Ecological and evolutionary dynamics of chlamydiae endosymbionts in social amoeba host communities

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Abstract

Endosymbiotic interactions have played fundamental roles in shaping the evolution of complex eukaryotes. However, how ecological processes shape endosymbioses that are still segregating in host populations have been less described. Here, we characterize the ecological and evolutionary dynamics of chlamydiae bacterial endosymbionts in dictyostelid social amoeba host communities. Our survey of over 1400 host isolates from across a 1200 km range revealed 41 unique chlamydiae haplotypes across 11 different host species, the prevalence of which significantly varied across host species and sampling locations. Patterns of chlamydiae phylogenetic divergence were not associated with geographic dissimilarity but were associated with patterns of host diversification, suggesting a shared evolutionary history and co-dispersal between some symbiont and host lineages. However, relatively few symbiont haplotypes showed both significant host specificity and high prevalence, indicating that most associations are not ecologically meaningful. Nonetheless, our laboratory passaging experiments showed that naturally occurring chlamydiae infections can be vertically transmitted across host generations. Finally, we found overall chlamydiae haplotype diversity was negatively associated with prevalence across host populations, mirroring the classic tension between selection and the influx of neutral variation due to ecological drift and stochastic environmental acquisition. To formalize this observation, we developed a stochastic differential equation model incorporating vertical and environmental symbiont transmission, as well as ecological selection and drift. This model recapitulated the observed diversity-prevalence relationship, and is consistent with a scenario in which most chlamydiae haplotypes are ecologically neutral and stochastically acquired, while relatively few are vertically transmitted and selectively relevant within their host populations.

Introduction

The evolution of complex eukaryotes cannot be fully understood without considering the endosymbiotic interactions that have shaped their evolutionary histories. While the most notable examples are the mitochondria and chloroplast, many other endosymbioses have independently arisen throughout natural history and played significant roles in shaping their respective lineages' evolutionary trajectories [1–5]. Significant progress has been made in describing the general molecular and genetic changes symbionts undergo throughout a long history of host association [6]. Said changes predominately accrue in heritable symbionts that have fixed in host populations and undergone extended periods of time evolving in intra-host environments [7, 8]. However, endosymbionts arise from free-living organisms, and how ecological processes govern the distribution, frequency, and ultimately fixation of endosymbionts that are still segregating in host populations has received less empirical attention.

General ecological theory describes the processes that drive changes in the distributions and frequencies of organisms as analogous to those that drive the distributions and frequencies of alleles within populations. That is, diversification (analogous to mutation) and dispersal (analogous to gene flow) introduce organisms into an environment where selection and drift drive subsequent changes in frequencies [9]. Patterns of symbiont distributions and frequencies among host populations are typically attributed to selection: selective pressures exerted by or on hosts favors symbiont residence or elimination due to differences in host fitness [10]. Although general ecological theory seeks to define the relative importance of the previously described processes in shaping the distributions of organisms, the shift from a selection-centric framework to one that integrates more general ecological processes (i.e., ecological drift and dispersal) has been fairly recent in investigations of host-symbiont interactions [11, 12].

Since it is not possible to study how fundamental ecological processes shaped the initial trajectories of ancient and now obligate host-endosymbiont interactions, we can instead study endosymbionts that are still segregating in host populations. The chlamydiae endosymbionts segregating within dictyostelid social amoeba host populations offers and excellent natural system for examining said dynamics. Bacteria in the phylum Chlamydiota are among the most successful intracellular parasites, having adopted this life style an estimated 1-2 billion years ago [13, 14]. Although chlamydiae bacteria are primarily known as human pathogens, other diverse lineages have been found in almost every environment on earth and infect a wide variety of mammals, fish, arthropods, and protists [15–17]. Social amoebae have recently been indicated as common hosts of diverse chlamydial endosymbiont lineages, where prevalence has been detailed in natural populations of *Dictyostelium discoideum* and incidental infection has been documented in several other species [18–21]. While chlamydiae infections are common in *D. discoideum*, no obvious fitness costs or benefits have been established in laboratory studies [22]. Consistent with their frequent association with dictyostelid hosts, some chlamydial lineages, such as those found in *D. discoideum* and *D. giganteum*, have reduced genomes and other apparent adaptations to intra-host environments [23, 24]. However, the natural frequencies at which different chlamydiae lineages associate with dictyostelid hosts, degrees of host specificity, biogeographic variation, and patterns of co-diversification have been less established but are important for understanding the relative importance of different ecological processes in shaping the evolutionary trajectory of these endosymbiotic interactions.

To begin establishing the ecological and evolutionary processes that govern the frequency and

distribution of chlamydiae-dictyostelid endosymbioses, we first conducted natural sampling of over 1400 dictyostelid isolates from across a 1200 kilometer range to quantify the prevalence and patterns of genetic diversity of chlamydial symbionts. This showed a diversity of chlamydiae haplotypes in 11 different social amoeba host species, adding further evidence that dictyostelids are a common host of chlamydiae endosymbionts. There was significant variation in prevalence across the sampled geographic range and among dictyostelid host species, and relatively few chlamydial haplotypes were both prevalent and host specific. We found that patterns of chlamydiae diversification were not related to geographic differences but did show significant congruence with patterns of their hosts' diversification. We then isolated 11 different naturally occurring symbiont-host pairings and show that chlamydial symbionts are reliably vertically transmitted across host generations. Finally, we used a simple but effective population genetics-inspired stochastic differential equation model of ecological selection, drift, and dispersal to show that observed patterns of symbiont diversity and prevalence across host populations correspond with those expected if most symbiont haplotypes were selectively neutral and relatively few were selectively relevant.

