A Tale of Two Phylogenies: Comparative Analyses of Ecological Interactions

Jarrod D. Hadfield,[1,*] Boris R. Krasnov,[2] Robert Poulin,[3] and Shinichi Nakagawa[3]

1. Institute of Evolutionary Biology, University of Edinburgh, United Kingdom; 2. Mitrani Department of Desert Ecology, Swiss Institute for Dryland Environmental and Energy Research, Jacob Blaustein Institutes for Desert Research at Ben-Gurion University of the Negev, Israel; 3. Department of Zoology, University of Otago, New Zealand

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ABSTRACT: The evolution of traits involved in ecological interactions such as predator-prey, host-parasite, and plant-pollinator interactions, are likely to be shaped by the phylogenetic history of both parties. We develop generalized linear mixed-effects models (GLMM) that estimate the effect of both parties' phylogenetic history on trait evolution, both in isolation but also in terms of how the two histories interact. Using data on the incidence and abundance of 206 flea species on 121 mammal species, we illustrate our method and compare it to previously used methods for detecting host-parasite coevolution. At large spatial scales we find that the phylogenetic interaction effect was substantial, indicating that related parasite species were more likely to be found on related host species. At smaller spatial scales, and when sampling effort was not controlled for, phylogenetic effects on the number and types of parasite species harbored by hosts were found to dominate. We go on to show that in situations where these additional phylogenetic effects exist, then previous methods have very high Type I error rates when testing for the phylogenetic interaction. Our GLMM method represents a robust and reliable approach to quantify the phylogenetic effects of traits determined by, or defined by, ecological interactions and has the advantage that it can easily be extended and interpreted in a broader context than existing permutation tests.

Keywords: phylogeny, host-parasite, comparative method, networks, mammals, fleas.

Most phylogenetic comparative analyses assume that evolution in a focal species is not influenced by evolutionary change in other taxa (Ives and Godfray 2006; Nunn 2011). However, for traits involved in ecological processes such as antagonistic interactions (e.g., interspecific competition and predator-prey and host-parasite interactions) and mutualistic interactions (e.g., plant-pollinator interactions), this assumption is unlikely to hold. Indeed, in many cases, it is hard to classify traits—such as virulence—as being the sole property of one species in an ecological interaction.

Dealing with such evolutionary processes requires the consideration of the phylogenetic histories of both sets of participants in an ecological interaction, either as main effects and/or as interactions. To take virulence in host-parasite systems as an example, we may consider the main effect of parasite history as one in which related parasites have similar virulences irrespective of the host they are in, and we may consider the main effect of host history as one in which related hosts have similar susceptibility irrespective of the parasite that infects them (Morand and Poulin 2003). However, it is also reasonable to suggest interaction effects. The most common form of interaction discussed is usually that between the two histories, which we call the coevolutionary interaction. Here, related parasites are expected to have more similar virulences when infecting related hosts than when infecting unrelated hosts even when the average susceptibility of hosts is invariant (cf. Poulin et al. 2011). However, other types of interaction are possible. For example, related parasites may have similar virulences when infecting a specific set of hosts irrespective of whether those hosts are related or not, and also related hosts may have similar susceptibilities when infected by specific sets of parasites irrespective of whether those parasites are related or not. For ease, we will refer to these effects as evolutionary interactions to distinguish them from the coevolutionary interaction which depends on the phylogenetic history of both sets of participants.

In some cases, it is only natural to consider the coevolutionary interaction. For example, if the focus of an analysis is to explore the incidence of parasite species across hosts, then main effects and the evolutionary interactions are absent in systems where parasites infect only a single host and hosts are infected only by a single parasite. In this case, there is no variation in the parasites' general ability to infect hosts, nor is there variation in the hosts'

* Corresponding author; e-mail: j.hadfield@ed.ac.uk.

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general ability to avoid parasitism. Moreover, the set of parasites infecting each host is of size 1 and unique, so related hosts cannot harbor more similar parasite assemblages than unrelated hosts. Nevertheless, processes such as cospeciation of parasites with their hosts will naturally lead to related parasites living on related hosts and the coevolutionary interaction term becomes important in predicting the incidence of parasitism. Many methods exist for analyzing such data (Brooks 1990; Page 1994; Charleston 1998; Huelsenbeck et al. 2000; reviewed in Stevens 2004).

For cases where hosts have multiple parasites and/or parasites have multiple hosts, fewer methods are available. Legendre et al. (2002) developed a statistic for measuring coevolution between hosts and parasites and used permutation to generate a null distribution to which it could be compared. Similarly, Hommola et al. (2009) developed an alternative statistic and permutation scheme that could be considered an extension of the commonly used Mantel test (e.g., Hafner and Nadler 1990) to situations where there is not a one-to-one correspondence. However, in both cases the permuted data are not exchangeable when main effects and/or evolutionary interaction effects exist. Such situations may lead to inflated Type I error rates if the chosen summary statistics depend on patterns generated by these additional processes. In an alternative nonparametric approach, Krasnov et al. (2012) used network analysis to assign host and parasite species to modules by maximizing the modularity statistic (Newman and Girvan 2004) developed for unipartite networks (see also Fortuna et al. 2010). The correlation between phylogenetic distance and the species co-occurrence index was used to measure phylogenetic signal in community structure. Although the approach cannot differentiate between a scenario where both evolutionary interaction effects exist and a scenario where only the coevolutionary interaction exists, we discuss it here because it has previously been applied to our test data set.

In this article, we develop a generalized linear mixed model (GLMM) approach to overcome some of the difficulties associated with previous methods. Although our results are applicable to a wide range of ecological interactions we focus on host-parasite associations and showcase our method with an analysis of data from 206 parasitic flea species collected from 121 host mammal species in 51 regions of the Palearctic. We separate patterns of incidence into three sources of phylogenetic signal: host/parasite specialism-generalism, host/parasite evolutionary interactions, and the coevolutionary interaction. Independently, Rafferty and Ives (2013) developed a linear mixed model of the same form and applied it to plants and their pollinators. The work presented here offers new empirical insights into host-parasite community structure and complements the methodological work of Rafferty and Ives (2013) by presenting strategies for dealing with non-Gaussian data, spatial replication, and sampling bias. In addition, we also use simulation to explore the properties of previously developed methods that test for host-parasite coevolution effects. We find that when phylogenetic signal in host/parasite specialism-generalism exists or the evolutionary interaction effects exist, the proposed statistics and permutation schemes of Legendre et al. (2002), Hommola et al. (2009), and Krasnov et al. (2012) do indeed result in high Type I error rates. In contrast, the framework that we present can adequately control for these additional effects if they are fitted and is still able to identify the coevolutionary interaction when it exists.

Material and Methods

The Study System

Data were obtained from published surveys that reported flea distribution and abundance on small mammals (Soricomorpha and Rodentia) in 51 different regions of the Palearctic (data files and analysis scripts are available from the Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.jf3tj; Hadfield et al. 2013). These sources provided data on the number of individuals of a particular flea species found on a given number of individuals of a particular host species. In total, 536,000 individuals from 121 mammal species were sampled and 1,692,000 individuals from 206 flea species. Notably, commensal rodents (*Mus* and *Rattus*) and fleas with cosmopolitan distributions (e.g., *Xenopsylla cheopis*, *Nosopsyllus fasciatus*) that were likely introduced to many regions with humans, domestic animals, and synanthropous rodents were omitted from the analysis. The same was done for introduced host species (e.g., *Ondatra zibethicus*).

