Integrating ecology and epidemiology using individual-based multi-species networks

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Abstract

Parasite transmission in host communities is a function of ecological factors that influence interspecific contacts and contact patterns within species. These two levels are studied with different kinds of networks – ecological networks and individual contact networks – and the integration of these levels is essential for effective understanding of parasite transmission. We combined these approaches by creating epidemiological networks based on parasite sharing from individual-based ecological host-parasite networks. We compared multi- to single-species networks to investigate the drivers of helminth infection in wild individual rodents of South-east Asia. Network modularity was higher in the multi-species than in the single-species networks. Phylogeny affected affiliation of individuals to modules. The importance of individuals differed between multi- and single-species networks, with species identity and individual traits influencing their position in the networks. Simulations revealed that a novel parasite spreads more slowly in multi- than in single-species networks and that this depended on network structure. Although the relative contribution of within- vs. between-species transmission rates to disease dynamics is important, using multi-host epidemiological networks improves our understanding of parasite dynamics as it further considers interaction structure between individuals.

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1 Introduction

Parasites play a major role in the lives of wild animals and humans. In attempts to understand the ecological processes leading to infection with a particular parasite, ecologists have investigated the factors influencing the interaction between the host species and the parasite in question (Fig. 1a). In recent years, the limits of the single-host-single-parasite perspective have become apparent due to the wealth of indirect effects that parasites and hosts exert on one other within a community (Fig. 1b), and given the recognized importance of understanding cross-species parasite transmission [1, 2, 3, 4]

Network analysis is the main approach taken to uncover the complexity underlying interactions among multiple hosts and parasites in a community [5, 6]. The biological interactions among hosts and parasites are depicted as a bipartite network, in which edges describe interactions between two disjointed groups of nodes (hosts and parasites) and in which nodes from one group (hosts) are allowed to interact only with nodes of the other group (parasites) (Fig. 1b). A network approach elucidates how properties of the whole network emerge from the properties of its nodes, allowing examination of the system at both the node and network levels.

Typically, the units of analysis in host-parasite networks are species rather than individuals. However, by aggregating individual observations into species-averages we lose valuable individual-based information [7]. This is especially important in disease ecology because parasite transmission necessarily occurs at an individual level (individuals are infected, rather than species). In addition, within an individual host, co-infection with multiple parasites can determine both infection with subsequent parasites and the transmissibility of parasites to other individuals [8]. The individual level is also important because large variation exists among individuals in traits that promote parasite transmission. For example, disease outbreaks may be promoted by a small fraction of well-connected individuals ('super-spreaders') which are responsible for the majority of transmission events [9, 10].

Unlike ecological networks, epidemiological networks characterize parasite transmission among host individuals of a single species [11, 12]. Epidemiological networks are unipartite (contain one set of nodes), with edges representing contact patterns or some other type of individual-based interaction meaningful for parasite transmission [13]. This approach is essentially a single-host-single-parasite approach (Fig. 1c,e). Hence, a great need exists to assess whether including interspecific connections in individual-based networks is important for epidemiological questions about parasite spread in host communities.

Previous works have highlighted the importance of considering multiple hosts and host heterogeneity for studies of parasite transmission [3, 4, 14, 15, 16] but none has adopted a network approach which considers the structure of the epidemiological network. Here, we examine the link between ecological and epidemiological networks by exploring the factors that determine host-parasite interactions (ecology) and characterize their dynamics (epidemiology) at the individual level. Building upon existing network analysis procedures, we compare multi- versus single-species individual-based networks (Fig. S1). In multi-species networks, heterogeneity exists at two levels: (i) species-level traits shared by all members of a species in the sampled population (e.g. niche breadth, sociality, and abundance) and (ii) individual traits associated with variation in parasite acquisition, such as variation in age [17], sex [18] or immunocompetence [19]. In contrast, in single-species networks heterogeneity is only a consequence of individual traits.

We tested three hypotheses. First, we hypothesized that the difference in sources of heterogeneity translates to structural differences between multi- and single-species networks. We examined this hypothesis using modularity, which is a network property crucial to the ecology and evolution of hosts and parasites [5, 20]. Modular networks are characterized by distinct net-

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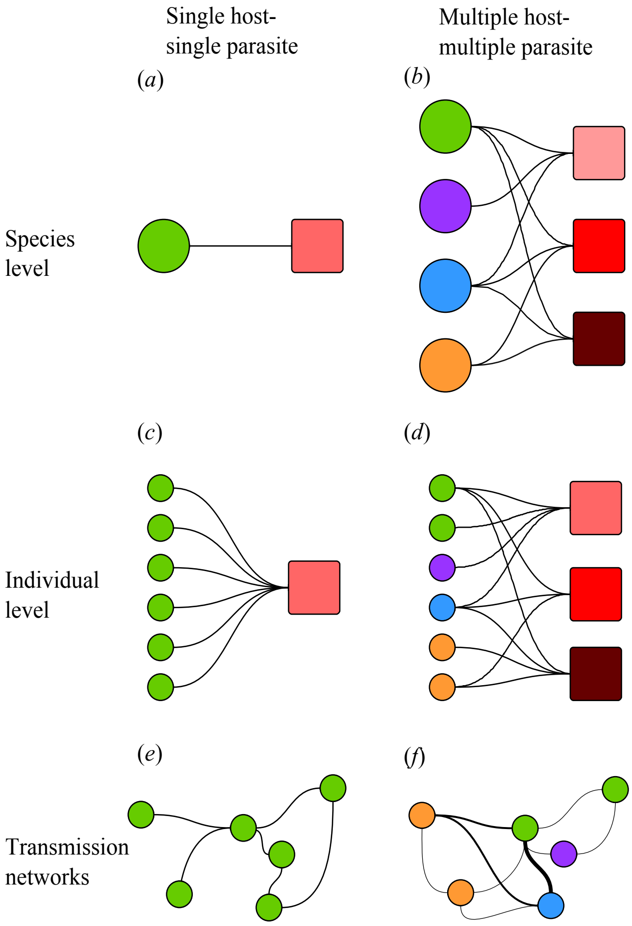

Fig. 1. Theoretical differences between the single host-single parasite (left) and multi host-multi-parasite (right) approaches. Hosts and parasites are depicted as circles and squares, respectively, with different colors representing different species. Large and small circles are host species or individuals, respectively. Networks in (a-d) are bipartite networks in which an edge represents infection of a host species or individual with a parasite species. (e) A single-species contact network between individuals of a single host. (f) A multi-species transmission-potential network obtained by connecting two individual hosts from the network in (d) if they share at least one parasite species. The weight of an edge between two individuals is the number of parasites shared (depicted as edge width).

work substructures (modules) composed of nodes interacting preferentially among themselves as compared to nodes of other modules. In ecological species-level networks, modules are composed of species similar in traits or

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phylogenetically related [5, 20]. In individual-based networks, we can expect the same phenomenon: the tendency for a pair of individual hosts to occur in the same module (i.e. to be infected by similar parasites) should increase as trait similarity between them increases. We also expected stronger modularity in multi- species networks than in single-species networks because individuals of closely related host species will tend to interact with similar parasites [21], resulting in modules composed of individuals of the same species.

