Linking community assembly and structure across scales in a wild mouse parasite community

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Abstract

Understanding what processes drive community structure is fundamental to ecology. Many wild animals are simultaneously infected by multiple parasite species, so host parasite communities can be valuable tools for investigating connections between community structures at multiple scales, as each host can be considered a replicate parasite community. Like free-living communities, within-host parasite communities are hierarchical; ecological interactions between hosts and parasites can occur at multiple scales (e.g. host community, host population, parasite community within the host), therefore both extrinsic and intrinsic processes can determine parasite community structure. We combine analyses of community structure and assembly at both the host population and individual scales using extensive datasets on wild wood mice (*Apodemus sylvaticus*) and their parasite community. An analysis of parasite community nestedness at the host population scale provided predictions about the order of infection at the individual scale, which were then tested using parasite community assembly data from individuals hosts from the same populations. Nestedness analyses revealed parasite communities

were significantly more structured than random. However, observed nestedness did not differ from null models in which parasite species abundance was kept constant. We did not find consistency between observed community structure at the host population scale and within-host order of infection. Multistate-Markov models of parasite community assembly showed that a host's likelihood of infection with one parasite was not impacted by previous parasite infection, suggesting there is not a deterministic order of infection among the species we investigated in wild wood mice. Our results demonstrate that patterns at one scale (i.e. host population) do not reliably predict processes at another scale (i.e. individual host), and that neutral or stochastic processes may be driving the patterns of nestedness observed in these communities. We suggest that experimental approaches that manipulate parasite communities are needed to better link processes at multiple ecological scales.

Key-words: *Bartonella*, coinfection, community assembly, community structure, *Eimeria*, helminths, Multi-state Markov model, nestedness, wild mice

Introduction

Ecological systems are fundamentally hierarchical, from individuals, to populations, communities, and the broader ecosystem. A major challenge in ecology is to understand the extent to which processes at one scale (e.g. within a population) affect patterns and processes at another (e.g. across the community). Specifically, a key issue is to investigate how ecological communities assemble, and the extent to which observed community composition reflects underlying processes occurring at finer scales. To assess the connection between community

47 assembly and structure, we need empirical systems at which processes at distinct scales can be
48 quantified, and for which a large number of replicates can be sampled. Within-host parasite
49 communities have recently been suggested to have potential for developing our understanding of
50 the processes underlying community assembly and structure (Blackwell, Martin, Kaplan, &
51 Gurven, 2013; Cobey & Lipsitch, 2013; Costello, Stagaman, Dethlefsen, Bohannan, & Relman,
52 2012; Dallas & Cornelius, 2015; Dallas, Park, & Drake, 2016). While host-parasite systems carry
53 some important differences to free-living systems, such as habitat patches being mobile (in the
54 case of animal hosts) and the host being an evolving habitat and food resource (Johnson, De
55 Roode, & Fenton, 2015; Poulin & Valtonen, 2001; Seabloom et al., 2015; Ulrich, Almeida, &
56 Gotelli, 2009), the typically large number of communities (infected hosts) and relative ease of
57 longitudinal studies of successive infections within individual hosts provides a great opportunity
58 to study the assembly of multiple replicate communities in an easily observable timespan.
59
60 Parasites are extremely common in nature and most wild hosts are coinfected by multiple
61 parasite species (defined here to include both macroparasites (e.g. helminths, ectoparasites) and
62 microparasites (e.g. viruses, bacteria, protozoans)) simultaneously and/or sequentially throughout
63 their life (Poulin, 1996). Each individual host can therefore be considered an ecosystem, with
64 many habitats for parasites and pathogens to infect, forming a clearly-defined within-host
65 ecological community (Pedersen & Fenton, 2007; Restif & Graham, 2015; Rynkiewicz,
66 Pedersen, & Fenton, 2015). Furthermore, host-parasite systems are inherently hierarchical; each
67 host is infected with its own community of parasites, and these hosts are linked by potential
68 dispersal via parasite transmission (Mihaljevic, 2012). Hence, both extrinsic (between-host)
69 factors, such as parasite exposure or variation in parasite species abundance, and intrinsic

(within-host) factors, such as host immune function and interactions between coinfecting parasite species, can combine to influence community structure at multiple scales (Joseph, Mihaljevic, Orlofske, & Paull, 2013; Lima, Giacomini, Takemoto, Agostinho, & Bini, 2012; Poulin, 2001; Ulrich & Gotelli, 2007; Zelmer & Arai, 2004). Hence, processes occurring at one scale can impact patterns and processes at another scale. For example, treating to reduce the burden of gastrointestinal worms in individual African buffalo increased survival of treated hosts, which could exacerbate the invasion and spread of bovine TB at the host population level (Ezenwa & Jolles, 2015). Scaling down, individual host and vector risk for infection with the agent of Lyme disease is influenced by the diversity and composition of the wider host community (Ostfeld & Keesing, 2000). It remains an open question to what extent patterns of community structure (e.g. community composition) at one scale reflect processes (e.g., assembly order) at another. The hierarchical nature of host-parasite systems, enabling measurements of between-host community composition to be coupled with data on community assembly (infection order) at the individual-host level, may provide a means to address this question.