Phylogenetic trees of fleas were based on the only available molecular phylogeny of fleas (Whiting et al. 2008), which includes 128 flea species belonging to 83 genera. We used the maximal parsimony version of flea phylogeny by Whiting et al. (2008). Our data sets included most of the genera present in the data set of Whiting et al. (2008), although it was not the case at the species level. Consequently, the positions of flea species that were not represented in the original tree of Whiting et al. (2008) were determined according to their morphologically derived taxonomy (see taxonomic references in Poulin et al. 2006). Because the only available information on the vast majority of fleas is limited to brief morphological descriptions and dichotomous identification keys, within-genus topology was established according to the subdivision of genera into subgenera and/or species groups and/or was based on morphological characters used for identification. All

branch lengths were arbitrarily set to an equal length of 1, and the tree was made ultrametric using the Mesquite (Maddison and Maddison 2011) function ultrametricise (as in Krasnov et al. 2012). In addition we also tested how robust our results were to alternative trees generated using different functions for calculating branch lengths (see the supplementary material, available online). For hosts, we used the global phylogenetic supertree for mammals of Bininda-Emonds et al. (2007) as a source of phylogenetic information (topology and branch lengths; an ultrametric tree).

The Model

Following Ives and Godfray (2006), imagine an $n \times m$ data matrix $\mathbf{Y}$ with rows indexed by n hosts and columns indexed by m parasites such that y_{ik} is some measured property of the interaction between host i and parasite k. Vectorizing $\mathbf{Y}$ ($\mathbf{y} = \text{vec}(\mathbf{Y})$) Ives and Godfray (2006) derived a model for the covariance structure of the data after conditioning on a set of linear predictors ($\boldsymbol{\eta} = \mathbf{X}\boldsymbol{\beta}$), $\mathbf{W} = \text{E}[(\mathbf{y} - \boldsymbol{\eta})(\mathbf{y} - \boldsymbol{\eta})']$, up to proportionality:

$$\mathbf{W} \propto \mathbf{U} \otimes \mathbf{V}, \tag{1}$$

where $\otimes$ is the Kronecker product and

$$\mathbf{U} = \frac{d_{\text{p}}^{2(\mathbf{J}^{(\text{p})} - \mathbf{A}^{(\text{p})})} - \mathbf{J}^{(\text{p})} d_{\text{p}}^2}{1 - d_{\text{p}}^2},$$

$$\mathbf{V} = \frac{d_{\text{h}}^{2(\mathbf{J}^{(\text{h})} - \mathbf{A}^{(\text{h})})} - \mathbf{J}^{(\text{h})} d_{\text{h}}^2}{1 - d_{\text{h}}^2} \tag{2}$$

are the matrix analogues of equation (A1) of Ives and Godfray (2006). Term $\mathbf{A}$ is a phylogenetic (co)variance matrix scaled to a correlation matrix, $\mathbf{J}$ is a matrix of all 1s (a unit matrix), and d is an estimated parameter which can take values between 0 and 1. The superscripts (h) and (p) refer to host and parasite, respectively.

Here we propose two extensions to this model. The first is to extend the method so that it is explicit about the distribution of the data, and specifically the incidence data of our real-world example. The second is to modify and extend the covariance structure proposed by Ives and Godfray (2006) so that it is a sum of components that can be given easier and distinct biological interpretations. The first extension is made straightforward by introducing the concept of a latent variable, which is an implicit feature of GLMM generally (Hadfield 2010). Here, we can consider a matrix of real-valued latent variables $\mathbf{L}$ that are mapped, via the inverse link function, onto the parameters of a distribution from which the matrix of outcomes ($\mathbf{Y}$) are assumed to be drawn. In the context of our incidence data, $\mathbf{Y}$ is an incidence matrix such that y_{ik} is 1 if host i is parasitized by parasite k and 0 otherwise, and applying the inverse-logit transformation to l_{ik} gives the probability that $y_{ik} = 1$. When the outcome is not Gaussian we assume the covariance structure, $\mathbf{W}$, applies to $\text{vec}(\mathbf{L}) - \boldsymbol{\eta}$ rather than $\text{vec}(\mathbf{Y}) - \boldsymbol{\eta}$.

The second extension is to propose a covariance structure of the form (see Rafferty and Ives 2013):

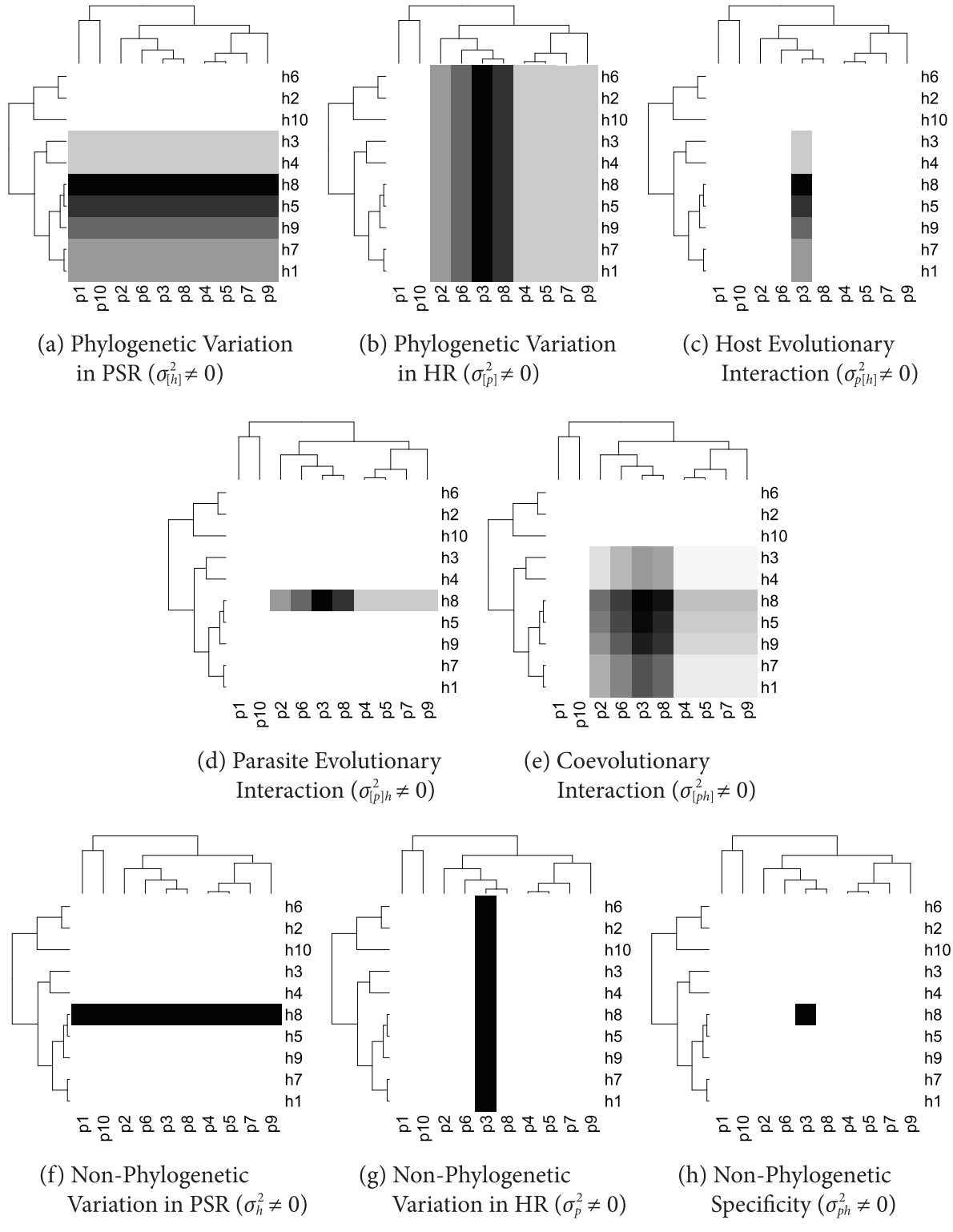
$$\begin{aligned}\mathbf{W}^{(a)} = {} & \sigma^2_{[\text{h}]}(\mathbf{J}^{(\text{p})} \otimes \mathbf{A}^{(\text{h})}) + \sigma^2_{[\text{p}]}(\mathbf{A}^{(\text{p})} \otimes \mathbf{J}^{(\text{h})}) \\ & + \sigma^2_{\text{p}[\text{h}]}(\mathbf{I}^{(\text{p})} \otimes \mathbf{A}^{(\text{h})}) + \sigma^2_{[\text{p}]\text{h}}(\mathbf{A}^{(\text{p})} \otimes \mathbf{I}^{(\text{h})}) \\ & + \sigma^2_{[\text{ph}]}(\mathbf{A}^{(\text{p})} \otimes \mathbf{A}^{(\text{h})}),\end{aligned} \tag{3}$$