Methods

Natural sample collection and symbiont screening

To study the prevalence and distribution of chlamydiae symbionts in natural Dictyostelid host communities, we first collected at least three different soil samples from just below the leaf litter on forest floors or decaying logs and stumps at 11 different sites across Arkansas and 1 site in Kentucky. We also included samples from a previous collection in Virginia [20], bringing our total to 13 sites from spanning just over 1200 km (Figure 1). To isolate amoebae, we plated soil samples on hay agar (Supplement section 1.1.1) no later than 96 hours after collection. We then identified amoebae based on fruiting body morphology [25]. Proliferation of individual amoebae cells gives rise to patches of several clonal fruiting bodies. Therefore, we collected clonal amoeba isolates from 2 to 5 sori (top of the fruiting body) from each patch, which allows for more sufficient DNA concentration for later analyses. We suspended collected isolates in 250 μL of KK2 spore buffer (Supplemental section 1.1.3) and used a Chelex/proteinase K approach to extract total DNA (described in [26]). We then confirmed that DNA extractions were successful by conducting a polymerase chain reaction (PCR) screen on all isolates using dictyostelid-specific primers (D307F and D862R) that target a $\sim$500 bp region of the 18S rRNA gene (Supplemental section 1.2.1) [27], and excluded isolates that failed to amplify. To further confirm dictyostelid identity, we sent a subset of samples for sequencing at Eurofins Genomics (Louisville, KY, USA). Here, we trimmed low quality or ambiguous base calls using Geneious (v8) [28] and classified isolates by using the NCBI BLAST web application (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to search for matching sequences in the standard nucleotide database. Of the 11 species we identified, 10 were able to be confidently classified, and we refer to the remaining unclassified species as "*Unclassified Dictyostelium*". Overall, our sampling effort yielded 1424 amoeba isolates from 11 different species.

To identify the presence of chlamydial symbionts in each amoeba isolate, we conducted a PCR screen using the Chlamydiota-specific CHL16SFOR2 and CHL16SREV2 primers, which amplify a $\sim$250 bp region of the 16S rRNA gene [29]. We then sequenced PCR products as described for dictyostelid isolates, which allowed us to identify unique haplotypes, which were defined as

unique sequences of the 220bp amplicon and were numbered by order in which they were isolated, continued from Haselkorn et al 2021. To gain deeper insights into patterns of symbiont diversification, we then used a variety of primers to amplify and sequence the full 16S rRNA gene for a representatives with unique haplotypes (Supplemental section 1.4). All primers and PCR designs are described in Supplemental section 1.2.

Sequence processing and phylogenetic analyses

We performed all sequence cleaning and quality control in Geneious as previously described for host sequence processing. After sequence processing, we used MEGA (v7) to align sequences with the MUSCLE algorithm, as well as construct several phylogenetic trees [30]. First, we constructed a tree from short Chalmydiota sequences to gain an initial overview of haplotype diversity (Figure 1A), where the data was best described by a K2+G substitution model [31, 32]. We also constructed a phylogenetic tree using chlamydiae haplotypes in which we were able to amplify the full 16S rRNA gene, where a GTR+G+I substitution model best described the data [33, 34]. Finally, we constructed a phylogenetic tree for hosts with corresponding full-length 16S rRNA chlamydiae sequences using host 18S rRNA sequences, where a T92+G substitution model best described the data [35]. We used maximum-likelihood to infer all trees and estimated branch support using 1000 bootstrap replicates.

To test for possible co-phylogenetic relationships between hosts and corresponding symbionts, we used the *rtc.test* function from the *manticore* R library (v.2025.12) to implement a random tree congruence test [36]. Briefly, a null co-phylogenetic model is generated by sampling trees ($i = 1000$ for our purposes) with random topologies and evaluating their congruence to the host tree, which is then used to statistically evaluate the observed degree of congruence between host and symbiont trees. Here, we evaluated tree congruence using mutual clustering information, which generally outperforms other congruence metrics when both quantifying congruence and when used with random tree congruence testing [36, 37]. We also examined how robust our statistical inference was to uncertainty in tree estimation using the *rtc.sensitivity.test* function in the *manticore* R library, which uses subtree pruning and regrafting to perturb observed trees and examine how the integrated $P(Null \geq Obs.)$ value changes as a function of topological perturbance (Supplemental Figure S1) [36, 38].

Evaluating host specificity

To quantify the degree of host specificity of different haplotypes, we first calculated the d' specialization index [39]. Letting $H = h_1, h_2, \ldots, h_k$ be the set of host species, $S = s_1, s_2, \ldots, s_m$ be the set of haplotypes, N_j be the number of hosts infected infected by haplotype s_j, and n_i be the number of total hosts sampled from host species h_i, the expected host frequency based on sampling effort is $q_i = \frac{n_i}{\sum_{l=1}^{k} n_l}$. For each haplotype s_j, let the proportional occurrence of s_j in host h_i be p_{ij}. The specialization index for haplotype s_j is then

$$d'_j = \frac{\sum_{i=1}^{k} p_{ij} \ln(\frac{p_{ij}}{q_i})}{\ln k} \tag{1}$$

where the sum is taken over only i where $p_{ij} > 0$. Here, $d' = 0$ indicates symbiont is a perfect generalist, while $d' = 1$ indicates symbiont is a perfect specialist.

We then employed a null model approach to statistically evaluate observed degrees of host specificity, which allowed us to account for variation in host sampling effort and symbiont prevalence. Here, the prevalence of each haplotype is fixed, such that haplotype s_j still occurs N_j times. Host assignments are then randomized, where N_j hosts are independently sampled with a probability q_i for each host species h_i, such that $\tilde{p}_{ij}^{(r)} \sim \text{Multinomial}(N_j, q_1, q_2, \ldots q_k)$. Proportions are then normalized to N_j, and the randomized $\tilde{d}_j^{\prime(r)}$ is calculated. This process is repeated for $r = 1, \ldots, R$ permutations to generate the null distribution for haplotype $s_j = \{\tilde{d}_j^{\prime(1)}, \tilde{d}_j^{\prime(2)}, \ldots, \tilde{d}_j^{\prime(R)}\}$. The observed $d_j^{\prime}$ is then compared to this null distribution to calculate the probability of observing null results greater than or equal to those observed, such that

$$P(N \geq O) = \frac{1}{n} \sum_{i=1}^{n} I(\tilde{d}_j^{\prime(r)} \leq d_j^{\prime}), \tag{2}$$

where I is the indicator function that equals 1 if the inequality is satisfied and 0 otherwise.