To investigate the relationship between the structure of within-host parasite communities and their assembly patterns over time, we used wild wood mice, *Apodemus sylvaticus,* and their species-rich endoparasite community. These datasets comprised longitudinal data (capture – mark – recapture) on individually-tagged mice, where infection with over 30 taxonomically-diverse parasite species was measured through time. These extensive within-host parasite community data allow for the quantification of the assembly order of these within-host parasite communities for each individual over the course of their life and across the same population over time. To analyse parasite community structure at the host population scale we used a nestedness

93 analysis approach. Nestedness describes the structure and co-occurrence of species in a
94 community, testing if less rich communities are perfect subsets of richer ones (Atmar &
95 Patterson, 1993). Nested communities can arise when some species rely on another for survival
96 or reproduction, such as mutualisms, food web or trophic interactions, from neutral processes
97 like variation in species abundance, patch colonization history, or through stochastic
98 colonization or extinction, which can be influenced by variation in species abundance or patch
99 quality (Amundsen et al., 2009; Bracken, Friberg, Gonzalez-Dorantes, & Williams, 2008;
100 Calatayud, Madrigal-Gonzalez, Gianoli, Hortal, & Herrero, 2017; McQuaid & Britton, 2013;
101 Ulrich et al., 2009). Notably, it has been suggested that nestedness in a community can imply a
102 fixed order of colonisation or extinction which structures communities in a predictable way
103 (Diamond, 1975; Ulrich et al., 2009), and nestedness theory has been used to analyse predictable
104 species loss or gain from islands or isolated patches (Atmar & Patterson, 1993; Ulrich et al.,
105 2009).
106
107 In the context of host-parasite systems, nestedness analyses have previously been used to
108 demonstrate significant structure of parasite communities in fish (Lima et al., 2012; Poulin &
109 Valtonen, 2002) and amphibian populations (Johnson & Hoverman, 2012). These findings
110 support epidemiological theory (e.g., Dobson, 1990) which predicts that parasite communities
111 may tend to show nested structures, with certain 'core' species (typically those with high basic
112 reproduction ratios, R_0) tending to be found in all communities, whereas 'satellite' species (those
113 with lower R_0 values) will typically be much rarer (Bush & Holmes, 1986; Holmes & Price,
114 1986; Stock & Holmes, 1987). Furthermore, it is well known that there is likely to be a strong
115 link between a parasite's R_0, its population-level prevalence, and the average age at which hosts

first become infected with that parasite (Anderson & May, 1991). This is similar to the pattern predicted by variation in the dispersal ability of species in their ability to move to new habitat patches in free-living systems (Leibold et al., 2004). Bringing these ideas together, we hypothesised that the patterns of community nestedness observed at the host population level should be predictive of the order of parasite assembly (i.e., infection order) at the individual host level (Götzenberger et al., 2012; Lima et al., 2012; Lindo, Winchester, & Didham, 2008). We tested this hypothesis using cross-sectional (population scale nestedness) and longitudinal data (individual scale order of infection) in the same populations of wild mice and their parasites. If the order of community assembly at the individual scale matches the predictions based on community structure at the population scale then we can conclude that patterns of nestedness at one scale predict the process of community assembly order at the other.

Methods

Sample collection

All parasite samples were collected from wild wood mice at three sites near Liverpool, UK: Haddon Wood (N 53 2716º, E -3 0297), Manor Wood (N 53 3301°, E -3 0516°,) and Rode Hall (N 53 1213°, E -2 2798°). There were five 70 x 70m grids among the sites, where each grid had 64 trap stations (10m apart), with 2 Sherman live traps (2 x 2.5 x 6.5-inch folding trap, H.B. Sherman, Tallahassee, FL, USA) at each trapping station, 128 traps per grid (Knowles, Fenton, & Pedersen, 2012; Withenshaw, Devevey, Pedersen, & Fenton, 2016). The traps were baited at dusk with crimped oats and carrot; bedding was also placed in the trap as nesting material. The following morning, all mice were given a numbered subcutaneous microchip transponder (PIT tag) at first capture. Faecal and small volume blood samples were collected from each individual

once per trapping session. Gastrointestinal 'gut' parasite infections (helminth worms and coccidial protozoans) were identified to species and burdens were measured (either faecal or oocyst egg counts (FEC/OEC) respectively) using salt flotation and microscopy. Infection with blood parasites (e.g. *Bartonella* spp., a flea-transmitted bacterium, and *Trypanosoma grosi*, a flea-transmitted protozoan) were identified using targeted, nested PCR assays on DNA extracted from blood (for more details on these methods see (Knowles et al., 2013; Withenshaw et al., 2016). Our previous research of these wild rodent and parasite communities have shown that most parasites are host-specific (Knowles et al., 2012; Withenshaw et al., 2016), therefore we focused our analysis on the parasite communities in wood mice only.