where σ^2 represents variances and $\mathbf{I}$ identity matrices of appropriate dimension. As before we use h and p to designate host and parasite, and when defining variance components, we use square brackets to designate terms that refer to phylogenetic effects. In order to understand the implications of this covariance structure it is perhaps easier to consider the covariance between two specific pairs of interacting species, host i and parasite k versus host j and parasite l:

$$\begin{aligned}w^{(a)}_{ik,jl} = {} & \sigma^2_{[\text{h}]} a^{(\text{h})}_{i,j} + \sigma^2_{[\text{p}]} a^{(\text{p})}_{k,l} + \sigma^2_{\text{p}[\text{h}]} \delta_{k,l} a^{(\text{h})}_{i,j} \\ & + \sigma^2_{[\text{p}]\text{h}} \delta_{i,j} a^{(\text{p})}_{k,l} + \sigma^2_{[\text{ph}]} a^{(\text{p})}_{k,l} a^{(\text{h})}_{i,j}\end{aligned} \tag{4}$$

where $\delta_{i,j}$ is 1 if i and j are the same species and 0 otherwise, and a is an element of $\mathbf{A}$.

Because the variances are constrained to be positive, each term makes a nonnegative contribution to the covariance and therefore the similarity in outcome between the two pairs of interacting species. In order to give a graphical explanation of these terms, we generated a hypothetical phylogeny of 10 hosts and 10 parasites and plotted heatmaps of the covariances that arise from the different processes between all 100 combinations of j and l, given $i = 8$ and $k = 3$ (fig. 1). Given the species identities and their phylogenetic relationships, the magnitude of each contribution is determined by the magnitude of the associated variance. The first term is the contribution of the main effect of host phylogeny to the covariance, and following earlier literature (Vázquez et al. 2005) we refer to $\sigma^2_{[\text{h}]}$ as the variation in parasite species richness (PSR) explained by the phylogeny (fig. 1*a*). The second term is the contribution of the main effect of the parasite phylogeny to the covariance, and $\sigma^2_{[\text{p}]}$ is the variation in host-range (HR) explained by the phylogeny (fig. 1*b*). The third term is the contribution of the host evolutionary interaction to the covariance, and $\sigma^2_{\text{p}[\text{h}]}$ captures the degree to which related hosts have similar parasite assemblages irrespective of parasite phylogeny (hence the Kronecker delta, δ, for parasites; fig. 1*c*). The fourth term is the contribution of the parasite evolutionary interaction to the covariance, and $\sigma^2_{[\text{p}]\text{h}}$ captures the degree to which related



(a) Phylogenetic Variation in PSR ($\sigma^2_{[h]} \neq 0$)

(b) Phylogenetic Variation in HR ($\sigma^2_{[p]} \neq 0$)

(c) Host Evolutionary Interaction ($\sigma^2_{p[h]} \neq 0$)

(d) Parasite Evolutionary Interaction ($\sigma^2_{[p]h} \neq 0$)

(e) Coevolutionary Interaction ($\sigma^2_{[ph]} \neq 0$)

(f) Non-Phylogenetic Variation in PSR ($\sigma^2_h \neq 0$)

(g) Non-Phylogenetic Variation in HR ($\sigma^2_p \neq 0$)

(h) Non-Phylogenetic Specificity ($\sigma^2_{ph} \neq 0$)

Figure 1: Expected patterns of covariance between the outcome for each pair of interacting species and the outcome for the interaction involving parasite 3 and host 8. Darker shades of gray indicate a greater covariance such that for incidence data darker shades represent a greater chance of occurrence if parasite 3 is present on host 8.

parasites have similar host assemblages irrespective of host phylogeny (fig. 1*d*). The final term is the contribution of the coevolutionary interaction to the covariance, and $\sigma^2_{[\mathrm{ph}]}$ captures the degree to which related parasites live on related hosts (fig. 1*e*).

In addition, the components in equation (3) all involve at least one phylogenetic term, but it may be reasonable to introduce analogous components that only involve nonphylogenetic terms:

$$\mathbf{W}^{(s)} = \sigma^2_{\mathrm{h}}(\mathbf{J}^{(\mathrm{p})} \otimes \mathbf{I}^{(\mathrm{h})}) + \sigma^2_{\mathrm{p}}(\mathbf{I}^{(\mathrm{p})} \otimes \mathbf{J}^{(\mathrm{h})}) + \sigma^2_{\mathrm{ph}}(\mathbf{I}^{(\mathrm{p})} \otimes \mathbf{I}^{(\mathrm{h})}), \tag{5}$$

where we can express the covariance between two specific pairs of interacting species as

$$w^{(s)}_{ik,jl} = \sigma^2_{\mathrm{h}}\delta_{i,j} + \sigma^2_{\mathrm{p}}\delta_{k,l} + \sigma^2_{\mathrm{ph}}\delta_{i,j}\delta_{k,l}. \tag{6}$$

Here σ^2_{h} captures interspecific variation in PSR not due to phylogeny (fig. 1*f*), σ^2_{p} captures interspecific variation in HR not due to phylogeny (fig. 1*g*), and σ^2_{ph} captures associations between specific host and parasite species not due to phylogeny (fig. 1*h*). It should be noted that for incidence data σ^2_{ph} is not identifiable unless replicate incidence matrices have been sampled (e.g., in time or space).

The most fully parameterized model would therefore have covariance structure $\mathbf{W} = \mathbf{W}^{(a)} + \mathbf{W}^{(s)}$, and the relative importance of each contributing term could be expressed in terms of intraclass correlations (ICC). For a Gaussian response this would simply be each variance over the sum, but for non-Gaussian data, the ICC cannot usually be expressed so simply and depends on whether it is to be calculated on the latent scale or the data scale (reviewed in Nakagawa and Schielzeth 2010). For Bernoulli data and logit link the intraclass correlation on the latent scale for component i is $\sigma^2_i/(\sum_j \sigma^2_j + \pi^2/3)$. Under some ecological scenarios estimates of the variance components may be expected to be bound at 0, and a full treatment of this issue is left for the "Underdispersion" section of the supplementary materials).

Our model coincides with that of Ives and Godfray (2006) under three conditions: (*a*) if only the host phylogeny contributes to the covariance ($d_{\mathrm{p}} = 0$) and $d_{\mathrm{h}} = 1$, then this is equivalent to a model in which all terms are 0 except $\sigma^2_{\mathrm{p[h]}}$; (*b*) if only the parasite phylogeny contributes to the covariance ($d_{\mathrm{h}} = 0$) and $d_{\mathrm{p}} = 1$, then this is equivalent to a model in which all terms are 0 except $\sigma^2_{[\mathrm{p}]\mathrm{h}}$; and (*c*) if only the interaction contributes to the covariance and $d_{\mathrm{h}} = 1$ and $d_{\mathrm{p}} = 1$, then this is equivalent to a model in which all terms are 0 except $\sigma^2_{[\mathrm{ph}]}$. If $0 < d < 1$ rather than 1 then the associated phylogeny does contribute to the covariance although evolutionary change no longer follows the Brownian motion model and the increase in variance is not linear in time.