Vertical transmission assays

To test the extent that naturally occurring chlamydial symbionts were able to be vertically transmitted by their amoebae hosts, we first isolated amoeba from soil samples collected in Woolly Hollow State Park, Arkansas. We grew amoebae directly from soil samples as previously described, and tested isolates for chlamydiae infection. We then re-plated positive samples with *K. pneumoniae* food bacteria on SM/5 media (Supplemental section 1.1.2), which allowed us to obtain plates full of amoeba descended from their original isolates. To test for chlamydiae prevalence and obtain individuals to passage for the next generation, we collected 8 amoeba fruiting bodies from different locations across each plate into 20 μL of KK2 spore buffer (Supplemental section 1.1.3). For each isolate, we plated 10 μL of spores to start the next generation and the other 10 μL for testing for chlamydiae presence as previously described.

Modeling symbiont prevalence and diversity dynamics

To model the prevalence dynamics of various symbiont haplotypes $X_{i,t}$ within host populations, we used a diffusion approximation (for the sake of computational practicality given large microbial population sizes) of Wright-Fisher sampling to consider symbiont spread via vertical transmission. Here, symbionts have some ecological selection coefficient s_i that facilitate their deterministic spread of decline in host populations. It is important to note that while the concepts are mathematically equivalent, ecological selection for a symbiotic interaction is not biologically equivalent to allelic selection in population genetics. That is, ecological selection for a symbiotic interaction can be driven by mutualistic interaction that benefits fitness (consistent with population genetics), or a proficient parasitic interaction. Thus, ecological selection for our purposes represents the degree of vertical transmission, regardless of specific (a)symmetric fitness dynamics. Here, the deterministic selection term $s_i X_i(1 - X_i)$ and ecological drift term $\sqrt{X_i(1 - X_i)/N} dW_t^{(i)}$, where $dW_t^{(i)}$ represents an independent Wiener process for drift dynamics in symbiont i, follow the standard Wright-Fisher diffusion limit for allele frequencies [31].

In addition to selective dynamics, we also include stochastic environmental acquisition of the symbiont by the host. Here, we consider the probability Y_i that a host a symbiont will occupy the same environment, as well as the rate m at which hosts acquire symbionts from the environment.

Therefore, environmental acquisition of a symbiont is represented as a mean flux $m(Y_i - X_i)$, which is analogous to how migration is treated in population genetic island models, plus an additive stochastic term $\sigma_{m,i} dV_i^{(t)}$ to represent stochasticity in environmental acquisition. Here, $dV_i^{(t)}$ represents an independent Wiener process and $\sigma_{m,i}$ represents the standard deviation in stochastic fluctuation in environmental acquisition, such that

$$m_t = m + \sigma_m \cdot \eta_t, \eta_t \sim \mathcal{N}(0, 1) \tag{3}$$

Taken together, changes in prevalence X of symbiont i are modeled by the stochastic differential equation

$$dX_{i,t} = s_i X_{i,t}(1 - X_{i,t})dt + \sqrt{\frac{X_{i,t}(1 - X_{i,t})}{N}} dW_t^{(i)} + m(Y_{i,t} - X_{i,t})dt + \sigma_{m,i} dV_t^{(i)} \tag{4}$$

Through the combination of vertical transmission and environmental acquisition, it is possible for hosts to become co-infected with multiple symbiont haplotypes, which has implications for how prevalence is estimated. For simplicity, we assume events that lead to co-infection are independent, and approximate the total prevalence $S(t)$ (fraction of hosts harboring at least one symbiont haplotype) as

$$S(t) \approx 1 - \prod_{i=1}^{k} (1 - X_i(t)) \tag{5}$$

where $X_i(t)$ is the marginal prevalence of haplotype i at time t. This approximation is exact under independence and provides a good approximation when marginal prevalence is small or co-infections are rare, which is supported by our data. Given that $S(t)$ is the fraction of hosts harboring any symbiont at time t, the conditional frequency of symbiont i among infected hosts is $p_i^c = \frac{X_i(t)}{S(t)}$. Therefore, we evaluate the diversity of haplotypes within a host population at time t using the Shannon-Wiener index H with conditional frequencies:

$$H(t) = -\sum_{i=1}^{k} p_i^c(t) \ln p_i^c(t) \tag{6}$$

When evaluating symbiont dynamics, diversity $H(t)$ is expected to initially increase because all haplotypes are rare and experience stochastic increases in frequency. If there are few haplotypes with meaningfully positive ecological selection coefficients, they will eventually rise in prevalence, while other remain at low prevalence due to stochastic environmental acquisition and loss. This creates an initial rapid increase in diversity which then declines as the selectively meaningful haplotypes rises in frequency. This decline continues until reaching a stable point (at which there is still stochastic variation). To estimate this stable point numerically, we fit an asymptotic exponential decay function

$$H = A + Be^{-\lambda t} + \epsilon_t \tag{7}$$

to simulated dynamics, where A is the asymptote that represents the stable diversity state. Due to the initial increase in $H(t)$ due to rarity, we only fit Equation 7 to the portion of dynamics that occurs after the peak in $H(t)$. Similarly, given any selectively meaningful symbiont haplotypes,

total prevalence S is expected to increase before plateauing at some stable value A around which stochastic fluctuations occur. To estimate this value, we fit an asymptotic growth function

$$S = S_0 + A(1 - e^{-\lambda t}) + \epsilon_t \tag{8}$$

to simulated prevalence dynamics.

Results

An overview of chlamydiae diversity and prevalence across space and host species

Our sampling effort of over 1400 dictyostelid isolates from 13 sites that span over 1200 km constitutes the most detailed characterization of dictyostelid-chlamydiae ecology to date. This revealed 41 unique chlamydiae haplotypes across 11 dictyostelid host species (Figure 1A). Within this, we identified 6 additional species of social amoebae harboring chlamydiae that had not been previously described, lending further support for dictyostelids being common hosts of these endosymbionts. There was significant variation in prevalence across sampling locations, with a maximum of 37%, a minimum of 3%, and a mean of 22% (Figure 1B). Similarly, there was significant variation in chlamydiae prevalence across host species, with a minimum of 9% in *R. minutum*, a maximum of 53% in *D. giganteum*, and a mean of 24% (Figure 1C). Taken together, these findings provide descriptions of the variation we seek to explain in the following sections.