Trapping took place between May and December across 4 years (2009-2012). In 2009-2011 the grids were sampled every 4 weeks, while in 2012 grids were sampled every 2 weeks. This leads a per year effort of 5,760 trap-nights per year in 2009-11 and 11,520 trap-nights in 2012. Data from 2009-11 and 2012 were considered separately, due to differences in sampling regimes; in 2012 grids were sampled every 2 weeks, while in 2009-2011, grids were sampled every 4 weeks) in 2012. The 2012 dataset had more repeat captures of individuals and thus made it more suitable for longitudinal, individual-scale analyses, while the 2009-11 datasets are better suited for cross-sectional, population-scale analyses. The unique nature of our dataset, with extensive longitudinal and cross-sectional data on the same populations of wild wood mice, gives us the ability to directly compare predictions of parasite community assembly based on population prevalence and order of infection likelihood to determine the concordance of population- and individual-scale patterns of parasite community structure.

Parasite community data

To test for nestedness, we used data from the first capture of each individual to avoid pseudoreplication due to repeat captures of the same mouse. In addition, we only included parasite species that were commonly found in all 4 years of sampling, which resulted in 16 parasite species for 2009-11 and 15 for 2012 (Table 1). For tests of community assembly at the individual-scale we analysed longitudinal data from the 2009-11 and 2012 datasets, limited to records with multiple captures per individual (2+ captures) to assess the temporal order of parasite species infection throughout each individual host's life. These analyses used either all parasites from the population-level analyses for a coarse assessment of individual-scale host parasite community assembly (rank order analysis; see below) or the three most abundant species for a finer-resolution assessment (Multistate-Markov models; see below).

Population scale - nestedness of parasite communities

To analyse the differences between observed and null model communities, the within-host parasite communities were arranged in an incidence matrix of individual hosts ('sites') and parasite species ('species occupying those sites') and analysed using Nestedness of Overlap and Decreasing Fill (NODF) method; this was implemented by the "oecosimu" function in the "vegan" package (Oksanen et al., 2013) in R (R Core Team, 2018). The Nestedness Matrix is the most efficient "packing" of hosts and parasites, with the most abundant parasite species in the left column and the most highly parasitized host (highest parasite species richness) in the top row, with the others hosts and parasites "ordered in a manner to minimize unexpected species absences and presences" (Atmar & Patterson 1993, p 375).

185 Three null models were constructed to correspond to alternative hypotheses of intrinsic (host
186 individual level)- or extrinsic-based (parasite identity or characteristics) mechanisms underlying
187 the community assembly process (Almeida-Neto, Guimaraes, Guimaraes, Loyola, & Ulrich,
188 2008): 1) completely random, where parasites were randomly assigned to hosts irrespective of
189 host or parasite identity (i.e. the same number of parasites are present in the null model as in the
190 observed community, but individual host (patch) species richness and parasite abundance are
191 drawn at random from the entire community), 2) random with respect to host identity, which
192 tests for whether population-level patterns are driven by individual (intrinsic) mechanisms
193 influencing host (patch) quality or exposure (i.e. parasite species richness in each host (row
194 totals) is the same as in the observed community but the species in each community are drawn at
195 random), and 3) random with respect to parasite species identity to test whether extrinsic
196 mechanisms drive parasite cooccurrence patterns, such as parasite species identity or abundance
197 (i.e. parasite abundance (column totals) in the null model is the same as in the observed
198 community but parasites are assigned to hosts at random) (see Fig. S1 for a visual example of
199 each null model). Testing these null models provides information about likely mechanisms
200 structuring the overall parasite community, i.e. host individual-level (intrinsic) variation,
201 population-level (extrinsic) variation in parasite abundance, both, or neither. One hundred
202 simulated null communities were constructed for each method to test against each dataset of
203 observed parasite infection in wild wood mice. The datasets used included the following: 1) the
204 combined three-year dataset (2009-2011), 2) each year of that dataset individually, and 3) the
205 dataset from 2012, in order to compare population and individual level community assembly
206 between years. We also analysed nestedness in adult hosts and young hosts (juveniles and sub-
207 adults) to test if parasite communities became more nested as hosts aged. Data from all trapping