Sampling Effort

An important issue with such data is that $\mathbf{Y}$ is in general not a complete inventory of all interactions and that greater sampling will usually lead to more interactions being observed. Indeed, strong relationships between PSR and the number of individuals of the host species sampled was evident in our data, as was a strong relationship between HR and the number of individuals of the parasite species sampled. However, in this study, sampling effort will be proportional to the species' abundance (and trapping probability), and so it is unclear whether these relationships exist solely because of sampling effort or whether biological processes also contribute, for example, if common species actually have a greater range of possible parasites. If parasite counts were available for each individual of a host species, then the effects of host sampling effort and biological variation could be separated because sampling effects do not result in a positive relationship at the individual level (all individuals are equally well sampled), whereas the effects of biological variation will often remain. Unfortunately, the flea counts for each individual mammal were not recorded and only the aggregate counts for all individuals of each species in each region are available.

Moreover, even when the relationships between PSR, HR, and species abundances are driven solely by sampling effort, the necessity of controlling for it depends on the question at hand. On one hand, if the question concerns differences in possible and impossible interactions then it is important to control for sampling effort. On the other hand, if the question concerns the relative frequencies of different interactions, then the incidence data should be seen as a (very) coarse measure of interaction frequency, and sampling effort should not be controlled for. We consider the focus of this study to be the former, and so our main analysis has the logarithm of region-specific species abundances fitted as covariates. However, we note that by doing this, we may also be controlling for biological causes underpinning the relationships between PSR, HR, and species abundances. Whether these relationships have biological causes is important in this instance because both host abundance and parasite abundance have reasonably high phylogenetic signals—phylogenetic heritability (sensu Lynch 1991): $H^2_{\mathrm{h}} = 0.535$ (0.263–0.686) and $H^2_{\mathrm{p}} = 0.395$(0.239–0.522), respectively, suggesting that controlling for abundance may alter the magnitude of the host and parasite phylogenetic signals. A model without the covariates was also fitted in order to assess the magnitude of change.

Spatial Replication

An additional issue of importance with the mammal-flea data is the fact that they are collected in 51 regions across the Palearctic rather than in a single region. Rather than consolidate interactions across regions we chose to retain this aspect of the data and thus analyze the 51 region-specific incidence matrices (in fig. 2, we plot the incidence matrix for the Volga-Kama region, the region with the highest diversity: 28 mammal species and 35 flea species). In this model the scalar definitions in equations (4) and (6) remain identical, but each term in the matrix equations (eqq. [3] and [5]) must be pre- and postmultiplied by a design matrix $\mathbf{Z}$ relating observations (rows) to host-parasite combinations (columns), since each host-parasite pair may be present in multiple localities. For example, the covariance due to the coevolutionary interactions would be

$$\sigma^2_{[\mathrm{ph}]}\mathbf{Z}(\mathbf{A}^{(\mathrm{p})} \otimes \mathbf{A}^{(\mathrm{h})})\mathbf{Z}'. \tag{7}$$

In the model, region effects were also fitted as random in order to capture any variation in the proportion of possible host-parasite incidences that are realized. It should also be noted that $\mathbf{Z} = \mathbf{I}$ when only data from a single region is available and equations (3) and (5) are recovered.

An important decision with such data is how to code interactions that are not observed because either the host and/or parasite are not present in a region. One possibility is to delete the rows/columns of each incidence matrix that pertain to species not recorded in that region (i.e., columns/rows of structural zeros) and our question then concerns differences in possible and impossible interactions among sympatric hosts and parasites. Alternatively, these zeros could be treated as real zeros and our question would then concern differences in possible and impossible interactions among all hosts and parasites. In this study, we chose to focus on the former although we acknowledge that, due to nonexhaustive sampling effort, some of these structural zeros may in fact be real.

In contrast to separate analyses for each region, this analysis still utilizes information from between-region patterns in incidence. For example, imagine an ancestral mammal species (A) that speciated in allopatry giving rise to species B and C, which continue to live allopatrically. Imagine then that an ancestral flea species (a) also diverges and speciates in the same manner, either directly because of host speciation or because the same ecological and geographical factors that facilitated host speciation also facilitate parasite speciation. In such instances, the presence of flea b on mammal B in one region is informative about the probability that flea c lives on mammal C in the other region. In order to assess how our conclusions would change by ignoring between-region patterns completely, we also fitted analyses where all between-region covariances were set to 0. For example, ordering host-parasite combinations within regions the covariance due to coevolutionary interactions has the form

$$\sigma^2_{[\mathrm{ph}]} \oplus_{k=1}^{51} (\mathbf{A}_k^{(\mathrm{p})} \otimes \mathbf{A}_k^{(\mathrm{h})}), \tag{8}$$

where $\oplus$ is the direct sum and $\mathbf{A}_k$ is the phylogenetic (co)variance matrix for species present in region k. This model is equivalent to fitting separate models to each region but under the constraint that the (co)variance parameters are identical. The term σ^2_{ph} is not however identifiable because each host-parasite combination is only observed once within a region and was omitted from the analysis.

Model Fitting

We fitted the models using the R (R Development Core Team 2012) package MCMCglmm (Hadfield 2010) and ASReml (Gilmour et al. 2002). In both cases it is necessary to work with the $\mathbf{S}^{-1}$ parametrization rather than the $\mathbf{A}^{-1}$ parametrization, where $\mathbf{S}$ is a phylogenetic (co)variance matrix like $\mathbf{A}$ but with ancestral nodes retained (Hadfield and Nakagawa 2010). Although $\mathbf{S}$ is approximately four times larger than $\mathbf{A}$, its inverse is orders of magnitude sparser. Indeed, even storing $(\mathbf{A}^{(\mathrm{p})} \otimes \mathbf{A}^{\mathrm{h}})^{-1}$, with $> 2 \times 10^8$ nonzero elements would require 1.7 Gb of memory at double precision compared to the $< 10^6$ nonzero elements in $(\mathbf{S}^{(\mathrm{p})} \otimes \mathbf{S}^{\mathrm{h}})^{-1}$, which would require 5.5 Mb. Nevertheless, the computing remained slow because of the large data set and particularly the large product of the phylogeny dimensions ($n \times m \approx 25{,}000$) although the ASReml analyses, which use PQL (penalized quasi-likelihood) methods (Breslow and Clayton 1993), were orders of magnitude faster than the MCMCglmm analyses, which use MCMC (Markov chain Monte Carlo) methods. For the Bayesian MCMC approach, parameter-expanded priors were used for all variance components to give scaled F-distributions with numerator and denominator degrees of freedom set to 1 and a scale parameter of 10^3. The chain was run for 10^6 iterations with a burn-in of 2×10^5 and a thinning interval of 400.

