




LETTER

Pathogens manipulate the preference of vectors, slowing disease spread in a multi-host system

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Abstract

The spread of vector-borne pathogens depends on a complex set of interactions among pathogen, vector, and host. In single-host systems, pathogens can induce changes in vector preferences for infected vs. healthy hosts. Yet it is unclear if pathogens also induce changes in vector preference among host species, and how changes in vector behaviour alter the ecological dynamics of disease spread. Here, we couple multi-host preference experiments with a novel model of vector preference general to both single and multi-host communities. We show that viruliferous aphids exhibit strong preferences for healthy and long-lived hosts. Coupling experimental results with modelling to account for preference leads to a strong decrease in overall pathogen spread through multi-host communities due to non-random sorting of viruliferous vectors between preferred and non-preferred host species. Our results demonstrate the importance of the interplay between vector behaviour and host diversity as a key mechanism in the spread of vectored-diseases.

Keywords

Barley/cereal yellow dwarf virus, behavioural ecology, disease ecology, host diversity, pathogen transmission, vector behaviour, vector manipulation hypothesis, vector-borne disease, vectored disease modelling.

Ecology Letters (2019)

INTRODUCTION

Vector-borne diseases are currently the most common emerging pathogens, with both new and resurgent vectored-pathogens infecting systems encompassing native flora, crops, wildlife, and humans (Gratz 1999; Taylor *et al.* 2001; Anderson *et al.* 2004; Malmstrom *et al.* 2007). Understanding the dynamics and forecasting the impacts of future epidemics of vectored-pathogens is therefore critical from ecological, agricultural, and public health perspectives. Yet, the spread of vectored-pathogens relies on a complex set of interactions among at least three agents: pathogen, vector, and host – each with their own behaviour, population and community dynamics (Johnson *et al.* 2015). Predicting vectored disease transmission and spread therefore depends on integrating key concepts and methodology from disease, community, and behavioural ecology (Lord 2004; Reisen 2010; Johnson *et al.* 2015; Seabloom *et al.* 2015; Shaw *et al.* 2017).

Vectors serve as critical links between pathogens and hosts. Yet for tractability, vectored transmission dynamics often are assumed to be relatively simple (Antonovics *et al.* 1995). When we recognise vectors as foraging animals rather than merely as necessary links in pathogen transmission, our expectations for disease dynamics are altered considerably. For example, vector foraging is the result of individual movement and feeding behaviour that is widely hypothesised to maximise vector fitness via optimal foraging theory (Charnov 1976; Pyke 1984). This foraging behaviour impacts vector fitness, population dynamics, and the spread of disease. Critically, optimal foraging dynamics for the vector may not optimise pathogen spread, creating the potential for a conflict between the fitness needs of the pathogen and vector.

To this end, an advantageous strategy for pathogens is to manipulate vector foraging behaviour such that pathogen fitness – rather than vector fitness – is optimized (Moore 2002; Hurd 2003).

As might be expected given this potential conflict, many vector-borne pathogens, including viruses (Stafford *et al.* 2011; Ingwell *et al.* 2012), fungal pathogens (Evans 1982), protozoa (Cator *et al.* 2012), and bacteria (Martini *et al.* 2015), have all been shown to alter vector behaviour in a manner hypothesised to promote pathogen spread. For example, viruliferous aphids carrying barley yellow dwarf virus prefer feeding on healthy wheat plants, while non-viruliferous aphids prefer infected hosts (Ingwell *et al.* 2012). Similarly, the bacteria *Candidatus Liberibacter asiaticus* alter vector (Asian citrus psyllid) dispersal behaviour and flight capacity such that vectors disperse earlier and travel greater distance, potentially promoting pathogen spread across larger geographic regions (Martini *et al.* 2015). In animal host systems, malaria sporozoite-infected mosquitos are more persistent at blood-feeding (Anderson *et al.* 1999) and probe more frequently than non-infected mosquitos (Rossignol *et al.* 1984; Koella *et al.* 2002). While pathogen manipulation of vector behaviour has been widely documented empirically, manipulation by pathogens has only recently been examined in a dynamical modelling framework to determine the implications for disease spread (Shaw *et al.* 2017; Gandon 2018).