208 grids were pooled in order to have as large a sample size as possible for testing against the null
209 models, while we recognize that there is possibly variation in parasite exposure between grid
210 locations.
211
212 *Individual host scale - order of parasite infection*
213 The nestedness analyses suggested that parasite community structure at the host population scale
214 was primarily driven by aspects relating to parasite species identity, such that there were highly
215 prevalent 'core' species found in most communities, and less prevalent 'satellite' species
216 occurring in fewer communities (see Results). As described previously, epidemiological theory
217 suggests there should be a strong link between a parasite's prevalence and the average age at
218 which hosts first become infected with that parasite (Anderson & May, 1991; see also
219 Supporting Information for a simulation model of this relationship; Fig. S2). We therefore
220 hypothesised that nestedness "rank" of each parasite in the nested matrix would be predictive of
221 the order of parasite assembly (i.e., infection order) at the individual host-scale. We tested these
222 predictions with two analyses at the individual host scale using longitudinal parasite community
223 assembly data.
224
225 First, we carried out a non-parametric analysis of ranks (Spearman's Rank) on all parasite
226 species, to analyse the concordance between the predicted rank order of infection from the
227 nestedness analysis at the host population-scale against the observed rank order of infection for
228 each individual host. For example, using the Nestedness Matrix from the 2009-11 combined
229 dataset (Fig. 1a), the parasite predicted to infect first is *H. polygyrus* (rank = 1), predicted second
230 to infect is *E. hungaryensis* (rank = 2), predicted third to infect is *B. taylorii* (rank = 3), etc. To

calculate observed rank orders of infection, longitudinal data were organized by host individual and date of capture to rank when each parasite infected the host over the course of the host's lifetime. The parasite observed at the earliest date was given a rank of 1, second a rank of 2, etc. If a host was re-infected with a parasite, we used only the first date of infection with that species to calculate its rank. These observed ranks were compared with the predicted ranks generated from the nestedness analyses from either the combined 2009-11 dataset or 2012 dataset with the degree of correlation between them measured by Spearman's Rho (r_s). We compared observed and predicted ranks of infection for all parasites from the two datasets to test if any patterns were generalizable enough to be consistent across years. Low p-values ($p < 0.05$) from the Spearman's Rank analysis indicate a statistically significant concordance between predicted and observed rank orders, implying the ability to predict the order of within-host community assembly from the results of a nestedness analysis of the whole host population.

For a finer resolution, and more robust analysis of the temporal orders of infection, we carried out Multi-State Markov models (MSM) of longitudinal order of infection to test whether infection by one parasite species tended to occur after prior infection by another species. MSMs use a maximum likelihood approach to quantify the rates or probabilities of individuals transitioning between different observable states (Meira-Machado, de Uña-Álvarez, Cadarso-Suárez, & Andersen, 2009), in our case each state corresponds to host infections. This approach is more powerful that other statistical approaches, such as general linear models (Fenton, Knowles, Petchey, & Pedersen, 2014) due to its use of longitudinal data to parameterize the likelihoods of one infection following another, not simply associations between infections. This analysis also assumes individuals transition between states in continuous time and estimates each

transition likelihood while taking into account all other possible likelihoods, as defined in the model, via a transition probability matrix (Jackson, 2011). This MSM approach has been used to study chronic disease progression in humans (Hoogenveen, van Baal, & Boshuizen, 2010; Huszti, Abrahamowicz, Alioum, & Quantin, 2011), but is starting to be used in ecological applications (Blackwell et al., 2013). We emphasise that no mechanisms are implied in this analysis, which simply quantifies whether the likelihood of a host transitioning to a state of being infected with one parasite species is more, less, or equally likely if they had been previously infected with another parasite species, compared to previously being uninfected. In other words, it provides a robust quantification of infection order (i.e., whether parasite B tends to infect before or after parasite A) among the parasite species tested. While this approach is more powerful than other forms of analysis, it requires very large datasets to parameterize all possible transitions between infection and coinfection states (Sofonea, Alizon, & Michalakis, 2015), so we restricted the models to the three most prevalent parasite species in the datasets and analysed transitions in infection and coinfection status between all possible pairs of these three parasites. MSMs were carried out with the 2012 data only, as this dataset had better longitudinal data from individual hosts, as grids were sampled every two weeks compared to every four weeks, which is needed for the calculation of transition likelihoods.

We ran the MSMs using the *msm* R package (Jackson, 2011) to quantify the transition intensity, or likelihood, of hosts transitioning between infection states per unit time (days). This intensity is the "instantaneous risk" of the host moving from one infection state into another given infection state (Jackson, 2011, p. 1). Using all possible pairs of the three most common parasite species, hosts were assigned to one of four infection states at each capture: uninfected with either

parasite, infected with parasite A, infected with parasite B, or co-infected with parasites A and B (Fig. 2a-c). To determine whether infection with one parasite is more likely to occur after prior infection with another, we compared the likelihood of host transitioning from an uninfected state to an infected state with a given parasite, compared to the transition from a singly-infected state to the coinfected state. Transition intensities between infection states in each of the three pairs of parasites were compared to the predicted order of infection for these parasites from the nestedness analysis. All possible transitions were allowed to occur between consecutive time points, meaning a host could gain or lose one or both parasites in any one transition (Fig. 2a-c). Starting conditions for the model were estimated from the data (using the function "crudeinits.msm") since we did not have prior assumptions about transition intensities. Transition intensities and 95% confidence intervals are presented. Sample size limitations did not allow for the addition of covariates in the MSM models. To assess the generality of parasite assembly rules in this community, we compared the observed order of infection to both to the predicted order of infection from the same year's (2012) population-scale nestedness results as well as those from 2009-11.