Second, at the node level, we hypothesized that the factors that affect the roles that individuals play in parasite transmission differ between multi- and single-species networks. This role can be quantified by indices of centrality where a central node is one that is highly connected to and reachable from other nodes. In epidemiological networks, central individuals can be considered as super-spreaders [13, 22]. We therefore used node centrality to capture a node's potential to spread parasites relative to other nodes in the network. We expected that species identity is a strong factor influencing centrality because some host species have been shown to be more central than others [23]. We also expected that individuals bearing traits that lead to greater parasite-sharing in single-species networks (making them more central) will also be more central in multi-species networks.

Finally, we hypothesized that the ecological differences between multiple- and single-species networks affect the dynamics of parasite spread. If multi-species ecological networks are more modular than single-species networks, then we expect that parasite transmission will be slower in multi-species networks because individuals from different species are less connected.

We examined our hypotheses exploring the structure of, and simulating parasite spread in, three networks of rodent individuals that interact with several gastrointestinal helminth parasites transmitted via fecal-oral pathways.

2 Methods

2.1 Data set

We used data on 104 individual rodents trapped at three human-disturbed localities: Buriram (14°89'N; 103°01'E; Thailand), Mondolkiri (12°12'N; 106°89'E; Cambodia) and Sihanouk (10°71'N; 103°82'E; Cambodia). Our data set was unique as it allowed us to test our hypotheses in three different communities with similar characteristics and contained information on individual traits as well as parasitism. Rodents were parasitized by 13 taxa of gastrointestinal helminths, five identified to genus level and eight to species level but as unique morpho-species. Trapping was conducted during the dry season in November 2008 (Sihanouk and Buriram) and November 2009 (Mondolkiri). Helminths survey for each rodent was conducted following [24] (see Fig. S1, Table S1 and Supplementary Information SI.1 for details on the study system). For each locality, we built one multi-species unweighted bipartite ecological network in which individual rodents were connected to parasites species (Fig. 1d). We then extracted from that network smaller single-species networks in which individual hosts belonged to the same species (Fig. S1).

We selected five individual traits potentially associated with variation in parasite acquisition: sex, age (adult versus young), immunocompetence, body mass and habitat in which an animal was caught (forest, lowland/upland agriculture and settlement) because the likelihood of exposure to parasites varies with habitat preference. As a proxy for immunocompetence, we used the ratio of spleen mass to body mass (RSM), with a larger ratio indicating higher immunocompetence [25]. We considered heterogeneity at the rodent species level by using either phylogenetic distance between species or a factor with species identities as levels.

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2.2 Network modularity

We identified modules of rodents that interact with similar parasites with an algorithm that finds the maximization of the modularity function M [26]. We tested for significance of M by comparing the observed value to those derived from 100 random networks constructed with a null model that assumed that the probability of drawing an edge between a rodent individual and a parasite species is proportional to the susceptibility of the rodent to parasites and to the infection potential of the parasite (Supplementary Information SI.3).

We tested the effect of individual traits on the affiliation of individuals to modules (module composition) with logistic multiple regression on distance matrices (MRM), following [5]. In the multi-species networks, we included an explanatory variable matrix that contained patristic distances (sum of phylogenetic branch lengths) as a measure of phylogenetic distance between a pair of rodents (Supplementary Information SI.2, SI.4). We used PCA-standardised coefficients to avoid effects of different scales of the explanatory variables [5]. Although we strived for an information-theoretic based analysis (as with centrality; see below), a likelihood function is unavailable for MRM. We thus interpret our results based on p-values and MRM coefficients.

2.3 Network centrality

A natural extension to the single host approach in epidemiology (Fig. 1e) is to build epidemiological networks with individuals belonging to multiple species. To achieve this, we projected each of our single- and multi-species bipartite 'ecological' networks to unipartite 'epidemiological' networks by connecting two individual hosts in the unipartite network if they shared at least one parasite species in the bipartite network [23] (Fig. 1f). The weight of an edge between two hosts was set as the number of parasites shared, as in

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earlier studies [23, 27]. We thus assumed a positive correlation between the number of parasites shared by a pair of individuals and the likelihood that a novel parasite would infect them both. We refer to the projected networks as 'transmission potential networks' (TPNs). By transmission potential, we mean the likelihood that a given individual will infect another individual, relative to other individuals in the network, with predictions based on observed sharing of parasites. We consider the advantages and limitations of assuming epidemiological linkage through parasite sharing in the Discussion.

We used eigenvalue centrality (EC) to quantify the role of a node in terms of promoting parasite transmission. With EC, a node's importance is increased when it has more connections to other nodes that are themselves important [28]; EC thus enables quantification of the transmission potential of an individual [29]. We examined the effect of individual traits on EC with a set of linear models for each of the multi-species TPNs and for single-species TPNs with > 10 individuals. Models within a set differed in the individual traits they had as explanatory factors and we included species identity as a factor in our models for multi-species networks (Table S3). We eliminated factors with no variation (e.g. when all individuals were the same sex), or with an excess of missing data (i.e. RSM in Mondolkiri). For each TPN, we compared models - including a null model with an intercept only – using model probabilities (w) based on AIC corrected for small sample size (AICc), which gives a measure of the plausibility, on a 0 to 1 scale, that a particular model is the best model [30]. We used a measure of coefficient importance, calculated as the sum of w across all the models in which the coefficient appears to quantify the importance of a trait in determining EC.

To quantify the effect of the inclusion of several species on the position of individuals in the network we correlated the centrality of individuals in a particular single-species network with their centrality in the corresponding multi-species network using a Pearson correlation for networks with > 5

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individuals. A positive, high correlation indicates that individuals with a more central position in the multi-species network are also more central in the single-species network.

2.4 Simulations

To link network structure to parasite dynamics and to put our results in an applied context, we simulated the spread of a novel parasite across the TPNs with a SI (susceptible-infected) epidemiological model in which an individual can be either susceptible to the disease or infected and thus infectious. Our model assumed that the novel parasite has similar characteristics to the parasites shared between the individual hosts, and that population densities of the rodent species were equal, although we considered the relative proportion of species abundances in the community (Supplementary Information SI.5).

At the start of each simulation, one rodent individual was randomly selected to be infected. In subsequent time steps, the parasite was allowed to spread across rodents in the network. The probability of parasite transmission from rodent individual i to its neighbour j in the next time step was calculated as $P_{i \to j} = 1-(1-\theta)^{\omega_{ij}}$, where ω_{ij} is the edge weight. We assumed that a stronger weight leads to an increased infection probability. The parameter θ is a fixed infection probability, characteristic to the novel parasite in the host species to which the parasite is spreading [27]. We set $\theta = 0.02$ in the single-species TPNs. The exact value of θ is irrelevant since we observe the system from a relative point of view (single vs. multi-species TPNs) and our sensitivity analysis showed that the results remained qualitatively similar for different values of θ.