Comparison with Other Methods

We calculated the test statistics ParafitGlobal (Legendre et al. 2002; S_{L}), the correlation between host and parasite shared branch lengths (Hommola et al. 2009; S_{H}) and MSEb (Ives and Godfray 2006; S_{I}). In the appendix we give the formal definitions of these statistics and show that S_{I} and S_{L} are closely related. In addition, we also followed a similar procedure to that in Krasnov et al. (2012)

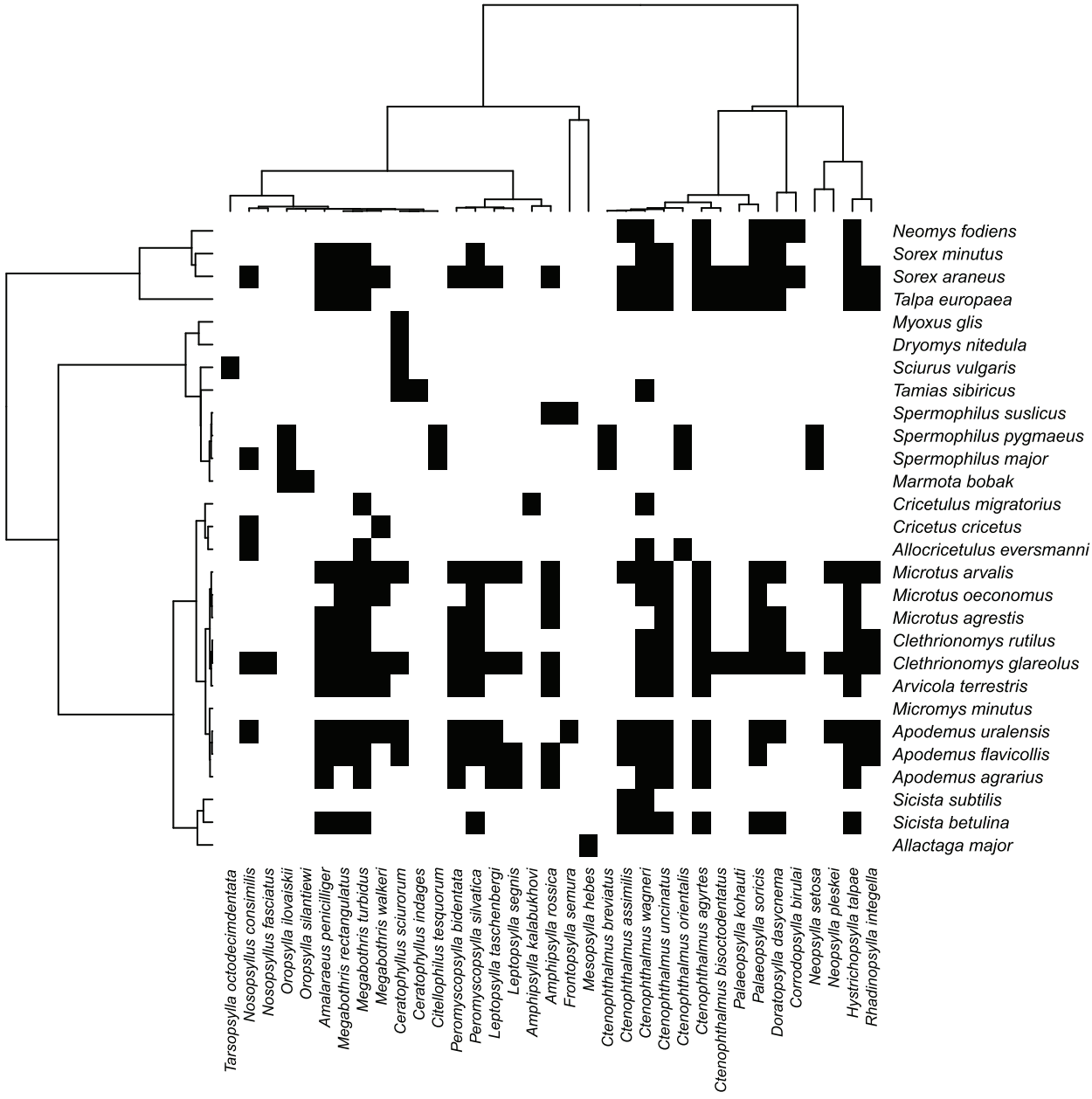


Figure 2: Incidence matrix of hosts (rows) and parasites (columns) in a single region (Volga-Kama), with the phylogenies of each plotted in the margins.

whereby species were assigned to modules by assuming the graph defined by the incidences is unipartite and then using the walktrap.community algorithm (Pons and Latapy 2005) as implemented in the R package igraph (Csardi and Nepusz 2006). We then calculated the correlation between module co-occurrence and host phylogenetic distance (S_{K_h}) and parasite phylogenetic distance (S_{K_p}) following Krasnov et al. (2012).

We generated null distributions using 1,000 permutations following the permutation scheme of Legendre et al. (2002) where rows of **Y** are permuted within columns and the permutation scheme of Hommola et al. (2009) where columns and rows of **Y** are permuted. The permutation scheme of Hommola et al. (2009) is such that the empirical distribution of row/column counts remains unchanged although the species labels are randomized. Consequently there is potential that the permutations result in a joint test for host-parasite coevolution, the phylogenetic signal

of generalism-specialism, and the evolutionary interaction terms in both hosts and parasites. The permutation scheme of Legendre et al. (2002) is such that the column counts remain unchanged but the order and empirical distribution of row counts are randomized. Thus, there is the potential of the permutation scheme to be a joint test for host-parasite coevolution, the evolutionary interaction terms, and the generalism-specialism of the hosts (irrespective of whether it has a phylogenetic signal or not). Given that assigning hosts to rows and parasites to columns is essentially arbitrary, it should be noted that the permutation scheme of Legendre et al. (2002) may also test the generalism-specialism of the parasites rather than the hosts.

We also calculated all statistics for each region in isolation, the results of which can be found in the supplementary materials. When some host species were present in a region but had no recorded parasites the resulting graph has isolated vertices so we removed these hosts when calculating the module-based statistics S_{K_h} and S_{K_p}. In addition, in some cases all hosts and/or all parasites were assigned to unique modules such that the correlation is not defined (because there is no variance in module co-occurrence).

Simulations Testing PQL versus MCMC

PQL methods (Breslow and Clayton 1993) for fitting GLMMs can behave poorly for Bernoulli data when there is little replication within levels of a grouping factor (Breslow and Lin 1995). For phylogenies, where the concept of a group is rather nebulous, the adequacy of PQL is questionable, but our results suggest that downward biases in the variance components are substantial, in accord with observations regarding pedigree analyses (Gilmour et al. 1985). However the speed of PQL over MCMC techniques is large (nearly 5,000 times faster). In order to verify that the discrepancies between the MCMC approach and the PQL approach were not due to programming error, we simulated 100 data sets using the posterior modes from the MCMC approach as parameters and refitted the model using ASReml. Engel and Buist (1998) extend results of Breslow and Lin (1995) and derive a correction factor for variance components estimated using PQL when random effects are correlated through a pedigree. We implement this for phylogenies and test its adequacy. In the supplementary materials, we provide results for simulating the interaction effects and computing the correction factor efficiently. Whether similar results could be employed to speed up the analyses (Van Loan 2000) remains an open problem.

Simulations Testing Other Methods

In order to compare our method against the properties of previously used statistics and permutation tests (Legendre et al. 2002; Hommola et al. 2009; Krasnov et al. 2012), we used the smallest simulation scheme of Legendre et al. (2002) with 10 hosts and 10 parasites. The trees were randomly generated using the rcoal function from the R package ape (Paradis et al. 2004), and the latent variables were simulated with zero mean and covariance structure given by equation (3). The incidence data were simulated from a Bernoulli distribution with probability equal to the inverse logit transformation of the latent variable. Four sets of parameter combinations were used. In the first we set all variances to 0 such that all null expectations are satisfied. In the second, we set all variances to 0 except those pertaining to phylogenetic signal in specialism-generalism which were set to 4 ($\sigma^2_{[h]} = \sigma^2_{[p]} = 4$). In the third, we set all variances to 0 except those pertaining to the evolutionary interactions which were also set to 4 ($\sigma^2_{p[h]} = \sigma^2_{[p]h} = 4$), and in the final set all variances were set to 0 except those pertaining to the coevolutionary interaction ($\sigma^2_{[ph]} = 4$).