Results

Population-scale nestedness

The nestedness analysis of parasite community structure was first conducted on 1,352 individual wood mice sampled from 2009-2011 (2009, n = 441; 2010, n = 403; 2011, n = 508), and separately on the 322 mice from 2012. The most common parasites were the gut nematode *Heligmosomoides polygyrus*, multiple species of the gut apicomplexan coccidial protozoans in the genus *Eimeria* (*E. hungaryensis* and *E. apionodes*), and vector-borne bacteria in the genus

Bartonella (*B. taylorii* and *B. grahamii*). Also present were other species of gut nematodes, cestodes, and less common *Bartonella* and *Eimeria* species (Table 1). The prevalence of each parasite differed across years; for example, infection prevalence of cestodes increased from 2009-11 to 2012 (Table 1), whereas *H. polygyrus*, *Eimeria* and *Bartonella* species were always highly prevalent. The majority of mice were infected with at least one parasite, 84% of individuals in the 2009-11 dataset and 82% in the 2012 dataset.

The wood mouse parasite community in the 2009-2011 dataset was significantly more nested than expected compared to a completely randomised community (Null model 1; SES = 77.248, p = 0.009), suggesting that the parasite community structure is indeed non-random (Fig. 1a, Table S1). The parasite community for each individual year, 2009-2012, was also more nested than a completely randomly-assembled community (Fig. 1b, Fig. S3, Table S1).

When we analysed community structure while maintaining individual host species richness (row totals within the matrix kept constant), the observed community was also significantly more nested than the null (Null model 2; SES = 337.08, p = 0.009). In contrast, when the overall prevalence for each parasite was maintained (column totals within the matrix kept constant), the observed degree of nestedness was not significantly different from the null (Null model 3; SES = -0.072, p = 0.960). Results of analysing each year separately showed the same patterns and significance (Table S1). Adults had richer parasite communities compared to young hosts, (Young mouse mean richness = 1.48 +/- 0.05, median = 1, max = 7; Adult mouse mean richness = 1.98 +/- 0.045 SE, median = 2, max = 7), however both age classes contained nested communities and showed the same patterns of significance as the tests on the whole host

323 population (Fig. S4, Table S1). The parasites present in the young hosts did not appear to be a
324 subset of those present in adults; young hosts could be infected with all parasites that infect adult
325 hosts. These results suggest that while parasite communities within individual hosts are non-
326 random, variation in parasite species prevalence is likely driving this pattern, not individual host-
327 level processes.
328
329 *Individual-scale community assembly*
330 The above analysis suggests that wood mouse parasite communities are nested across the host
331 population, and that the degree of nestedness is related primarily to differences between
332 parasites, rather than differences between hosts. As explained in the Methods section (see also
333 Supporting Information; Fig. S2) we hypothesised that individual-scale parasite communities
334 would assemble in accordance to their ranks in the nestedness matrices. To test this, we analysed
335 the individual-level longitudinal data, first using rank order analyses of all parasite species used
336 in the nestedness analyses, then using Multi-state Markov models of the three most prevalent
337 species.
338
339 The Spearman's Rank analysis, which tested the concordance between parasite's predicted rank
340 order of infection, from the nestedness analysis at the host population-scale (Table 2), against the
341 observed rank order of infection of each parasite at the individual scale, revealed a significant
342 positive relationship between the predicted and observed rank orders of parasite infection (for all
343 comparisons $p < 0.01$, except when testing the relationship between the predicted ranks from
344 2012 and observed data from 2009-11; Table 3). Hence there is evidence for some ability to
345 predict individual-level assembly order from patterns of nestedness at the population level.

346 However, Spearman's r_s correlation values were relatively low, with between 2-12% of the
347 variation in observed ranks being explained by predicted ranks (Fig. 3).
348
349 The MSMs used the three most prevalent parasite species from the 2009-11 dataset:
350 gastrointestinal parasites *H. polygyrus* (33% infection 2009-11, 22.4% 2012) and *E.*
351 *hungaryensis* (28.4% infected 2009-11, 18% 2012), which have been found to interact within
352 coinfected mice (S. C. L. Knowles et al., 2013), and the flea transmitted, blood-borne bacterium
353 *Bartonella taylorii* (23.3% infected, 31.7% 2012; Withenshaw et al., 2016). Hence, the
354 predictions for individual-level community assembly based on the population-level nestedness
355 analysis from the analysis using 2009-11 data were: Uninfected →*E. hungaryensis* → *E.*
356 *hungaryensis* + *H. polygyrus* → *E. hungaryensis* + *H. polygyrus* + *B. taylorii* (Fig. 2d);
357 predictions from the 2012 data were: Uninfected → *B. taylorii* → *B. taylorii* + *H. polygyrus* →
358 *B. taylorii* + *H. polygyrus* + *E. hungaryensis* (Fig. 2e). The pairwise associations tested with the
359 MSMs were: *H. polygyrus-E. hungaryensis, H. polygyrus-B. taylorii,* and *E. hungaryensis-B.*
360 *taylorii*. We then tested whether the outcome of the analyses of parasite community assembly at
361 the individual host-scale were consistent with the predictions from the nestedness analyses at the
362 host population-scale.
363
364 Contrary to our predictions, none of our MSM analyses revealed cases where an individual was
365 more likely to become infected with a parasite after previously being infected with a different
366 parasite species, compared to becoming infected from an uninfected state (Table 4). For
367 example, in the 2012 dataset, the parasite with the first nestedness rank and highest prevalence
368 was *B. taylorii* which would therefore be expected to be the parasite most likely to infect first.