Previous studies have shown that in homogeneously-mixed systems the ability of parasites to spread in multi-host (versus single-host) species depends on the relative contribution of within-species transmission to cross-

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species transmission [1, 3, 14]. In addition, a common assumption of multi-host models is that within-species transmission is lower than between-species transmission. We acknowledged these issues in our model: In the multi-species TPNs, we set $\theta = 0.02 \times (1 - \beta_{mk})$, where β_{mk} is the Jaccard index of shared parasites between species m and k [21, 31], assuming that the infection probability of individuals of different species was linearly proportional to the number of parasites shared by the host species [21]. Because β_{mk} ranges between 0 and 1, infection probability was at a maximum of 0.02 for individuals from species with completely overlapping parasite communities (same species) and 0 for individuals from species with no shared parasites. Our model thus allowed us to account for parasite sharing both at the individual and the species levels and took phylogeny into account because closely related species tend to share more parasites [21, 31]. To separate the effect of network structure from that of differential infection probability, we also repeated these analyses with a fixed $\theta = 0.02$ in the multi-species networks, simulating a constant infection probability for all species.

The time steps required to infect all individuals in the network was used as a measure of parasite spread efficiency that we defined as time to global infection (TGI), sensu [27]. To eliminate the effects of network size and connectance when comparing TGI between a multi-species TPN and a single-species TPN within the same locality, we created 100 multi- and single-species sub-TPNs of equal size and connectance derived from the original TPNs within a locality (Supplementary Information SI.5). We ran the algorithm 100 times per sub-TPN and used the average value of each sub-TPN ($\overline{TGI}$) to obtain a distribution of 100 $\overline{TGI}$ values corresponding to 100 sub-TPNs. We then visually compared the distributions (density plots) of single- and multi-species sub-TPNs. We expected a difference in infection patterns (shape and position of distributions) between multi-species TPNs

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and each of the single-species TPNs due to the greater individual heterogeneity in the multi-species networks and the heterogeneity in θ in individuals from different species.

2.5 Statistical analyses

Analyses were done within the R environment (version 3.0; [32]) with aid of the 'bipartite' package (version 2.00; [33]). We calculated EC with the evcent function from the igraph package (version 0.6-3; [34]). Multi-model inference was done with package MuMIn in R [35]. Modularity analyses were done with software bipartmod [26] and MRM analysis in MatLab.

3 Results

3.1 Network modularity in ecological networks

For ecological networks with > 10 nodes, all but one single-species network (*Bandicota savilei* in Buriram) were significantly modular (Table 1). The three multi-species networks were evenly fragmented with 4 modules in each but modularity (M) of the multi-species networks of Buriram and Sihanouk was ≈ 1.8 times stronger than in Mondolkiri. Modularity was higher in the multi-species than in the single-species networks in Buriram and Sihanouk, but not in Mondolkiri (Table 1). Differences in M between multi- and single-species networks were generally not a result of differences between network size or connectance (Supplementary Information SI.3).

The phylogenetic distance between individuals was a significant predictor of affiliation to modules in Buriram and Sihanouk (but not in Mondolkiri): the closer two individuals were phylogenetically, the more likely that they occurred in the same module (Fig. 2a,c). Individual traits like habitat and body mass were also significant predictors of the affiliation of individuals to modules in the multi-species networks of Buriram (Fig. 2a) and

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Table 1. **Information on multi- and single-host bipartite networks.** Parasite richness is the number of helminth taxa infecting an individual rodent. C – Network connectance – is the number of realized interactions divided by the number of possible ones. Statistical significance of modularity: $^{*}P < 0.05$; $^{*} * P < 0.01$; $^{*} * *P < 0.001$.

	# Individuals	# Helminth taxa	Parasite richness (range, mean±SD)	C	M (# modules)
BURIRAM					
Multi-species	27	10	1-3, 1.63±0.63	16.30%	0.53 (4) ***
Bandicota savilei	15	7	1-3, 1.93±0.59	27.60%	0.24 (5)
Mus cervicolor	6	4	1-2, 1.33±0.52	33.30%	
Rattus exulans	6	3	1-2, 1.17±0.41	38.90%	
MONDOLKIRI					
Multi-species	37	8	1-4, 1.95±0.85	24.30%	0.29 (4) ***
Bandicota savilei	23	7	1-4, 2.13±0.87	30.40%	0.24 (3) *
Rattus tanezumi	14	6	1-3, 1.64±0.74	27.40%	0.33 (3) *
SIHANOUK					
Multi-species	40	6	1-3, 1.32±0.57	22.10%	0.54 (4) ***
Rattus argentiventer	5	3	1-3, 1.8±0.84	60%	
Rattus exulans	11	3	1-3, 1.45±0.69	48.50%	0.25 (3) **
Rattus norvegicus	9	2	1, 1±0	50%	
Rattus tanezumi	15	6	1-2, 1.27±0.46	21.10%	0.52 (4) ***

Sihanouk (Fig. 2c). When considering only single-species networks, none of the individual traits that we proposed was a significant predictor of module affiliation (except sex in *B. savilei* in Mondolkiri). Looking more closely at the standardized coefficients, a large difference between the coefficient of the multi-species network and that of a single-species network indicates that the effect of the trait on the probability that two individuals will co-occur in the same module changes upon inclusion of other species. In Sihanouk, for example, the effect of immunocompetence was stronger when considering only *Rattus exulans* than when considering all species. In contrast, sex had a relatively constant effect when considering all species and for each species in particular (Fig. 2c).

3.2 Network centrality in epidemiological networks

Results of model selection (Table S3) indicated that species identity was a strong determinant of the position of individuals in the multi-species TPNs in Buriram and Sihanouk, and to a lesser extent in Mondolkiri (Fig. 2d-f). Thus, individuals of particular species were consistently more central (we used eigenvalue centrality). We found differences among the multi-species networks in the importance of traits. For example, in Buriram the body mass

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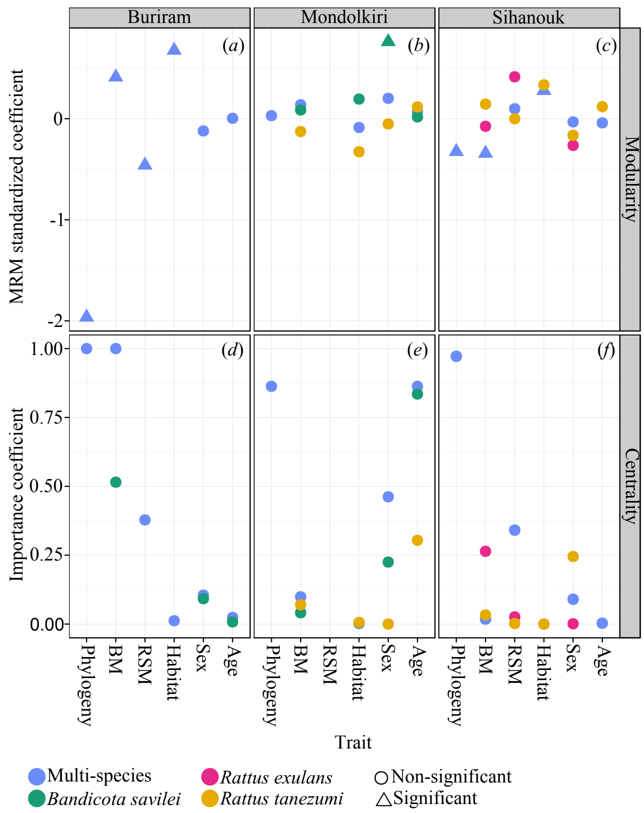