We generated 500 data sets for each parameter set and calculated the three test statistics S_L, S_I, and S_H, defined in the appendix. Significance testing was performed using the permutation schemes of both Legendre et al. (2002) and Hommola et al. (2009), as described above. In addition, three models were fitted to each data set using MCMCglmm. The first model only included a coevolutionary interaction term, the second model included a coevolutionary interaction term and phylogenetic main effects for both hosts and parasites, and the third model included coevolutionary and evolutionary interaction terms. Parameter-expanded priors for the variance components were used as in the main analyses, but a weakly informative prior on the intercept (Gaussian with mean 0 and variance $5 + \pi^{2/3}$) was also employed because of instability when the number of incidences was extreme (>90/100 or <10/100). The chain was run for 65,000 iterations with a burn-in of 15,000 and a thinning interval of 50. Although this resulted in rather low effective sample sizes for the posterior coevolutionary interaction variance in the third model (~200), it allowed all models to be fitted to all data sets in a reasonable amount of time.

Using the mammal-flea data set, Krasnov et al. (2012) found evidence for host phylogenetic signal, but not parasite phylogenetic signal in module membership, suggesting that the host evolutionary interaction was the only source of phylogenetic effect. Because we found that the coevolutionary interaction dominated host-parasite interactions (see below), we performed three sets of simulations identical to those above where all variances were set to 0

except one (either $\sigma^2_{[h]} = 4$, $\sigma^2_{p[h]} = 4$, or $\sigma^2_{[ph]} = 4$). We generated 500 data sets for each parameter set and calculated the two statistics S_{K_h} and S_{K_p} and tested their significance using the permutation schemes of both Legendre et al. (2002) and Hommola et al. (2009).

Results

Mammal-Flea Data

In the main analysis of the mammal-flea incidence data, the phylogenetic variances through both the mammal and flea phylogenies were close to 0, indicating that related fleas do not have similar host ranges and related mammals do not have similar levels of parasite species richness. Whereas the flea evolutionary interaction term was close to 0, the mammal evolutionary interaction was moderately large, indicating that related mammals have similar assemblages of flea after taking into account the flea phylogeny. However, the mammal-flea coevolutionary interaction contributed the greatest variation suggesting that related fleas tend to be found on related mammals. (These results are summarized in table 1, MCMC-a.) In the supplementary materials we show that these conclusions are broadly similar if we use alternative methods to make the host phylogeny ultrametric, despite the resulting trees being quite different (tables S1, S2; tables S1–S8 available online).

When the model was fitted without controlling for sampling effort, the ICCs for interspecific variation in PSR (both phylogenetic [$r_{[h]}$] and nonphylogenetic [r_h]) increased and the ICCs for the mammal evolutionary interaction and the coevolutionary interaction decreased (table 1, MCMC-b). However, the coevolutionary interaction remained the dominant term. When the model was focused at smaller geographical scales by ignoring between-region information the relative importance of the coevolutionary interaction term dropped below that of the mammal evolutionary interaction term, although both had reasonably large ICCs (table 1, MCMC-c). When sampling effort was not controlled for in the analysis ignoring between-region information, the results were broadly consistent with the corresponding full model in that the magnitude of the main effects increased substantially and actually exceeded the magnitude of the coevolutionary interaction term (table 1, MCMC-d).

The ASReml estimates for the ICC were generally smaller than the MCMC estimates, with the coevolutionary ICC in particular being 77.8% that of the MCMC estimate in the main analysis (table 1, PQL-a). The means of the estimates from the simulated data were in reasonable agreement with the ASReml estimates from the actual data, suggesting that the discrepancy between the PQL and MCMC approach is primarily due to the known biases for PQL (table S3). These biases appeared particularly strong for the model that ignored between-region information (table S2) presumably because of the reduced amount of replication per random effect. Indeed, the variance parameters were very close to their true values if the latent variable was analyzed as a Gaussian trait (table S3). The correction factor for the coevolutionary variance parameter was 1.01, suggesting that the method of Engel and Buist (1998) may not be useful in this context. Extending results in Breslow and Lin (1995) and Engel and Buist (1998) to models with more than one variance component may reduce the bias further, although more accurate approxi-

Table 1: Estimates of the intraclass correlations (ICCs; designated as *r*) from the mammal-flea data

	MCMC-a	PQL-a	MCMC-b	MCMC-c	MCMC-d
Geographical region	.086 (.056–.130)	.089**	.062 (.042–.099)	.151 (.097–.237)	.000 (.000–.060)
Host phylogeny ($r_{[h]}$)	.000 (.000–.083)	.012	.062 (.000–.197)	.040 (.000–.070)	.082 (.032–.164)
Parasite phylogeny ($r_{[p]}$)	.000 (.000–.014)	.000	.000 (.000–.043)	.000 (.000–.007)	.000 (.000–.029)
Host evolutionary interaction ($r_{p[h]}$)	.114 (.062–.189)	.099**	.077 (.028–.124)	.226 (.183–.299)	.245 (.200–.311)
Parasite evolutionary interaction ($r_{[p]h}$)	.000 (.000–.009)	.000	.000 (.000–.006)	.000 (.000–.017)	.000 (.000–.013)
Coevolutionary interaction ($r_{[ph]}$)	.373 (.277–.443)	.290**	.333 (.252–.401)	.159 (.111–.224)	.126 (.080–.178)
Host species (r_h)	.019 (.000–.034)	.016*	.058 (.029–.105)	.036 (.016–.053)	.189 (.140–.230)
Parasite species (r_p)	.000 (.000–.015)	.000	.000 (.000–.045)	.000 (.000–.008)	.077 (.055–.110)
Species interaction (r_{ph})	.025 (.000–.051)	.000	.016 (.000–.035)		
ICC denominator	1.319 (9.241–11.552)	6.657	1.608 (9.434–12.225)	1.135 (8.858–11.597)	15.553 (13.679–18.114)

Note: The Markov chain Monte Carlo (MCMC) columns contain the posterior modes (with 95% credible intervals in parentheses) for the ICCs, and the PQL column contains the ASReml point estimates. The suffix a refers to the main analysis, and b is an equivalent model but without controlling for sampling effort; c and d are equivalent models to a and b, respectively, but ignoring between-population information. Asterisks on the penalized quasi-likelihood (PQL) estimates denote estimates deemed significant.

* $P < .05$.

** $P < .001$, using a two-tailed *t*-test on the approximate *Z*-score for the variance component.

mations to the likelihood such as Laplace approximations may prove more useful.

Consolidating the data over regions gave S_L = 1,115.483, S_I = 154,325.872 and S_H = 0.025, all of which were significantly different from the null distribution under both Legendre and Hommola permutation schemes. The correlations between module co-occurrence and host phylogenetic distance ($S_{K_h} = -0.000$) and parasite phylogenetic distance ($S_{K_p} = -0.023$), however, were very small for the consolidated data, and neither were significantly different from the null distribution. The region-specific statistics were broadly comparable to their global statistics (after accounting for the dimension changes), and in most cases, the number of regions in which the statistic significantly differed from the null distribution considerably exceeded the null expectation of $51 \times 0.05 \approx 2.5$. The exception to this rule were the modularity metrics which showed many highly significant negative correlations between module co-occurrence and phylogenetic distance for hosts (S_{K_h}) but not for parasites (S_{K_h}), consistent with the results presented in Krasnov et al. (2012). The full results are presented in the supplementary material (table S5).

Properties of Other Methods

To test the properties of the other methods we used various simulated data sets for which $m = n = 10$. When all variances were set to 0 ($\sigma^2_{[h]} = \sigma^2_{[p]} = \sigma^2_{p[h]} = \sigma^2_{[p]h} = \sigma^2_{[ph]} = 0$) all statistics under all permutation schemes had appropriate rejection rates at the 5% level (first row of table 2). However, when phylogenetic signal in PSR and HR was present ($\sigma^2_{[h]} = \sigma^2_{[p]} = 4$), all statistics and permutation schemes gave high Type I error rates ranging from 0.19 to 0.296 (second row of table 2). When evolutionary interaction effects were present ($\sigma^2_{p[h]} = \sigma^2_{a_s Ph} = 4$), all statistics and permutation schemes gave even higher Type I error rates, with rejection rates ranging from 0.186 to 0.552 (third row of table 2). When only the coevolutionary interaction was present ($\sigma^2_{[ph]} = 4$), the statistics of Hommola et al. (2009; S_H) and Legendre et al. (2002; S_L) had comparable power to reject the null hypothesis under both permutation schemes (an average of 49% of cases), but the MSEb statistic of Ives and Godfray (2006; S_I) had lower power (an average of 23% of cases).