However, uninfected hosts were more likely to become infected with *E. hungaryensis* (0.036, 95% CI: 0.0015, 0.892) from an uninfected state compared to *B. taylorii* (0.017, CI: 0.0098, 0.030), and equally likely to become infected with *H. polygyrus* (0.017, CI: 0.0089, 0.033) compared to *B. taylorii* (0.017, CI: 0.0092, 0.032). To compare to the 2009-11 predictions, *H. polygyrus* was the most prevalent parasite and would therefore be expected to be the first to infect. However, uninfected hosts were more likely to become infected with *E. hungaryensis* first (0.0329, CI: 0.0016) compared to *H. polygyrus* (0.0066, CI: 0.00014, 0.3165), and, as stated above, hosts were equally likely to become infected with either *H. polygyrus* or *B. taylorii* from an uninfected state. Hence, while parasite abundance seemed to be the driving mechanism structuring parasite communities at the host population scale, the inconsistency of infection order from these individual-scale results suggest there is not a deterministic order of infection among these three parasites.

Discussion

By combining analyses across scales, from host population to individual, we show 1) that there is clear non-random structure to the parasite communities of wild wood mice, 2) this non-randomness is not related to systematic differences between hosts, but 3) this observed structure does not translate to predicting within-host parasite community assembly over time. Overall our results do not provide evidence for patterns at one scale directly predicting patterns at another in this system, suggesting the observed patterns of community structure may be arising from neutral or stochastic processes. We suggest targeted experiments are needed to fully elucidate the intrinsic and extrinsic mechanisms behind such observed patterns of parasite community structure (Boughton, Joop, & Armitage, 2011; Pedersen & Fenton, 2015).

392

393 Our population-scale analyses showed that parasite communities are highly nested across hosts,
394 such that species-poor parasite communities (i.e., hosts with relatively few coinfecting species)
395 tended to be subsets of species-rich parasite communities (i.e., hosts harbouring many
396 coinfecting species). Hence there tended to be highly prevalent 'core' parasite species found in
397 most communities, and less prevalent 'satellite' species found in fewer communities (Bush &
398 Holmes, 1986; Holmes & Price, 1986; Stock & Holmes, 1987). Furthermore, we showed that
399 parasite species identity, rather than factors relating to host identity, appeared to be the key
400 driver of the observed degree of nestedness. Community ecology theory predicts four
401 mechanisms generally drive community nestedness: selective colonisation among species,
402 selective extinction, habitat nestedness, or neutral, stochastic sampling (Atmar & Patterson,
403 1993; Azeria, Carlson, Part, & Wiklund, 2006; Ulrich et al., 2009). Given we found no signal of
404 host-related factors driving the observed nestedness, we focussed on processes relating to the
405 colonisation process (i.e., the acquisition of infections) in driving these patterns. In particular, we
406 tested the hypothesis from free-living community ecology (Atmar & Patterson, 1993; Diamond,
407 1975; Ulrich et al., 2009), that observed nestedness arises from a fixed, predictable order of
408 colonisation (infection order). Epidemiological theory, supported by our simulations (Supporting
409 Information, Fig. S2), predicts there should be an inverse relationship between population-level
410 prevalence and order of infection (parasites with higher population prevalence have shorter times
411 to first infection in an individual host; Anderson & May, 1991), thereby providing an explicit
412 link between patterns of parasite community structure at the host population scale with the
413 process of parasite infection order at the individual host scale. However, we found very little
414 support for a relationship between the order of infection among individual mice and the

predictions arising from the nestedness analysis. We also did not observe young hosts to have a less-rich subset of the parasite communities observed in older adult mice. Together these results suggest that although parasite community composition at the host population-scale is driven by parasite species-specific variation, parasite community assembly within individual hosts is not predictable from population-scale analyses.