The importance of geographic structure and host diversification in explaining patterns of symbiotic chlamydiae diversification

Given the significant chlamydiae diversity and variation in patterns of host association, we were then interested in the potential for geography to explain patterns of diversification. Limitations on dispersal can create patterns where individuals from more geographically distant locations tend to be more distantly related, thus creating a positive relationship between geographic distance and phylogenetic distance. Such barriers to dispersal can have important (negative) consequences on symbiont fixation dynamics. However, we found that patterns of chlamydiae phylogenetic dissimilarity among haplotypes was not correlated to their geographic dissimilarity ($r_M = -0.252$, $p = 0.791$) (Figure 2).

Our previous analysis suggests patterns of chlamydiae diversity are not explained by their geographic dissimilarity. Therefore, we then examined the potential for patterns of host phylogenetic divergence to explain patterns of chlamydiae divergence. We found a significant degree of congruence between the chlamydiae phylogeny and that of their host (Mutual Clustering Information = 5.27, $p \leq 0.001$) (Figure 3). Furthermore, our sensitivity analysis suggests these findings are robust to reasonable degree of uncertainty in phylogenetic estimation (Supplemental Figure S1).

A small portion of chlamydiae haplotypes show both host specificity and high prevalence

Host-specificity is often considered a hallmark of a shared evolutionary history between host and symbiont lineages. However, host-specificity in the absence of a high prevalence could indi-

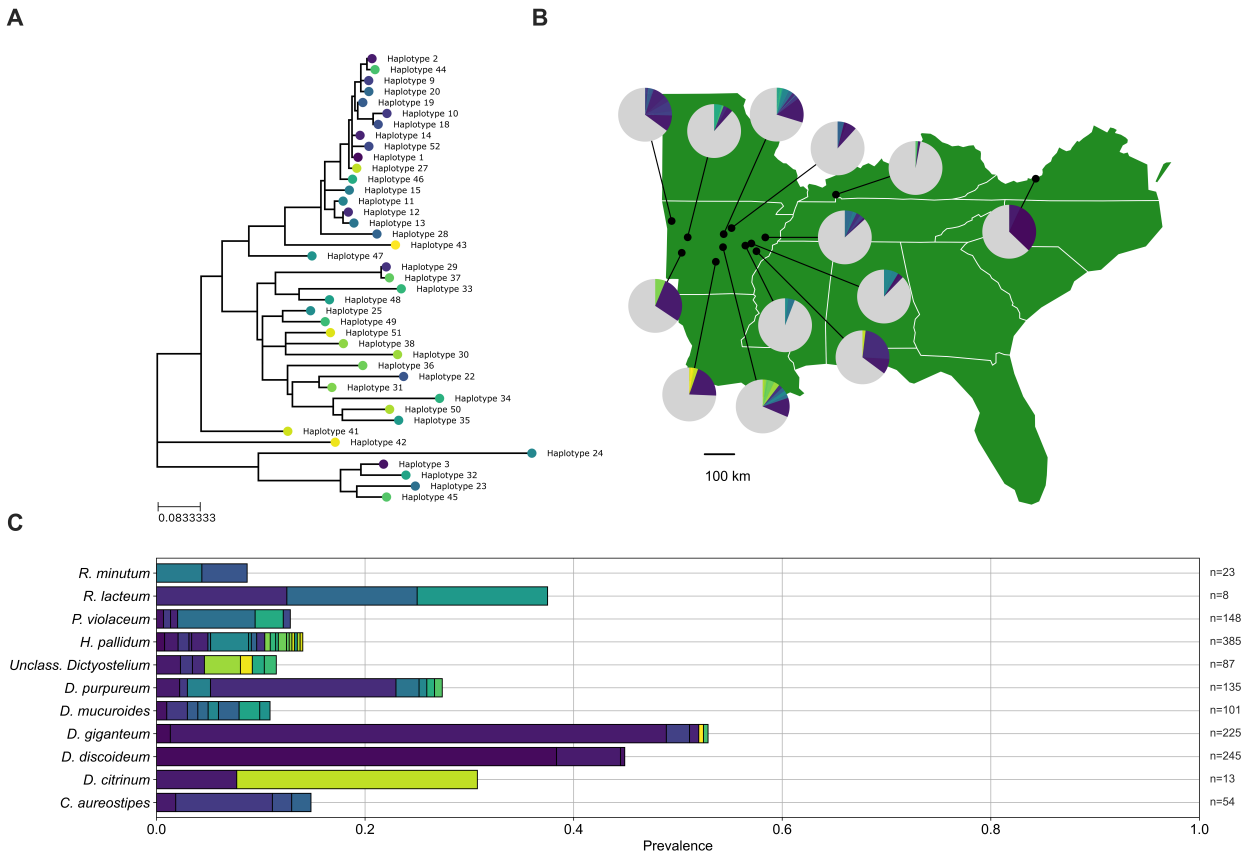

Figure 1: An overview of symbiotic chlamydiae diversity and geography in Dictyostelid hosts. A) A maximum-likelihood estimated phylogenetic tree showing patterns of chlamydiae diversity. Each tip is arbitrarily colored to represent different haplotypes. B) A map showing the distribution and prevalence of chlamydiae haplotypes across sampling locations. Each pie graph represents the prevalence of each haplotype in the corresponding location, and the gray portion represents the prevalence of uninfected host isolates. C) A bar graph showing the prevalence of each chlamydiae haplotype in each host species. Numbers at the ends of each bar indicate the number of corresponding host species sampled.

cate spurious or poorly established host-symbiont pairings. Therefore, we quantified the host-specificity and maximum prevalence of each symbiont haplotype within host populations to identify evidence of more ecologically relevant host-symbiont pairings. This showed that relatively few (12/41) chlamydiae haplotypes were more host-specific than expected due to sampling effects (Figure 4). Among these, only two haplotypes, designated as 1 and 2, were highly prevalent within their host's populations. Specifically, haplotype 1 was present in 38% of *D. discoideum* isolates, and haplotype 2 was present in 48% of *D. giganteum* isolates (Figure 4). Haplotype 27 also showed a significant degree of specificity and prevalence (23%) in *D. citrinum* hosts, but our low sample size of *D. citrinum* hosts (n=13) warrants caution (Figure 4).