Using lower 95% credible intervals greater than 0.01 as a criterion for supporting the presence of the coevolutionary interaction, the MCMCglmm models found little support for the coevolutionary interaction when it was absent and the actual sources of variation were included in the model ($\leq$1.6% of simulated data sets). If the additional sources of variation were not modeled, then strong support was found for the coevolutionary interaction despite it not contributing to the covariances in the data. When the coevolutionary interaction was present, MCMCglmm models that included it alone showed strong support for the coevolutionary interaction (in 89% of cases), but power was reduced when additional sources of variation were controlled for despite not being present (52% of cases when $\sigma^2_{[h]}$ and $\sigma^2_{[p]}$ were estimated and 33% of cases when $\sigma^2_{[p]h}$ and $\sigma^2_{p[h]}$ were estimated). However, we note that parameter-expanded priors place high prior densities at extreme values of the ICC (de Villemereuil et al. 2013) and that the small sample sizes and binary data used in the simulations resulted in the posterior distributions being sensitive to the prior. The results are more fully summarized in the supplementary material (table S4), but focusing on the simulations where data were generated under a coevolutionary interaction the true ICC, $r_{[ph]}$, was equal to $4/(4 + \pi^2/3) \approx 0.55$, and yet the means and standard deviations of the posterior modes for $r_{[ph]}$ across simulations were 0.66 $\pm$ 0.26 when only $\sigma^2_{[ph]}$ was estimated, 0.29 $\pm$ 0.28 when $\sigma^2_{[h]}$ and $\sigma^2_{[p]}$ were also estimated and 0.31 $\pm$ 0.34 when $\sigma^2_{p[h]}$ and $\sigma^2_{[p]h}$ were also estimated. Moreover, the coverage (the percentage of analyses in which the true value was contained in the 95% credible interval) was

Table 2: Proportion of tests deemed significant at the .05 level for 500 simulated data sets

	Legendre permutations			Hommola permutations			MCMCglmm		
Model	Hommola	Legendre	Ives	Hommola	Legendre	Ives	$s^2_{[p]}\&s^2_{[h]}$	$s^2_{[p]h}\&s^2_{p[h]}$	-
-	.044	.048	.062	.052	.058	.062	.000	.000	.014
$\sigma^2_{[p]} = \sigma^2_{[h]} = 4$	.131	.124	.228	.144	.172	.134	.016	.424	.854
$\sigma^2_{[p]h} = \sigma^2_{p[h]} = 4$	.190	.224	.296	.260	.278	.222	.320	.014	.626
$\sigma^2_{[ph]} = 4$	.454	.492	.282	.480	.552	.186	.516	.332	.894

Note: Five hundred data sets were simulated from four different models each defined by equation (3) but with all variances set to zero except those in the Model column. The test statistics S_H (Hommola et al. 2009), S_L (ParafitGlobal: Legendre et al. 2002), and S_I (MSEb: Ives and Godfray 2006) were calculated for each data set and significance evaluated using permutation tests with the permutation schemes used by Legendre et al. (2002) and Hommola et al. (2009). Three MCMCglmm models were also fitted to each data set, each conforming to the model defined by equation (3) but with all variances set to zero except the variances defined in the subheading and the coevolutionary interaction variance ($\sigma^2_{[ph]}$), all of which were estimated. A lower 95% credible interval greater than 0.01 for $\sigma^2_{[ph]}$ was used as the criterion for statistical support of the coevolutionary interaction.

rather poor in the three cases (88%, 81%, and 90%, respectively) and was less than the expected 95%.

When phylogenetic signal in PSR was present ($\sigma^2_{[h]} = 4$), there was on average a host phylogenetic signal in community structure ($S_{K_h} = -0.092 \pm 0.013$, $P < .001$) but not a parasite signal ($S_{K_p} = 0.003 \pm 0.009$, $P = .721$). When a host evolutionary interaction was present ($\sigma^2_{p[h]} = 4$), the host phylogenetic signal was strong ($S_{K_h} = -0.268 \pm 0.013$, $P < .001$) but the parasite signal weak ($S_{K_p} = 0.014 \pm 0.008$, $P = .084$). When a coevolutionary interaction was present ($\sigma^2_{[ph]} = 4$), both host and parasite phylogenetic signals were strong ($S_{K_h} = -0.232 \pm 0.014$, $P < .001$ and $S_{K_p} = -0.220 \pm 0.014$, $P < .001$).

Discussion

In this study, we developed a model-based approach for characterizing the effects of phylogenetic history on bipartite ecological interactions (see Rafferty and Ives 2013). In particular, we decompose phylogenetic effects into those associated with each set in isolation and those associated with interactions between sets. We apply this model to extensive data on the incidence of mammals and fleas across the Palearctic. Here, sets are parasites and hosts, and the phylogenetic effects fall into three categories: a main effect of host and/or parasite specialism-generalism, an evolutionary interaction effect where community structure depends only on host and/or parasite phylogeny in isolation, and a coevolutionary interaction effect where communities are structured by the combined effect of host and parasite phylogenies. We find that the importance of these different processes depends critically on the geographical scale at which the analysis is focused and whether or not sampling effort is controlled for. When sampling effort is controlled for, we find that at broad geographical scales, the coevolutionary interaction effect dominates, suggesting related flea species live on related mammal species. At small geographical scales, we find that although the coevolutionary interaction effect remains, its magnitude is considerably reduced, and the host evolutionary interaction effect is dominant. This effect captures the degree to which related hosts harbor the same sets of parasites irrespective of parasite phylogeny. When sampling effort is not controlled for the importance of both host specialism/generalism (PSR) and parasite specialism/generalism (HR) is elevated since abundant hosts have more recorded parasites, and abundant parasites are recorded on more hosts. Since host abundance, and to a lesser extent parasite abundance, have high phylogenetic signal, this effect results in greater phylogenetic effects on PSR and HR. At small geographical scales and without controlling for sampling effort we find that the combined phylogenetic and nonphylogenetic effects for PSR are the dominant processes.

The fact that the coevolutionary interaction was strongest when between-region information was retained suggests that pairs of closely related host/parasites are found, albeit in allopatry. Although we emphasize that our model is one of coassociation rather than cospeciation, this finding is consistent with allopatric speciation of both hosts and parasites but is silent on whether the speciation of parasites is concomitant with the speciation of hosts. Although the pioneering studies on parasite-host cospeciation (Hafner and Nadler 1988 1990) led to the notion that cospeciation events are highly prevalent in parasite-host coevolution, subsequent cophylogenetic studies of host-parasite associations demonstrated that congruence of phylogenies is usually not the case and that the shared history of hosts and parasites is complicated by a variety of other coevolutionary events (Paterson et al. 1993 2000; Beveridge and Chilton 2001; Roy 2001). Regarding fleas, some evidence of flea-host cophylogeny has been derived from both flea morphology (Traub 1980 1985) and flea-host biogeographic patterns (Traub 1980 1985; Jameson 1999), but quantitative attempts to reveal whether fleas have cospeciated with their hosts indicated that related hosts are mainly parasitized by unrelated fleas and that current flea-host associations are better explained by ecological and geographic factors (Krasnov and Shenbrot 2002; Liang and Houyong 2005; Krasnov et al. 2012) Our analyses are broadly in line with these findings in that the host evolutionary interaction was found to be the dominant term when the between-region information was discarded. Waxman et al. (D. Waxman, L. A. Weinert, and J. J. Welch, unpublished manuscript) show that this is likely to be the case if host switches are biased toward closely related host species and rates of host switching are very high compared to rates of speciation. Given that widespread correlative (e.g., Roy 2001; Charleston and Robertson 2002; Davies and Pedersen 2008) and experimental (e.g., Perlman and Jaenike 2003; de Vienne et al. 2009; Longdon et al. 2011) evidence already exists for biased host switching, we suggest that this is likely to be a major cause of the patterns we observe in the within-region analyses. However, unlike Krasnov et al.'s (2012) analysis of the same data, we do still find some evidence for the coevolutionary interaction even at the within-region scale. Given the limited empirical evidence for both sympatric speciation (Coyne and Orr 2004) and concomitant host-parasite cospeciation (de Vienne et al. 2013), it seems likely that the within-region coevolutionary interaction is generated by either allopatric speciation followed by secondary contact and/or host switches occurring between closely related taxa at rates comparable to speciation rates, resulting in host-shift speciation (de Vienne et al. 2013).