Given the lack of predictability in infection order, our results suggest that neutral or stochastic processes may be generating the levels of community nestedness observed (Higgins, Willig, & Strauss, 2006; Ulrich et al., 2009; Ulrich & Gotelli, 2013). Some aspects of this system, such as differences in parasite infection prevalence among years, suggest that there is natural variation in the force of parasite infection, which will likely impact both host exposure and the likelihood of successful infection. We acknowledge that while we have parasites that span a range of taxonomic groups and transmission modes, we do not have data on parasite variables outside of the hosts, such as the infection prevalence in vectors or abundance of infectious stages in the environment. An important next step in assessing the extrinsic mechanisms that impact parasite community structure would be to integrate these system-specific details integral to each parasite's life cycle. In addition, we focused on species gains in our analysis of parasite community assembly, but species losses, through processes such as host clearance of infection, are also an important factor driving parasite community patterns. It is likely that parasite communities experience succession-like dynamics, with early and late colonizers, which would compete for habitat and resources, with some species ultimately being lost in this process (Rynkiewicz et al., 2015). However, from our longitudinal sampling we rarely observe the loss of any of these parasites from an individual. Of course, there may be losses followed by

438 reinfection occurring, but these are difficult to distinguish with the resolution of sampling
439 methods used here. So, while we focused on colonisations (gains of infection) rather than losses,
440 we acknowledge that integrating both would be needed to differentiate these processes in future
441 analyses.
442
443 As stated above, our results found no evidence for host-related factors playing a significant role
444 in shaping parasite community structure. One explanation for this is that host-level processes that
445 were not measured in our study may influence the outcome of parasite community assembly.
446 Variation in an individual host's immune response to infection, or cross-reactivity between
447 parasite-specific antibodies, may determine the outcome of a parasite infection in an individual
448 host (Cobey & Lipsitch, 2013; Graham, Cattadori, Lloyd-Smith, Ferrari, & Bjornstad, 2007).
449 The dynamic nature of the host immune response may lead to fluctuations of the within-host
450 immune environment, such as switching between being dominated by inflammatory or anti-
451 inflammatory components, in shorter periods of time than the sampling regime used in the
452 longitudinal dataset. For example, after infection with *H. polygyrus* laboratory mice show a shift
453 towards an anti-inflammatory immune profile in a matter of days (Monroy & Enriquez, 1992).
454 Finer-scale monitoring of the host's response to parasite infection or experimental manipulation
455 of the immune response could better describe these interactions to further investigate within-host
456 processes as mechanisms impacting community structure.
457
458 Our analyses quantified infections in terms of their presence or absence; it may be, however, that
459 there are more subtle, quantitative effects driven by variation in infection burdens. Wild host
460 populations show significant variation in parasite burdens (Shaw & Dobson, 1995), and extrinsic

461 factors, such as resource availability (Budischak et al., 2015; Pedersen & Greives, 2008; Ramiro,
462 Pollitt, Mideo, & Reece, 2016), as well as intrinsic factors, such as immune phenotype (Cobey &
463 Lipsitch, 2013; Reese et al., 2014), can impact host-parasite interactions and coinfection
464 susceptibility. This could mean that analyses that include only parasite presence (i.e., whether a
465 host is infected or uninfected) may not fully describe the interaction between host and parasite.
466 Furthermore, theory suggests that the magnitude, and even direction (net positive or negative) of
467 within-host parasite interactions can vary depending on the burden of infection (Fenton, 2013).
468 As such it may be that our analyses using infection status, and not burdens, is too coarse to detect
469 any signal of prior, burden-dependent infection by one parasite species on subsequent infection
470 by another. However, most data collected on wild parasite infections are in the form of
471 presence/absence, therefore there are practical reasons to test the ability, or inability, of these
472 sorts of data to inform community processes at multiple scales.
473
474 Overall, we found little evidence for deterministic assembly order at the individual-scale driving
475 the observed non-random structure seen in the wild wood mouse parasite communities. While
476 there is a growing appreciation that ecological tools and concepts developed for free-living
477 communities can be applied to understanding the hierarchical nature of host-parasite
478 communities, there are still many challenges to successfully integrating ecological information
479 among scales (Handel & Rohani, 2015; Johnson et al., 2015; Sofonea et al., 2015). Practically,
480 most data collected from parasite-host systems are cross-sectional and are often used to make
481 predictions of disease dynamics at scales beyond the original individual, population, or
482 community scale at which it was originally collected. Our results show this can lead to false or
483 spurious conclusions concerning individual-level parasite community assembly. The diversity of