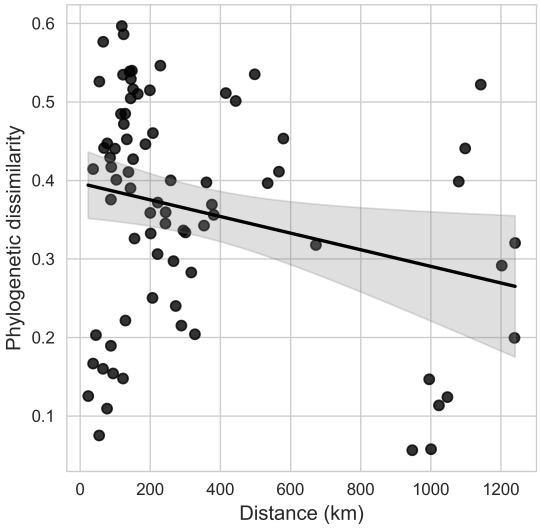

Figure 2: Patterns of chlamydiae phylogenetic dissimilarity do not correlate with patterns of geographic dissimilarity. The y-axis represents the phylogenetic distance between a given pair of haplotypes and the x-axis represents the distance between the center of their geographic distribution. The fit linear model displayed along side points is used to provide a visualization of the lack of a correlation, but we made statistical inferences using the Mantel test (not parameter estimates from the linear model).

Chlamydiae isolates are reliably vertically transmitted in hosts they naturally infect

Symbionts with a long history of host-association tend to evolve increased rates of vertical transmission. To examine the degree to which chlamydiae symbionts are vertically transmitted in hosts they naturally infect, we passaged naturally infected hosts for 8 generations and quantified the proportion of infected offspring. Overall, this showed that across 11 naturally occurring host-symbiont pairings, chlamydiae symbionts were vertically transmitted with near perfect fidelity after initial stochasticity in isolation (Figure 5). In other words, 100% of offspring were infected with chlamydiae from generations 3-8 across all host-symbiont pairings. Notably, we saw this general pattern when chlamydiae haplotype 2, which our previous analyses suggest is highly prevalent and host specific, was paired with 6 different *D. giganteum* isolates (Figure 5A). We also found that chlamydiae haplotypes 19 and 45 were reliably vertically transmitted in *D. mucoroides* and haplotypes 11 and 13 were reliably vertically transmitted in *D. purpureum*, though none of these haplotypes are very prevalent and host-specific (Figure 5B and C). Finally, we found that chlamydiae haplotype 10, which was somewhat host specific but had relatively low prevalence, was reliably vertically transmitted in *C. aureostipes* hosts (Figure 5D).

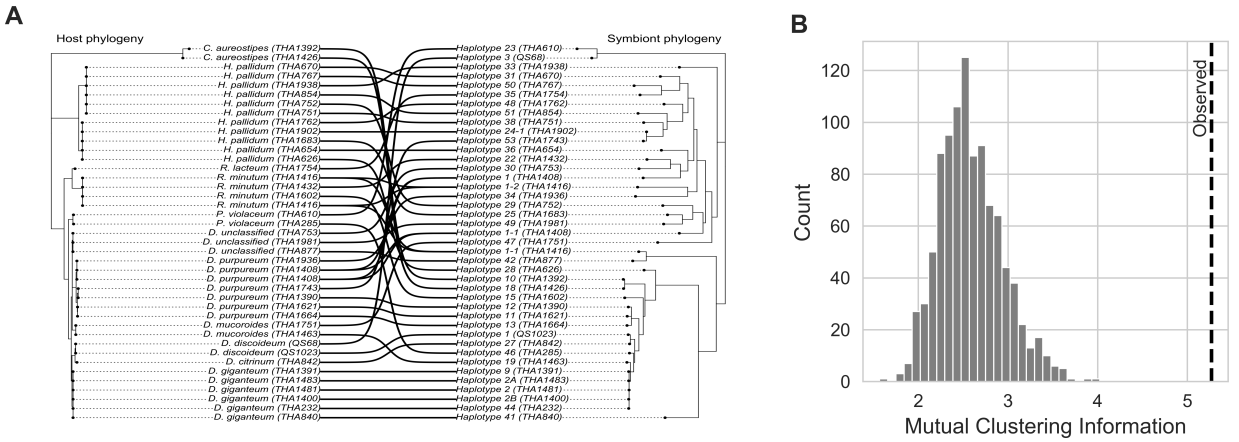

Figure 3: Patterns of chlamydiae diversification are somewhat associated with patterns of host diversification. A) A tanglegram showing the alignment of tips between the dictyostelid host phylogeny (left) and the chlamydiae symbiont phylogeny (right). Note that tanglegrams only show the alignment of tip positions but does not necessarily reveal the more deeply rooted shared structure that is considered by mutual clustering information. B) A null model showing the null congruence distribution between the chlamydiae phylogeny and a sample of 1000 trees with random topologies. The histogram shows null congruence values and the dashed line shows the observed congruence between the host and symbiont phylogenies.

Patterns of chlamydiae diversity and prevalence within host populations are consistent with predominately neutral dynamics paired with few selectively relevant haplotypes

Our previous analyses recovered a diversity of chlamydiae haplotypes that infect Dictyostelid host communities, the majority of which were at low prevalence and were not very host specific. However, we also found some degree of phylogenetic congruence between chlamydiae symbionts and their hosts, some symbiont haplotypes that were both host-specific and highly prevalent, and a high-degree of vertical transmission of naturally occurring chlamydiae infections. Therefore, we were then interested in describing how ecological processes could produce such patterns.