Fig. 2. Differences between multi- and single-species networks in traits that determine co-occurrence in modules and centrality of individuals (rows) for three localities (columns). (a-c) The PCA-standardized coefficients of a multiple-regression on matrices (MRM) procedure. (d-f) The importance of coefficients calculated from a multi-model inference procedure as the sum of model weights across all the models in which the coefficient appears (see Table S3). In (a-c) phylogeny is the taxonomic distance between two individuals and in (d-f) it is a factor depicting rodent species. BM body mass; RSM relative spleen mass to body mass (see Materials and Methods for details). Note that: (1) in Buriram the single-species network was not analyzed because it was not statistically significantly modular; (2) RSM was not included in the analyses in Mondolkiri due to an excess of missing cases; (3) Statistical significance is relevant only for (a-c).

of individuals was an important predictor of centrality whereas in Mondolkiri age was important (Fig. 2d-f). As with modularity, we found inconsistencies between the multi- and single- species networks in the importance of traits that affect centrality within a locality. For example, in Mondolkiri, age was

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an important predictor of centrality in the multi-species network and in the single-species network of *B. savilei* but not in that of *Rattus tanezumi*. In contrast, body mass was a poor predictor of centrality in the multi-species network and in both single-species networks (Fig. 2e).

The position of specific individuals in the single-species networks in relation to their respective multi-species networks was maintained for some host species but not for others as indicated by a correlation between the centrality of individuals in a particular single-species network and their centrality in the corresponding multi-species network (Fig. S2). For example, individuals of *Rattus norvegicus* in Sihanouk, which were very central in the multi-species network (high centrality), were peripheral (low centrality) in the single-species network, as indicated by a negative correlation coefficient (Fig. S2).

3.3 Parasite transmission dynamics

When controlling for network size and connectance, the density plots differed greatly between the single- and multi-sub-TPNs (with differential θ, the infection probability) in all five comparisons made: *B. savilei* in Buriram, *B. savilei* and *R. tanezumi* in Mondolkiri, and *R. exulans* and *R. tanezumi* in Sihanouk (Fig. 3). Specifically, the proportion of simulations with faster parasite spread (lower $\overline{TGI}$; see Methods) was greater in the single-species sub-TPNs. When not considering differential infection probability (i.e. fixed θ), the spread of the parasite became similar to that of the single-species network in Sihanouk (Fig. 3d,e), but not in Buriram and Mondolkiri (Fig. 3a-c).

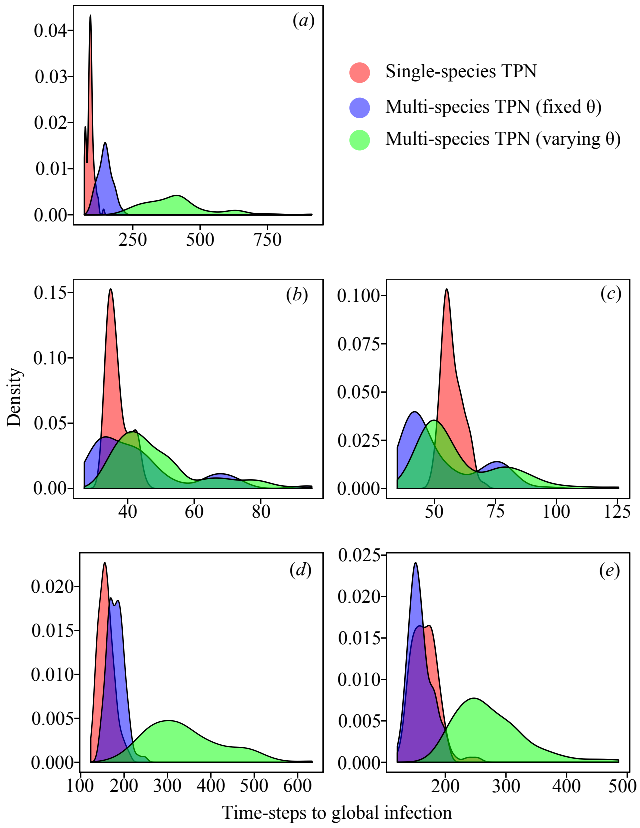

Fig. 3. Density plots depicting the distribution of time to global infection for transmission-potential networks (TPN) of equal size and connectance under three conditions: single-species, multi-species with θ varying among species (see Materials and Methods for details) and multi-species with fixed $\theta = 0.02$. Each panel describes comparison of single-species network in a certain locality: (a) *Bandicota savilei*in Buriram; (b) and (c) *B. savilei* and *Rattus tanezumi* in Mondolkiri, respectively; (d) and (e) *R. exulans* and *R. tanezumi* in Sihanouk, respectively. Plots skewed to the left indicate a faster infection and plot height is indicative of the probability that global infection occurs at a certain pace. Lack of overlap between the single-species and the multi-species plots means that the inclusion of other species changes the velocity of parasite spread.

4 Discussion

A primary aim of disease ecology is to understand host-parasite interactions and parasite spread in a particular environment [36]. At the species level, studies of host-parasite networks have provided insights into parasite sharing and the inter-dependence among hosts in the parasites that infect them

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[6, 23]. At the individual level, epidemiological network models have shed light on the dynamics of parasite spread among individuals within a host species (reviewed in [13]). Here, we integrated individual- and species-level network approaches. We found that a combined approach improves our understanding of host-parasite interactions and dynamics in host communities. By examining indices common to network ecology and using a simulation model, we offer a tractable framework to investigate the impact of multiple species in individual-based host-parasite networks. Below, we discuss our results in the light of the connection between ecology and epidemiology, we consider the assumptions and limitations of our study, and we identify avenues for future research.

4.1 Single-species vs multi-species networks

We found that the structure of bipartite individual-based ecological networks differed between multi- and single-species networks, partially supporting our first hypothesis that predicted stronger modularity in multi-species networks. This indicates that species-related traits – such as diet – shape the structure of individual-based ecological networks. Individual heterogeneity in some traits – such as body mass – had similar effects in both multi-and single-species networks, while other traits had different effects in the different networks such as habitat. When scaling up from individual to species-level networks, this may affect the structure of the species-level network [7]. In addition, differences between localities point to the context-dependence of the network itself.

At the node level, the difference between single- and multi-species networks was more striking, indicating that the role individuals played in parasite transmission was a function of both sources of heterogeneity. Previous studies have emphasized the importance of transmission heterogeneity and the identification of super-spreaders [10, 22]. In our study and others, char-

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acteristics of super-spreaders were also identified [37]. Our results suggest that another source of heterogeneity involves differences among species. In support, individuals that were more central in the single-species networks were generally also more central in the multi-species networks.

4.2 Parasite dynamics in multi-host systems

Host heterogeneity is known to be important for parasite infectiousness [4, 15, 16, 38]. For example, the introduction of Grey squirrels infected with parapoxvirus caused a severe decline in a diseae-free population of Red squirrels in England [2]. Recently, Streicker et al. [16] have identified major sources of heterogeneity among host species (host abundance, infection rates and egg-shedding rates) which can help identify key host species for parasite transmission. Similarly, there were also previous efforts to understand parasite dynamics in multi-host systems, and these indicated that the ability of parasites to spread in multi-host systems depend on the relative contribution of within-species to cross-species transmission [1, 3, 14]. Therefore, dynamics is affected by both individual- and species-level sources of heterogeneity. However, current multi-host models of parasite dynamics assume a homogeneously-mixed population. Here, we made a first attempt to include multiple host species in an epidemiological network model based on ecological observations of host-parasite interactions.