484 combinations of extrinsic and intrinsic processes mean that trying to infer what mechanisms
485 drive the interactions at one scale, such as the impacts of competing parasites in coinfected
486 individuals, from patterns occurring at another scale, such as the force of infection driving
487 parasite prevalence, is difficult and more research is needed to understand the connections
488 between these ecological processes across multiple scales. We suggest the best approach to deal
489 with these complexities is to integrate data from the same system at multiple scales with
490 experiments to directly elucidate the directionality of processes at one scale and their
491 consequences at another. Further utilization of host-parasite systems as models for community
492 assembly will be a critical tool in this pursuit.
493
494 **Data Accessibility**
495 All data associated with this study have been deposited in the Dryad Digital Repository. As data
496 analyses are ongoing, release of data has been embargoed for 1 year following publication of this
497 manuscript.
498
499 **Acknowledgements**
500 We would like to thank Godefroy Devevey, Sarah Knowles, Owen Petchey, and all the
501 technicians and field assistants who collected the years of data used in this study. We also thank
502 Sarah States for discussion of NODF methods and Aaron Blackwell for discussion of MSM
503 methods, as well as the anonymous reviewers for their thoughtful comments on an earlier version
504 of this manuscript. This work was funded by grants from the National Science Foundation
505 (Postdoctoral Research Fellowship in Biology (DBI-1306608)) to ECR, NERC standard grants
506 (NE/G006830/1, NE/G007349/1, NE/I024038/1 and NE/I026367/1) to ABP and AF, and a

Wellcome Trust CIIE Advanced Fellowship (095831) and University of Edinburgh Chancellors Fellowship to ABP.

Authors' contributions

ECR and ABP conceived of the idea for the study and developed the methodology along with AF. ECR conducted the data analyses and production of figures. AF developed the simulation model. ECR lead the writing of the manuscript but all authors contributed significantly to its writing. All gave final approval for publication.

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Figures

Fig. 1. Nestedness matrices for the parasite community used in the analysis of the a) 2009-11 dataset, and b) the 2012 dataset. Each row in the y-axis represents an individual host with all parasites included in the analyses along the x-axis. A horizontal line represents if the host is infected with a parasite. In each nestedness matrix, the host coinfected with the most parasites is located in the top row and the most abundant parasite is located in the left column. The rest of hosts and parasites are then arranged to minimize unexpected species presences or absences (i.e. to create the most efficiently packed matrix). All data used in nestedness analyses was from a host's first capture.

Fig. 2. a-c) Illustration of all possible pairwise infection transitions in the MSM analyses; d) The predicted order of infection (community assembly) based on the nestedness analysis of the 2009-11 dataset; and e) the predicted order of infection based on the analysis of the 2012 dataset.

Fig. 3. Concordance of predicted and observed parasite ranks from a) 2009-11 and b) 2012. Predicted parasite ranks are along the x-axis, observed ranks (order of infection within individual hosts) are along the y-axis. Boxplots illustrate the distribution of observed ranks for the predicted rank of each parasite (median, interquartile range). The black line illustrates the linear relationship between predicted and observed ranks.

736 **Tables**

737

738 Table 1. Total number and infection prevalence for each parasite species in the wild wood mouse
739 populations in the 2009-11 and 2012 datasets.

740

741 Table 2. Predicted ranks, derived from the results of the nestedness analyses, used in Spearman
742 Rank analyses to compare to observed parasite rank order of infection in each individual wood
743 mouse host.

744

745 Table 3. Results of Spearman Rank analyses. Results presented are those of the observed ranks
746 (order in which a host was infected with each parasite) compared to the predicted ranks from
747 either the same dataset (e.g. 2012 predicted ranks and 2012 observed ranks) or different dataset
748 (e.g. 2012 predicted ranks and 2009-11 observed ranks). Both comparisons were done to test the
749 generality of the predictions generated from each dataset.

750

751 Table 4. Transition likelihoods with confidence intervals for all pairwise MSM infection models.
752 Hosts were able to transition between any two states per unit time (day). If a transition likelihood
753 is "0" this is due to there being no records of a host transitioning between those two states in the
754 dataset.

755

756

Supporting Materials

Table S1. Results of nestedness analyses of all datasets against the three null models: 1) fully random, 2) row totals (host parasite species richness) kept constant, 3) column totals (parasite abundance) kept constant.

Fig. S1. Nested matrix examples for each of the 3 null models used in the NODF analysis: a) random model (number of parasites total is the same, but they are assigned to parasites species and host individuals at random), b) host richness constant (host richness is identical to what is observed, parasite identity is randomized), c) parasite abundance constant (parasite species abundance constant, which hosts are infected is randomized). Each figure represents one of the 100 null communities generated from the, in this case, 2012 dataset for comparison to the observed nestedness of the wood mouse population.

Fig. S2. Results of the simulation exploring relationship between host population-scale parasite prevalence and time to first infection. Parasites with higher prevalence have shorter times to first infection, such that they are expected to infect an individual host faster than less-prevalent parasites.

Fig. S3. Nestedness plots for the parasite communities from the years 2009, 2010, and 2011 individually.

778 Fig. S4. Nestedness plots for a) adults and b) young hosts. Hosts in both age classes had similar
779 levels of nestedness and did not show patterns of young hosts containing subsets of the parasites
780 infecting older, adult hosts.
781
782 **Supporting Information**: Simulation model linking parasite prevalence to order of infection.
783
784