Examining Figure 1C, it appears that host species with a high overall prevalence of chlamydiae symbionts harbor a lower diversity of symbionts. Conversely, host species with low chlamydiae prevalence tend to harbor a greater diversity of symbionts. We found that this relationship was quantitatively supported, where diversity (Shannon-Weiner Index) decreased with increasing prevalence across host species ($\beta = -2.8$, 95% CI = [-5.45, -0.142], $p = 0.041$) (Figure 6A). Although it is not possible to discern the specific ecological drivers, this pattern mirrors the classic tension between selection and the influx of new variation in population genetics. That is, when one or few symbionts are selectively favored within a host population, it can increase in frequency and dominate the symbiont composition of the population, reducing diversity in a manner similar to a selective sweep. In contrast, when no selectively relevant symbionts are present, stochastic immigration (symbiont diversification may also contribute, but over shorter time scales host sampling from the same pool of symbionts is likely the more important driver of variation) may play a more dominant role in shaping the composition of symbionts within a host population, resulting in many

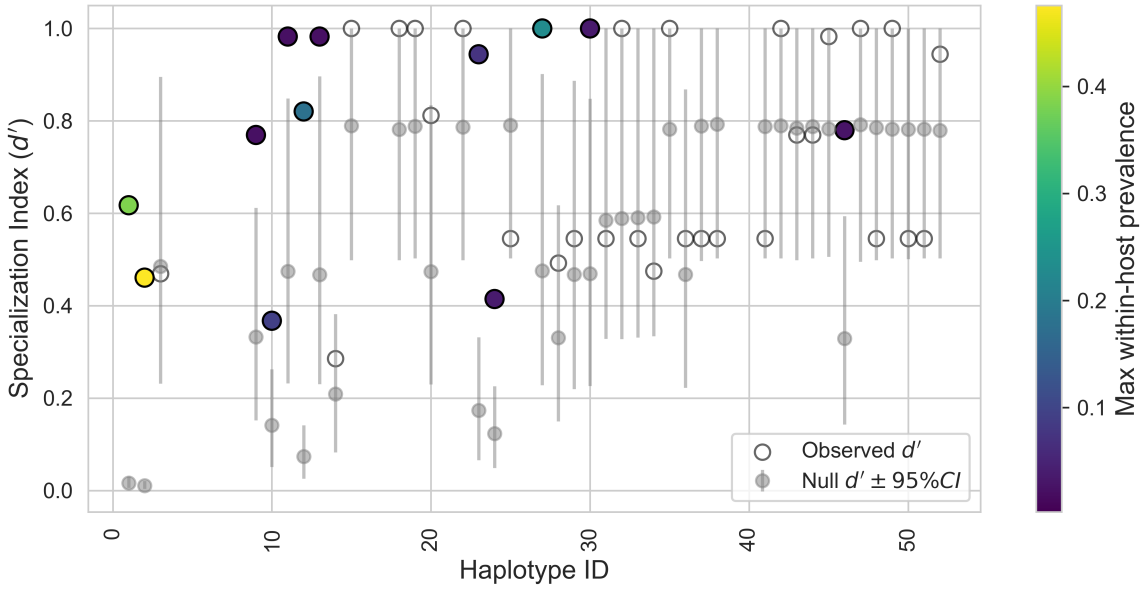

Figure 4: Relatively few chlamydiae haplotypes show significant host specificity and high prevalence. The y-axis represents the specialization index d', which ranges from 0 (no host-specificity) to 1 (entirely host specific), and the x-axis denotes the unique haplotype identifier (arbitrarily numbered). Large points represent the observed specialization index of the corresponding haplotype, where points that are colored in were statistically more specialized than expected under a null model. Likewise, points that are not colored represent haplotypes that were not more specialized than expected under the null model. Haplotypes that were statistically more specialized that colored by their prevalence in the host species that they were most prevalent in. Each smaller gray point and confidence interval represent the mean and 95% CI of the null model.

low-frequency symbionts but higher overall diversity.

To formalize this hypothesis, we constructed a stochastic differential equation model of vertical symbiont transmission, ecological selection, and symbiont acquisition from an environmental pool. Within this framework, we considered a symbiont pool of size k, which for our purposes represents the number of symbiont haplotypes the host community could sample from. We then set all symbionts to be selectively neutral except for one, which we assigned a progressively increasing ecological selection coefficient. Numerical analysis of this model showed the same pattern we observed in our natural data: overall symbiont diversity decreased with increasing prevalence, and increasing overall symbiont prevalence was driven by stronger ecological selection for the selectively relevant haplotype (Figure 6B). The amount of symbiont diversity present varied by the size of the symbiont pool, but the same general pattern was consistent across symbiont pool sizes (Figure 6B). Overall, these analyses are consistent with a model where most chlamydiae symbionts are selectively neutral in most host populations, while some host populations with high chlamydiae prevalence harbor one or few selectively relevant symbiont haplotypes.

