989) Spatial and temporal variation in the infracommunity structure of helminths of *Apodemus sylvaticus* (Rodentia: Muridae). Parasitology 98: 145–150

Pence DB (1990) Helminth community of mammalian hosts: concepts at the infracommunity, component and compound community levels. In: Esch GW, Bush AO, Aho JM (eds) Parasite communities: patterns and processes. Chapman & Hall, New York

Poulin R (1996) Richness, nestedness, and randomness in parasite infracommunity structure. Oecologia 105: 545–551

Prokopic J (1968) A description of the cysticercoid of cestode *Vigisolepis spinulosa* (Cholodkowsky, 1906) found in Collembola. Folia Parasitol 15: 266

Prokopic J (1969) Three species of the genus *Ctenophtalmus* (Siphonaptera) as new, natural intermediate host for *Hymenolepis scutigera*. Folia Parasitol 16: 264

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

Stone L, Roberts A (1990) The checkerboard score and species distributions. Oecologia 85: 74–79

Wilson JB (1987) Methods for detecting non-randomness in species co-occurrences: a contribution. Oecologia 73: 579–582

Wilson JB, Roxburgh SH (1994) A demonstration of guild-based assembly rules for a plant community, and determination of intrinsic guilds. Oikos 69: 267–276