Our simulations clearly showed that in networks of the same size and connectance, a parasite (potentially) spreads faster in single-species networks, supporting our prediction. This result was not only due to the relative contribution of within-species vs. cross-species transmission but also due to network structure because it was maintained in two of the three sites (Buriram and Mondolkiri), even when assuming that the parasite has an identical infection probability in different species. Studies that consider transmission only within a single species, as is common in current network models (e.g.

[39, 40]), may thus be overestimating the velocity of parasite spread, in line with previous studies that demonstrated a decreased infection probability with increasing species richness under certain conditions [15, 38]. However, this aspect should be further investigated because incorporation of several host species may have several effects on the system, depending on the particular dynamics of the pathogen in each of the species [15] and species traits related to their potential to encourage transmission [16].

Interestingly, the process of infection was context-dependent: While in Sihanouk assuming a constant infection probability (θ) for all species made parasite transmission velocity equal in single- and multi-species networks, in Mondolkiri the distributions of the multi-species networks greatly overlapped regardless of that assumption. This indicated that different networks may be affected by different processes: in Sihanouk individuals of different species shared parasites to a large degree, and thus a constant infection probability essentially transformed the multi-species network to a single-species one. Furthermore, there was a striking similarity in the shape of the curves between species within a locality in Mondolkiri and Sihanouk, indicating that network structure is probably more important than the species involved in the process of infection.

It is equally important to consider the parasite's perspective. For instance, the transmission potential of a parasite may differ across host species. Likewise, parasite virulence may be very high in one host species but low in another [4]. This may affect the dynamics of between-species transmission for different parasites. In multi-host systems in general [4], and networks in particular, a parasite may spread mostly among nodes of a single host species in the network regardless of their connectivity to nodes of other species. Yet, high connectivity between individuals of particular species in the network increases the likelihood of transmission to other host species. Such considerations can be incorporated into the multi-host network model

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by changing the parameters of cross-species transmission rates.

4.3 The use of transmission-potential networks

Using network models to study parasite dynamics in multi-host systems is advantageous because individuals of different species may not be homogeneously mixed as previously assumed (e.g. [2, 3]). We captured this effect by using parasite sharing as a predictor of parasite spread on our TPNs. At the interspecific host level parasites may be shared through processes occurring at different time scales: cross-species transmission (ecological time scales) and co-inheritance (evolutionary time scales). However, the mechanisms underlying parasite sharing at the host level are irrelevant for our TPNs because once two individuals of different species do share at least one parasite it is clear that they posses similar physiological (e.g. immune response) and ecological (e.g. diet and habitat preferences) traits that would most likely allow them to share a novel one.

One limitation of our approach is that for a widespread parasite, individuals may be connected on the TPN yet have no actual contact. A second limitation is that TPNs do not represent true individual contacts. However, a recent study by VanderWaal et al. [12] showed that a network based on shared *Escherichia coli* strains matched a network of social contacts in giraffes (*Giraffa caelopardalis*). This finding supports the assumption in our study that shared parasites reflect transmission pathways. However, this study was conducted on one host species. Thus, comparisons between a TPN and a true contact network are needed to further validate our approach. For multiple species, we are not aware of any data set that includes both individual contacts and parasite survey, but such data can be obtained. For example, spillover is possible between primate species sharing a physical space [41]. Following primate groups to document potential transmission edges (e.g. shared space, common food), while simultaneously

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collecting their faeces for a parasite survey would enable construction of a multiple-species network based on shared space use and a TPN.

When working at the individual level, several criteria must be satisfied. First, data collection should occur over a rather narrow time window and limited geographical space, depending on the animal's life history and the goal of capturing individual heterogeneity. For example, data aggregated over a reproductive and a non-reproductive season may lead to biased results because parasitism and social contacts may be affected by reproduction [37]. Second, capture probability should be equal among species, an assumption that in practice is difficult to achieve (although can sometimes be corrected statistically). Finally, transmission mode of parasites included in the ecological network should be taken into account. For instance, a TPN derived from an ecological network in which parasites are sexually-transmitted is likely to differ from one based on environmental transmission.

The method chosen for quantifying edge weights can also affect the results. Here, we assumed a linear proportion between the number of parasite species shared and parasite transmission potential. Other quantitative methods, such as those based on similarity indices (e.g. Jaccard), could also be applied. For many of these issues, computer simulations can be usefully applied to investigate options for constructing TPNs from patterns of parasite sharing.

4.4 Applicability and future directions

From a modelling perspective, applying other structural indices will undoubtedly provide new insights into individual-based multi-species networks. For example, the degree of specialization of individuals and parasite species in the host-parasite network can be measured [42]. On the epidemiological side, extensions of our SI model could allow for recovery or an exposed but non-infectious period. From a disease control perspective, understanding multi-species networksis essential because it provides a way to model spread across individuals of parasites or pathogens that can switch hosts. Insights from individual-based multi-species models may aid disease control efforts by identifying both individuals and species that require a tighter control, making the efforts more effective [22]. Furthermore, in systems where individual data has already been collected, such as in many parasite surveys (e.g. [43]), TPNs provide an immediate and low-cost method to understand individual heterogeneity and multiple species in disease transmission. Overall, TPNs provide a prediction for routes of disease spread, which is based on parasite sharing and can be investigated for additional parasites or over time.

Some previous studies have looked at mutualistic networks, limited to a single species [44, 45, 46, 47]. The main conclusion of these studies was that the individual level provides new insights to how mutualistic networks operate. Our view is more focused towards parasite transmission yet our conclusion is similar: by downscaling to the individual level, we can gain new insights into the ecological mechanisms that underlie host-parasite interactions. From an epidemiological perspective, our method provides an indication of transmission pathways, with differences in parasite dynamics between single and multi-species networks. We therefore advocate the use of multiple species in individual-based ecological and epidemiological networks due to potential effects on parasite transmission.

In conclusion, the greatest advantage of our approach is that insights can be gained from ecological and epidemiological perspectives alike for a complete picture of the infection process. In this way, we provide novel insights into how disease is transmitted within a community or assemblage, thereby opening a new avenue of research in the interface between ecology and epidemiology.

Acknowledgments

We thank Miguel A. Fortuna for helpful comments and for providing code for modularity analysis. We thank Henrique C. Giacomini for providing MatLab scripts for MRM analysis and Julien Claude for helping with phylogenetic tree construction. We thank the CERoPath Project team for sample collection and analysis. This work was supported by the French ANR 07 BDIV 012 CERoPath Project and by the ANR 11 CPEL 002 BiodivHealthSEA. SP was funded by the Kreitman Foundation Fellowship, Ben-Gurion University of the Negev. This is publication XXX of the Mitrani Department of Desert Ecology.