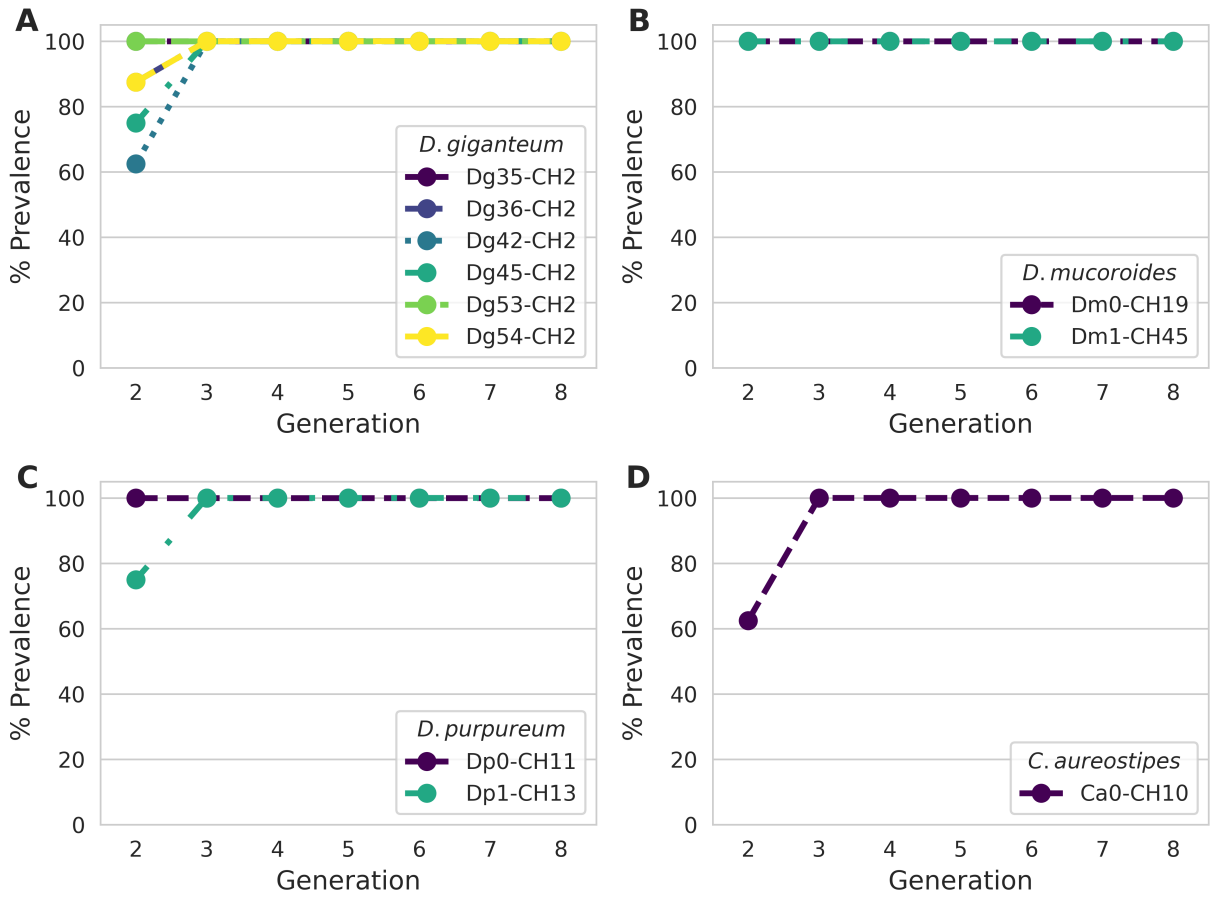

Figure 5: Chlamydiae haplotypes are reliably vertically transmitted in hosts they naturally infect. The y-axis for each panel represent the percent of sampled offspring that were infected with chlamydiae, and the x-axis represents this value across subsequent generations. Different lines represent different naturally occurring host-symbiont pairings, which include A) *D. giganteum*, B) *D. mucoroides*, C) *D. purpureum*, and D) *C. aureostipes*.

Discussion

Understanding the evolution of endosymbiotic interactions requires explaining how ecological processes shape the frequency and distribution of endosymbionts that are still segregating in host populations. To further this goal, here we studied the ecological and evolutionary drivers of chlamydiae bacterial endosymbionts in Dictyostelid social amoeba host communities. Our survey of natural host communities revealed a diversity of chlamydiae haplotypes with prevalences that vary significantly across host taxa (Figure 1). We found that patterns of chlamydiae diversification were not explained by their geographic dissimilarity (Figure 2), but were somewhat associated with patterns of host diversification (Figure 3). However, relatively few chlamydiae haplotypes showed both significant degrees of host specificity and high prevalence within a host population (Figure 4). Nonetheless, when we isolated several naturally occurring dictyostelid-chlamydiae pairings, we found that chlamydiae was reliably vertically transmitted across host generations in laboratory

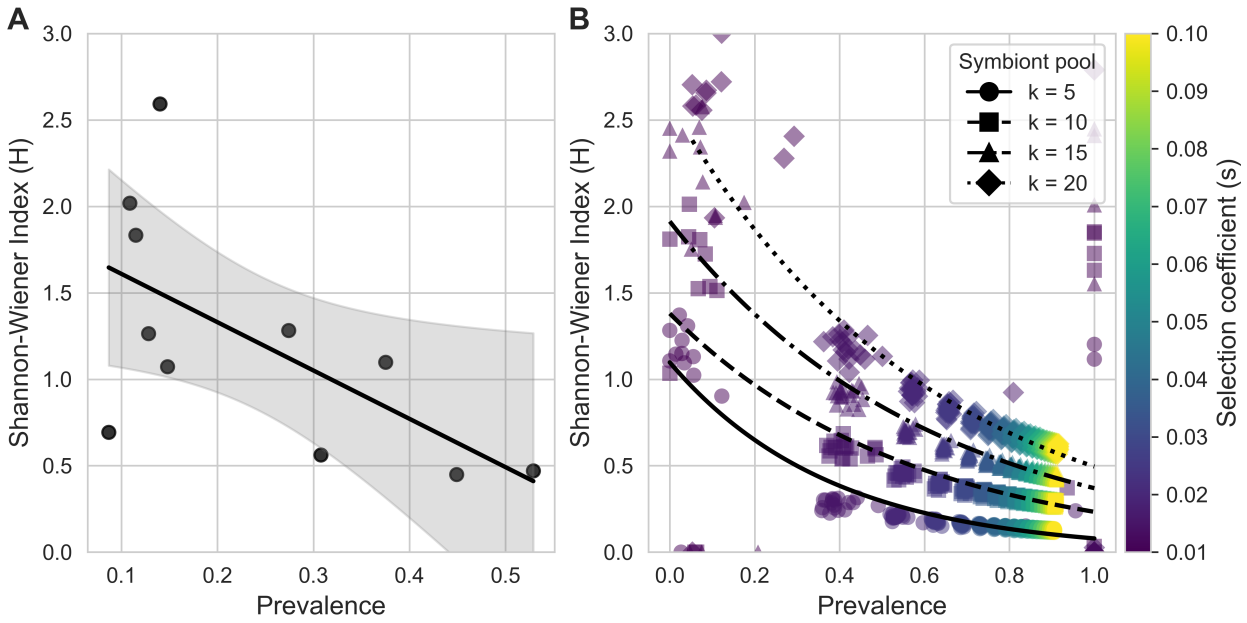

Figure 6: The diversity and prevalence chlamydiae symbionts within Dictyostelid host populations is consistent with predominately neutral dynamics paired with few selectively relevant symbiont haplotypes. A) Overall symbiont haplotype diversity as a function of overall symbiont prevalence. The y-axis represents the Shannon-Weiner index of symbiont haplotypes within a host population, and the x-axis represents the overall chlamydiae prevalence within the corresponding host population. Each point represents a different host population, the solid line shows the fit linear model, and the shaded area represents the 95% confident interval. B)

conditions in all species, even those with a lower incidence in nature (Figure 5). Finally, we found that overall chlamydiae diversity was negatively associated with their overall prevalence across host populations (Figure 6A). Considering this and our previously described findings, we hypothesized that the ecological dynamics of chlamydiae endosymbionts can be explained by most chlamydiae haplotypes being stochastically acquired and selectively neutral, paired with relatively few haplotypes that are vertically transmitted and selectively relevant. We formalized this hypothesis with a population genetics-inspired stochastic differential equation model, which recapitulated the observed relationship between symbiont diversity and prevalence in natural host communities (Figure 6B).