Waxman et al. (unpublished manuscript) show that host switching between closely related hosts is expected to result in higher incidences of parasite species in dense parts of the host tree, something that is not expected from speciation models and that could be brought to bear on assessing the relative importance of each process.

We suggest that the discrepancies between our results and those of Krasnov et al.'s (2012) network analysis are due to the increased power of our approach for detecting the coevolutionary interaction and the ability to control for the phylogenetic main effects that might inflate the estimates of the host evolutionary effect. The possibility of phylogenetic signal in PSR inflating the evidence for host-evolutionary interaction effects in the network approach was confirmed using simulations and is probably exacerbated by variation in sampling effort caused by variation in host abundance, which has a relatively strong phylogenetic signal. More generally, we show that many other methods for analyzing these types of data do not distinguish between phylogenetic main effects, evolutionary interactions, and the coevolutionary interaction and as a consequence are susceptible to ascribing a pattern to a process for which there is little statistical support. For example, previous methods that test for the presence of the coevolutionary interaction had Type I error rates of 16% when only phylogenetic main effects existed and 25% when only evolutionary interaction effects existed. Although our approach had Type I error rates closer to the nominal 5%, we acknowledge that getting precise estimates of the contribution of each process to the global pattern will require extensive data collected on a large number of host species and parasite species. Even for moderate-sized data sets it may prove difficult to separate all effects (Waxman et al., unpublished manuscript), and care must be taken to understand and explain the impact that particular choices of prior and model structure have on any inferences.

In this article we have focused on a very specific application of our method; binary data from a host-parasite system. However, the approach is applicable to a range of data types and could be applied fruitfully to a range of quantitative measures of interaction strength, such as parasite replication rate, host fecundity, host growth rate, and immune gene expression. Moreover, the approach can be used to analyze the outcomes of any bipartite ecological interactions where interacting species can be placed in disjoint sets that are reciprocally monophyletic. Indeed, while this article was in revision, Rafferty and Ives (2013) published an equivalent model and applied it to plant-pollinator interactions, and Henry et al. (2013) have applied a similar model to understand the distribution of host-symbiont interactions. Extending the approach to unipartite interactions, such as interspecific competitive interactions, may prove difficult because the tractability of likelihood-based comparative methods depends on conditional independence whereby two daughter species evolve independently given their ancestor (Felsenstein 1985; Freckleton 2012). Given that causes of nonindependence such as character displacement are likely to be the focus of models of interspecific competition, we suggest that a GLMM framework may be difficult to implement for such scenarios (but see Ives and Helmus 2011). Nevertheless, we envisage plenty of opportunities for modeling numerous ecological systems with this method, and we hope that the richer class of models that we and Rafferty and Ives (2013) present should allow researchers to ask more nuanced questions of their data in a more robust way.

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APPENDIX

Derivations of Coevolutionary Interaction Metrics

For the appendix and supplementary material, four matrix identities will be useful: (i) $\text{vec}(\mathbf{CBA}') = (\mathbf{A} \otimes \mathbf{C})\text{vec}(\mathbf{B})$, (ii) $\text{tr}(\mathbf{AB}) = \text{vec}(\mathbf{A}')\text{vec}(\mathbf{B})$, (iii) $\text{tr}(\mathbf{AB}) = \mathbf{1}(\mathbf{A} \odot \mathbf{B})\mathbf{1}'$, and (iv) $(\mathbf{C} \odot \mathbf{ab}')\mathbf{d} = \mathbf{a} \odot \mathbf{C}(\mathbf{b} \odot \mathbf{d})$, where $\odot$ is the Hadamard product.

Relationships between Former Models

Legendre et al. (2002) use the metric $\text{tr}(\mathbf{D}'\mathbf{D})$ where $\mathbf{D} = \mathbf{L}'^{(\text{h})}\mathbf{Y}\mathbf{L}^{(\text{p})}$, $\mathbf{L}$ is a matrix of principal coordinates of $\mathbf{J} - \mathbf{A}$ and tr is the trace operator. However, if we take $\mathbf{L}$ to be the nonnormalized eigenvectors of $\mathbf{A}^{-1}$, then using (i) we find $\text{vec}(\mathbf{D} = (\mathbf{L}'^{(\text{p})} \otimes \mathbf{L}'^{(\text{h})}\mathbf{y}))$ and using (ii) we obtain $\text{tr}(\mathbf{D}'\mathbf{D}) = \mathbf{y}'(\mathbf{L}^{(\text{p})} \otimes \mathbf{L}^{(\text{h})})(\mathbf{L}'^{(\text{p})} \otimes \mathbf{L}'^{(\text{h})})\mathbf{y} = \mathbf{y}'(\mathbf{L}^{(\text{p})}\mathbf{L}'^{(\text{p})} \otimes \mathbf{L}^{(\text{h})}\mathbf{L}'^{(\text{h})})\mathbf{y} = \mathbf{y}'(\mathbf{A}^{\text{p}} \otimes \mathbf{A}^{\text{h}})^{-1}\mathbf{y}$. If $\mathbf{y}$ is mean centered, then $\text{tr}(\mathbf{D}'\mathbf{D})$ is proportional to the mean sum of squares of Ives and Godfray (2006) under a pure Brownian model with $d_{\text{h}} = d_{\text{p}} = 1$ (MSE_b). The constant of pro-

portionality $(|\mathbf{A}^{(h)}|^n|\mathbf{A}^{(p)}|^m)/(nm - 1)$ does not depend on the data.

Hommola et al. (2009) create two $N \times N$ matrices, $\mathbf{C}^{(h)}$ and $\mathbf{C}^{(p)}$, where N is the number of interactions. Term $c_{i,j}^{(h)}$ is the element of $\mathbf{J} - \mathbf{A}^{(h)}$ corresponding to the hosts in interaction i and j, and $c_{i,j}^{(p)}$ is the element of $\mathbf{J} - \mathbf{A}^{(p)}$ corresponding to the parasites in interaction i and j. Their statistic is the correlation between the elements in the upper triangles of $\mathbf{C}^{(h)}$ and $\mathbf{C}^{(p)}$. It should be noted that the distance matrix $\mathbf{J} - \mathbf{A}$ can be replaced with $\mathbf{A}$ and the resulting statistic is identical.

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"Everything indicated that the mouse was perfectly dead, excepting the fact that it was not as rigid as perhaps a dead mouse would be in the winter.... By holding it in my hand and thus warming it, the mouse soon began to show signs of life, and although it was nearly the whole day in coming back to activity, at last it was as lively as ever, and afterward, on being set free in the room, it moved about so swiftly by means of its long leaps, that it required two of us a long time to capture it uninjured." From "Hibernation of the Jumping Mouse" by Sanborn Tenney (*The American Naturalist*, 1872, 6:330–332).