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KEY: Barton2013

ANNOTATION: R package version 1.9.0

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SI Supplementary Information

Supplementary Figures

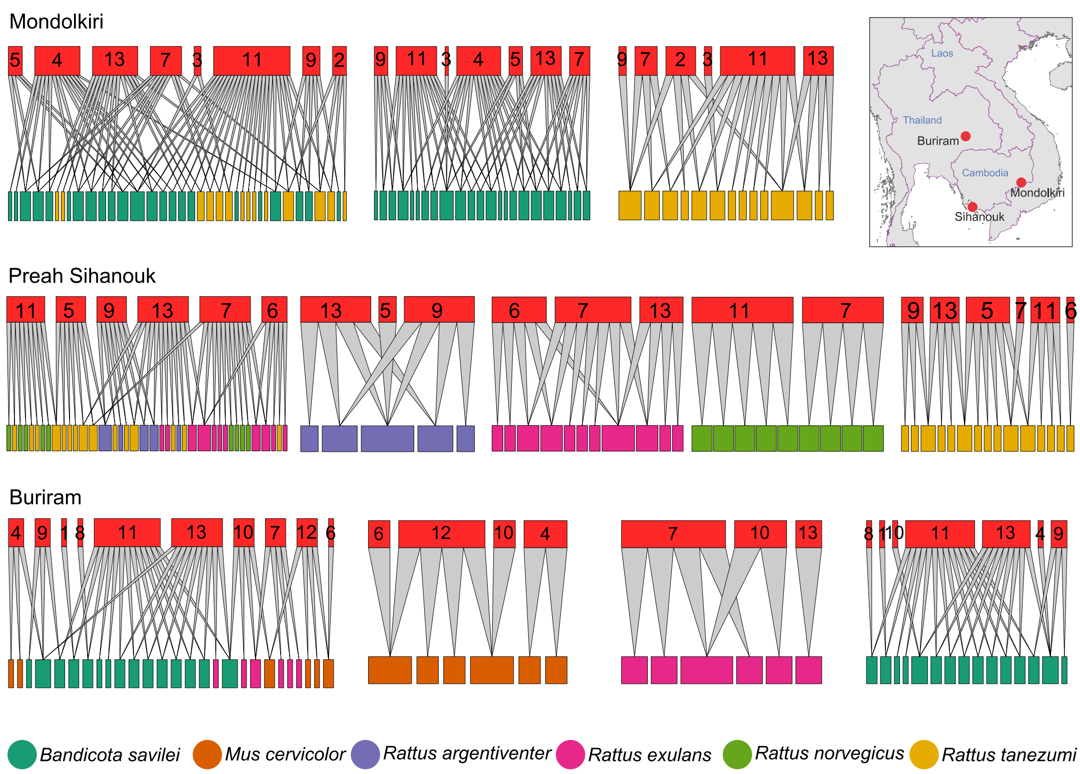

Fig. S1. **Networks at the three localities.** The leftmost network at each locality is the multi-species network. In each network helminth taxa (upper nodes) are in red and their ID numbers correspond to Table S1. Lower nodes are individual rodents, and their color represents their species. Width of rectangles is proportional to the number of individuals infected by a parasite (higher rectangles) or the number of parasite species an individual is infected by (lower rectangles). Inset: a map of the general region of the capture localities. Bipartite graphs were made using package 'bipartite' in the R environment.

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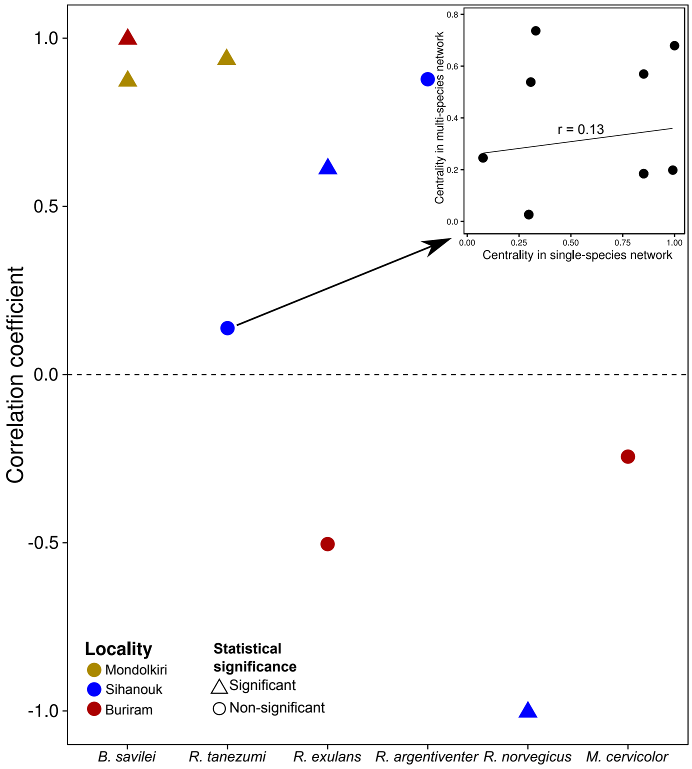

Fig. S2. **Coefficients of Pearson correlation between the rescaled eigenvalue centrality of individuals of a particular species in the single-species network and in the multi-species network.** Inset: an example for Rattus tanezumi in Sihanouk. Note that in the inset data points represent individuals, with some overlapping data points (i.e. individuals with identical centrality values).

SI.1 Study system and data set

We used data from the Community Ecology of Rodents and their Parasites (CERoPath) project. An extensive description of the field and laboratory methodology (including helminths surveys) applied in the project can be found in refs. [24, 48, 49] and in the CERoPath website (www.ceropath.org). Briefly, rodents were trapped at three human-disturbed localities: Buriram (14°89'N; 103°01'E; Thailand), Mondolkiri (12°12'N; 106°89'E; Cambodia) and Sihanouk (10°71'N; 103°82'E; Cambodia) (Fig. S1). Trapping was conducted during the dry season in November 2008 (Sihanouk and Buriram) and November 2009 (Mondolkiri). At each locality, 30 lines of ten traps, distanced 1 to 5 km from one other were set over four days. The traps were evenly distributed among four habitat types: forest (natural forest and tree plantations); non-flooded upland (shrub, orchards and upland agriculture); lowland flooded areas (rice paddies); and peridomestic locations (houses and immediate surrounding areas).

Trapped rodents were euthanized and dissected. The stomach, small intestine and large intestine were separated and examined for helminth infection under a stereo-microscope. The collected helminths were preserved in 70% alcohol and identified according to general helminth identification keys as referenced in [24, 49]. All work with animals was approved by the French National Research Agency, project ANR 07 BDIV 012. Animals were treated in accordance with the guidelines of the American Society of Mammalogists and with the European Union legislation (Directive 86/609/EEC). Each trapping session was validated by the national, regional and local health authorities, and including the oral agreement of local land owners. Approval notices for trapping and investigating rodents were given by the Ethical Committee of Mahidol University, Bangkok, Thailand, number 0517.1116/661 based on the validation of the rodents trapping book protocols of CERoPath.

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Across the three localities, the three multi-species networks had 27-40 individuals from 2-4 rodent species infected by 6-10 helminth taxa. The single-species networks had 5-23 individuals infected by 2-7 helminths. Helminth richness (number of helminth taxa infecting an individual rodent) ranged between 1 and 4. When averaged across individuals within each network, mean helminth richness ranged between 1 and 2.13. The prevalence of each helminth in each rodent species is indicated in Table S2. Network connectance was higher in the single-species compared to the multi-species networks within each locality in most cases (Table 1).