One of the most striking host-symbiont pairings we identified was between *D. giganteum* and chlamydiae haplotype 2, which has been recently named *Reclusachlamydia socialis* [24]. This endosymbiont was highly prevalent in *D. giganteum*, infecting approximately 50% of isolates, and showed significant degrees of specialization to *D. giganteum*. Interestingly, *R. socialis* shows phenotypic and genomic patterns consistent with adaptation to the social amoeba multicellular lifecycle. The standard chlamydiae lifecycle consists of a reticulate body stage that replicates within the host cell and an elementary body stage that is stable in the environment and is responsible for infecting new hosts [15, 40, 41]. *R. socialis* has lost this elementary body stage [24], which would likely make it more dependent on vertical transmission across social amoeba hosts and potentially lead to the attenuation of virulence often seen associated with this transmission mode in chlamy-

diae [42]. Taken together, the *R. socialis* ecological dynamics and model predictions we have presented here are consistent with these previously described host-symbiont dynamics in laboratory settings. However, horizontal transmission of *R. socialis* through cell-to-cell contact during the dictyostelid social cycle has been documented, and loss of the elementary body stage has not been identified as a general property of the wide diversity of chlamydiae symbionts identified in this study [24]. Therefore, it is likely *R. socialis* and other chlamydial symbionts have mixed transmission modes in natural populations, which is supported by our co-phylogenetic and host specificity analyses. Therefore, future work focused on understanding variation in rates of horizontal/vertical transmission across these chlamydial endosymbionts will provide further understanding of their ecological and evolutionary dynamics.

Our findings regarding the negative relationship between chlamydiae haplotype diversity and prevalence across host populations parallel other natural systems undergoing selective sweeps, which have been primarily described in Wolbachia-driven sweeps in various insect taxa [43–47]. Said studies describe sweeping dynamics with respect to the host population, where Wolbachia prevalence is thought to drive the increase in frequency of compatible host haplotypes. However, it is important to note that here we describe how patterns of symbiont diversity relate to their distribution across host taxa, which represents a more mirrored perspective of dynamics that have been previously described. It is also important to note that a selective sweep of haplotypes within a host population and a "selective sweep" of symbiont haplotypes segregating in host populations are driven by different aspects of fitness. That is, the selective sweep of a host haplotype is driven by its increased fitness [48]. The selective sweep of a symbiont haplotype may also be driven by its increased fitness, but it may also be driven by ecological selection. Here, ecological selection describes the increase in frequency of an interaction beyond what would be neutrally expected [9, 49]. Therefore, being a better mutualist or a better parasite could drive the selective sweep of different chlamydiae haplotypes [10, 50].

This points towards the outstanding question of what the ecological roles of potentially selectively relevant haplotypes could be. Fitness studies have only been conducted in two species, which only measured host parameters like developmental time and spore production under standard laboratory conditions and found no effect [22, 24]. Some hosts have facultative symbionts that confer context-dependent benefits, such as thermal tolerance, nutritional supplementation, or defense against other parasites, the latter of which been associated with chlamydiae symbionts of more distantly related amoeba and other hosts [51–56]. However, it is important to re-emphasize that the ecological dynamics of potentially selectively relevant chlamydial symbionts we present here are also applicable to symbionts that are more parasitic and proficient and ensuring their transmission. Therefore, future work establishing the specific functional aspects of these relationships, particularly in natural settings, would help contextualize these ecological dynamics.

Overall, these findings illustrate a stark contrast to the dynamics of *Paraburkholderia* endosymbionts within the same host communities, which show evidence of significant dispersal limitation and lack signatures of a history of co-dispersal with Dictyostelid hosts [57, 58]. Furthermore, while the signal was weak, we did find some detectable degree of phylogenetic congruence between chlamydiae symbionts and their hosts, a pattern that has also not been supported in *Paraburkholderia* symbionts [58]. These ecological patterns are consistent with the frequently observed fitness detriments of *Paraburkholderia* symbionts in Dictyostelid hosts [58–60]. However, the fitness consequences (positive or negative) associated with chlamydiae infection, if any, are less established, making comparisons of ecological and evolutionary dynamics based on associated fitness differ-

ences difficult [22]. Nonetheless, there seems to be a stark contrast in ecological and evolutionary dynamics between chlamydiae and *Paraburkholderia* symbionts, which are known parasites of dictyostelids, adding some credence to the hypothesis that (at least some) chlamydiae lineages have a long history of ecologically meaningful associations with Dictyostelid hosts.

More generally, our findings suggest relatively few symbiont haplotypes are both host specific and ecologically meaningful, while the remaining appear ecologically neutral. This hypothesis, as described and supported by our model, is consistent with a growing body of literature suggesting that most microbial symbionts are ecologically neutral, while relatively few show evidence of their ecological selection [61–65]. While these insights have been primarily derived from studies of host-associated microbial communities, the model we present here to describe our findings is structurally similar to those previously described [66] (barring adjustments for vertical transmission and explicit modeling of stochasticity). However, it is important to emphasize that the patterns we observed and that were produced by our model could also arise from weak ecological selection against most host-symbiont pairings that occurs at a longer time scale than transmission or stochastic environmental acquisition (i.e., symbionts could be quickly acquired by take some time to selectively remove), and it is not possible to discern these dynamics from neutrality without both temporal sampling and experimental validation. This issue is not only a feature of our study, but many others that investigate the importance of neutral dynamics in shaping the ecology of microbial symbioses. Therefore, future studies that seek to describe temporal symbiont dynamics and experimentally verify ecological relevance (or lack thereof) have the potential to significantly improve our account of how ecological process shape the evolution of long-term endosymbiotic interactions.

Data and code availability

All sequences generated for this project have been deposited into GenBank and will be made available upon publication. All code written to perform analyses and run model simulations can be accessed at https://github.com/gabe-dubose/chlam_ecology_initial_description, which has been archived under the DOI: 10.5281/zenodo.20261623.

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Conflicts of interest

The authors declare no conflicts of interest.

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