Table S1. **Information on helminth taxa used in this study.** Data are from [49] and Palmeirim et al. (unpublished). B Buriram; M Mondolkiri; S Sihanouk. Helminths in the table are gastrointestinal parasites transmitted via fecal-oral pathways. We included helminths with direct mode of transmission because our preliminary work indicated that removing those helminths did not change our results, but decreased the viability of the analysis due to low parasite richness. ID corresponds to that in Fig. S1

ID	Species	Locality	Group	Transmission	Vector	Zoonotic
1	*Aonchotheca sp*	B	Nematoda	Direct		
2	*Capillaria sp 1*	M	Nematoda	Direct		
3	*Echinostoma malayanum*	M	Trematoda	Indirect	Snail	+
4	*Eucoleus sp*	M,B	Nematoda	Direct		
5	*Ganguleterakis spumosa*	M,S	Nematoda	Indirect	Arthropod	
6	*Gongylonema neoplasticum*	S,B	Nematoda	Indirect	Arthropod	
7	*Hymenolepis diminuta*	M,S,B	Cestoda	Indirect	Arthropod	+
8	*Notocotylus sp*	B	Trematoda	Indirect	Snail	
9	*Physaloptera ngoci*	M,S,B	Nematoda	Indirect	Arthropod	
10	*Protospiura siamensis*	B	Nematoda	Indirect	Arthropod	
11	*Raillietina sp.*	M,S,B	Cestoda	Indirect	Arthropod	+
12	*Rodentolepis nana*	B	Cestoda	Indirect	Arthropod	
13	*Syphacia muris*	M,S,B	Nematoda	Direct		

Table S2. **Prevalence of helminths in rodent species in the three localities.** Bs–*Bandicota savilei*; Mc–*Mus cervicolor*; Re–*Rattus exulans*; Rn–*Rattus norvegicus*; Rt–*Rattus tanezumi*. Empty cells indicate that the helminth taxa did not occur in the locality.

	Buriram			Mondolkiri		Sihanouk			
	Bs	*Mc*	*Re*	*Bs*	*Rt*	*Ra*	*Re*	*Rn*	*Rt*
Aonchotheca sp	0.067	0	0						
Capillaria sp 1				0	0.29				
Echinostoma malayanum				0.04	0.07				
Eucoleus sp	0.067	0.333	0	0.57	0				
Ganguleterakis spumosa				0.17	0	0.2	0	0	0.4
Gongylonema neoplasticum	0	0.167	0			0	0.45	0	0.07
Hymenolepis diminuta	0	0	0.667	0.26	0.21	0	0.64	0.44	0.07
Notocotylus sp	0.067	0	0						
Physaloptera ngoci	0.200	0	0	0.17	0.07	0.8	0	0	0.2
Protospiura siamensis	0.067	0.167	0.333						
Raillietina sp	0.867	0	0	0.52	0.71	0	0	0.56	0.27
Rodentolepis nana	0	0.667	0						
Syphacia muris	0.600	0	0.167	0.39	0.29	0.8	0.36	0	0.27

SI.2 phylogenetic tree construction

We built a phylogenetic tree (Fig. S3) based on molecular data of the cytochrome b mitochondrial gene. We compiled cytochrome b sequences from the NCBI gene bank and used a maximum likelihood analysis with the GTR+G+I substitution model of molecular evolution with the aid of the function phymltest in the R package ape [50]. To ensure that our results were not affected by the way we constructed the tree, we re-ran analyzes with a tree from [51], but that did not include *Mus cervicolor*. The results were qualitatively the same.

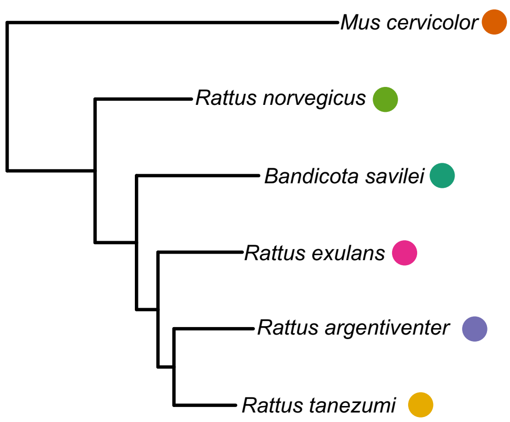

Fig. S3. Phylogenetic tree. Colours match those of Fig. S1.

SI.3 Analysis of network modularity

In the ecological networks, we identified modules of rodents that interact with similar parasites with an algorithm that finds the maximization of the modularity function M [26]. We tested for significance of M by comparing the observed value to those derived from 100 random networks generated with the probabilistic model used by [52, 53]. This null model suited our study system because it assumes that the probability of drawing an edge between a rodent individual and a parasite species is proportional to the susceptibility of the rodent to parasites (i.e. it considers the number of parasites infecting the individual) and to the infection potential of the parasite (i.e. it considers the number of individuals infected by the parasite).

The value of the modularity function M may be affected by network size or connectance. In our case, in each locality, the multi-species network was larger than each of the single-species networks and its connectance was lower (see Table 1 for exact number of individuals). To ensure that M of the multi-species network (M_m) was not affected by network size or connectance, we sub-sampled each of the multi-species networks 100 times as follows. In each of the 100 iterations we randomly chose n individuals, where n corresponds to the number of individuals in a single-species network to which comparison was made. We held the proportion of species constant. For example, in the original multi-species network in Mondolkiri, *Bandicota savilei* accounted for 62% of the individuals (23 of 37) and *Rattus tanezumi* for 38%. These proportions were kept for each sub-network.

Connectance of the sub-network was equalized to that of the single-species network by randomly removing edges from the sub-network. It was impossible to set the number of parasites equal to the original multi-species network because removal of individuals entailed removal of parasites. However, only sub-networks with at least six parasites were considered.

Under these conditions, we made four comparisons: *B. savilei* in Buriram; *B. savilei* and *R. tanezumi* in Mondolkiri; and *R. tanezumi* in Sihanouk. We then calculated M for each of the 100 sub-networks in each comparison to produce a distribution of 100 values of M per locality. We examined where in the distribution M_m falls. If M_m does not fall beyond the 2.5% or 97.5% extremes, then our conclusions hold (i.e. a two-tailed permutation test). Below are the four histograms, with a red arrow indicating M_m. Only in Buriram was M_m affected by network size/connectance, but this can be discarded since the single-species network of *B. savilei* in Buriram was not significantly modular (see Table 1 in Main Text).

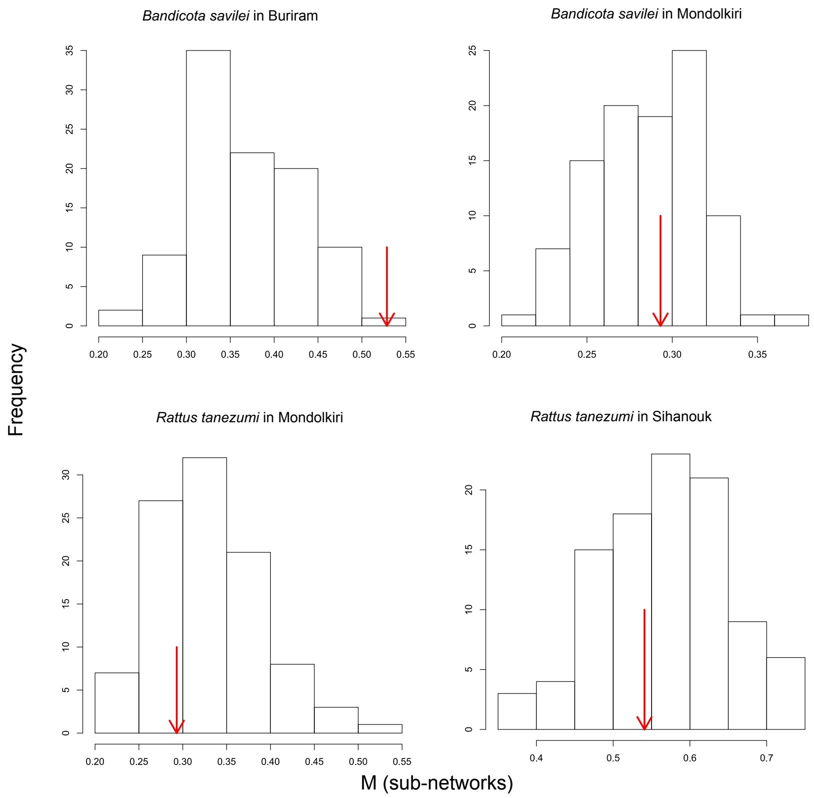

Fig. S4. Comparisons of modularity values M for multi-species networks with the same size and connectance as single-speceis networks.

SI.4 Effect of individual traits on modularity

We tested the effect of individual traits on module composition with logistic multiple regression on distance matrices [54]), following [5]. Briefly, we defined the response matrix $\mathbf{R}$ as a binary adjacency matrix where $\mathbf{R}_{ij}$ received a value of 1 if rodents i and j occurred in the same module and 0 otherwise. Each of the explanatory matrices described pairwise differences between individuals in a certain trait. For continuous traits, the difference was calculated as an absolute difference; for a discrete trait, 1 was assigned if the two individuals had the same trait value (e.g. both were males), and 0 if they differed in the trait. In the multi-species networks, we included an explanatory variable matrix that contained patristic distances (sum of

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phylogenetic branch lengths) as a measure of phylogenetic distance between a pair of rodents. We only analysed networks with > 10 individuals and omitted RSM from analyses in Mondokiri because data were missing for seven individuals.

SI.5 Construction of sub-TPNs

Our goal was to compare TGI between a multi-species TPN and a single-species TPN (with > 10 individuals) within the same locality. It is inappropriate, however, to compare networks of different sizes and connectance (i.e. the number of realized interactions divided by the number of possible ones). To control for different size and connectance while comparing multi-species TPNs to their respective single-species TPNs (within the same locality) we built 100 multi-species sub-TPNs by randomly sampling the original one to match the number of individuals of the single-species TPN. We kept the proportion of individuals of different species in the sub-TPN equal to that of the original multi-species TPN. For example, in the original multi-species network in Mondolkiri, *Bandicota savilei* accounted for 62% of the individuals (23 of 37) and *Rattus tanezumi* for 38%. These proportions were kept at each of the 100 sub-networks.

We also kept the connectance of the sub-TPNs constant to that of the original TPN. The connectance of the single-species TPN was always higher than that of the multi-species TPN (Table 1 in Main Text). Therefore, we built 100 single-species sub-TPNs by randomly removing edges from the original one to adjust for the connectance of the original multi-species network. The result was a set of 100 multi-species sub-TPNs and a set of 100 single-species sub-TPNs of equal size and connectance. For each of these 200 sub-TPNs we generated a distribution of 100 TGI values by randomly selecting individuals as starting points. We used the distribution of 100 mean TGI values (averaged for each sub-TPN) to examine differences

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between the single- and multi-species TPNs.

Table S3. Comparison of models used for multi-model inference. Models were obtained with a backwards stepwise regression starting from the global model and are ranked from the most to the least supported according to corrected Akaike information criteria (AICc). The global models contained all possible variables. Variables with missing cases (e.g. RSM in Mondokiri) or with no variation (e.g. when all individuals belonged to one sex) were excluded. ΔAICc – difference in AICc between the current and best model; w_i – model probabilities. Species – host species; BM – body mass; RSM – relative spleen mass to body mass (see Methods for details); EC – eigenvalue centrality; ^{G} – global model.

Rank	**Model structure**	**ΔAICc**	w_i
Buriram multi-species			
1	*EC Species + BM*	0	0.62
2	*EC Species + BM + RSM*	1.651	0.27
3	*EC Species + BM + RSM + Sex*	4.078	0.08
4	*EC Species + BM + RSM + Sex + Age*	7.887	0.01
5^G	*EC Species + BM + RSM + Sex + Age + Habitat*	7.887	0.01
6	(Null)	29.644	0
Buriram *Bandicota savilei*			
1	(Null)	0	0.49
2	*EC BM*	0.272	0.42
3	*EC BM + Sex*	3.511	0.08
4^G	*EC BM + Sex + Age*	8.176	0.01
Mondolkiri multi-species			
1	*EC Species + Age*	0	0.4
2	*EC Species + Sex + Age*	0.2	0.36
3	(Null)	2.146	0.14
4	*EC Species + BM + Sex + Age*	2.843	0.1
5^G	*EC Species + BM + Habitat + Sex + Age*	10.407	0
Mondolkiri *Bandicota savilei*			
1	*EC Age*	0	0.61
2	*EC Sex + Age*	2.399	0.18
3	(Null)	2.621	0.17
4	*EC BM + Sex + Age*	5.586	0.04
5^G	*EC BM + Habitat + Sex + Age*	10.211	0
Mondolkiri *Rattus tanezumi*			
1	(Null)	0	0.7

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Table S3 – continued from previous page

Rank	Model structure	ΔAICc	w_i
2	*EC Age*	2.174	0.24
3	*EC BM + Age*	4.768	0.06
4	*EC BM + Habitat + Age*	9.658	0.01
5^G	*EC BM + Habitat + Sex + Age*	16.144	0
Sihanouk multi-species			
1	*EC Species*	0	0.63
2	*EC Species + RSM*	1.84	0.25
3	*EC Species + RSM + Sex*	4.348	0.07
4	(Null)	6.197	0.03
5	*EC Species + BM + RSM + Sex*	7.496	0.02
6	*EC Species + BM + RSM + Sex + Age*	10.88	0
7^G	*EC Species + BM + RSM + Habitat + Sex + Age*	15.052	0
Sihanouk *Rattus exulans*			
1	(Null)	0	0.74
2	*EC BM*	2.254	0.24
3	*EC BM + RSM*	6.731	0.03
4^G	*EC BM + RSM + Sex*	14.034	0
Sihanouk *Rattus tanezumi*			
1	(Null)	0	0.76
2	*EC Sex*	2.533	0.21
3	*EC BM + Sex*	6.423	0.03
4	*EC BM + RSM + Sex*	11.743	0
5^G	*EC BM + RSM + Sex + Habitat*	28.103	0