While most of the evidence for pathogens manipulating vector behaviour focuses on single-host systems, host community composition and diversity can strongly alter disease load and pathogen spread. Manipulation of vector preference seems especially likely to influence disease spread in multi-host communities, as vectors and pathogens may each benefit from

different conditions. For example, grain aphids feed on both annuals and perennial grasses, yet their fecundity is almost double on annual compared to perennial hosts (Borer *et al.* 2009). However, perennials can serve as reservoirs for pathogens vectored by aphids across growing season. Thus, while a foraging preference for annual plants is likely optimal for vectors, foraging on perennials may provide long-term advantages for pathogens. Similarly for ticks vectoring Lyme, vector acquisition rates and realised host competence varies widely among host species. Mice and shrews have higher host competence than opossum, deer, and birds, making them likely optimal hosts for the Lyme pathogen, though not necessarily for the tick vectors (Mather 1993; Ostfeld & Keesing 2000; LoGiudice *et al.* 2003). Given the potential for conflict between optimal foraging strategies of vectors and pathogens in multi-host systems, pathogen manipulation of vector behaviour may be widespread, leading to a preference for hosts with either higher transmission rates or the capacity to serve as reservoirs. Therefore, the interplay of host diversity and pathogen manipulation of vector behaviour, though untested, could profoundly alter the projected spread of vector-borne pathogens in multi-host systems.

Here, we integrate experimental tests of pathogen manipulation of vector behaviour with a novel model quantifying the role of vector preference across single and multi-host communities. The model accounts for potential pathogen-induced changes in vector preference, both for non-infected and infected hosts and for preference between host species. We predicted that pathogen manipulation of vector preference has evolved to increase disease spread both in monocultures and multi-host systems, where we expected a stronger effect in multi-host communities as both preference for host infection status and species could be manipulated by the pathogen. To test this hypothesis we use the aphid-vectored barley and cereal yellow dwarf virus complex (B/CYDV), where we experimentally examine if pathogens manipulate vector preference across single and multi-host systems. Coupling experiment and model, we subsequently project the consequences of such altered preferences through time, comparing (1) single- and multi-host systems and (2) host community composition.

MATERIALS AND METHODS

Barley and cereal yellow dwarf viruses, B/CYDV (family Luteoviridae) are economically important viral pathogens that infect cereal crops and over 150 grasses (Poaceae family) across the globe, including both annuals and perennials (Irwin & Thresh 1990; Darcy 1995). Perennials serve as reservoirs for B/CYDV, maintaining the pathogen in their roots while dormant (Malmstrom *et al.* 2005), and B/CYDV has been hypothesized to have mediated one of the largest plant invasions worldwide – the displacement of native perennial grasses with European annuals across California's grasslands (Borer *et al.* 2007).

In this study, we used BYDV-PAV as it is one of the most common strains (Borer *et al.* 2010) and provides one of the original examples of pathogen manipulation of vector foraging behaviour (the vector manipulation hypothesis) (Ingwell *et al.* 2012). We tested the generality of vector manipulation by pathogens by reproducing single-host results using oat

cuttings (*Avena sativa*, rather than winter wheat plants) and by extending empirical tests to multi-host systems. We used the principle aphid vector *Rhopalosiphum padi* (*R. padi*) which has a worldwide distribution (Ingwell *et al.* 2012). We additionally place empirical results into a predictive framework by coupling experiments and epidemiological theory explicitly incorporating vector preference in a multi-host context. Our model tracks transmission dynamics in hosts and vectors under different preference scenarios. We parameterised the model using our experimental preference assays for both non-viruliferous and viruliferous aphids and extended our single host model to a multi-host system, incorporating the experimentally observed vector preference for annual and perennial grasses.

Preference experiment

We determined the preference of non-viruliferous and viruliferous vectors for (1) non-infected vs. infected hosts and (2) annual vs. perennial hosts. We first planted 12 pots, each with four *Avena sativa* individuals and grew them in the greenhouse for 17 days without aphids. On day 17, we randomly chose half of the pots to be infected with BYDV-PAV by placing 50 viruliferous *R. padi* in each pot. We introduced aphid colonies on the remaining six pots using 50 non-viruliferous *R. padi* per pot. We grew colonies for an additional 15–25 days (depending on temporal block and preference experiment) before beginning behavioural experiments. RNA extractions confirmed infection status (Supplement S1).

The first experiment determined viruliferous and non-viruliferous aphid preference for non-infected vs. infected *Avena sativa* host tissue using a dual-choice assay. We randomly picked a non-viruliferous and viruliferous colony for each paired replicate, using the colony plants for clippings and the aphids in the preference experiments. We removed all aphids from the plant tissue and placed a pre-weighed 4 cm non-infected and infected plant tissue clipping at opposite edges of a petri dish (15.25 cm in diameter) lined with moist filter paper, which prevented clippings from desiccating during the experiment. We used plant cuttings to standardise the orientation of leaves and the amount of plant tissue available. Specifically, in the second experiment for annual vs. perennial plants, leaf architecture varied significantly between species, and cuttings allowed us to control for different architectures. We then placed seven apterae (wingless) aphids in the centre of each petri dish, covered the dish with insect netting, and allowed aphids to forage for four hours, the optimal time for aphid settlement pre-determined from a preliminary time-series experiment (Fig. S2). We then recorded the number of aphids on non-infected tissue, infected tissue, and living aphids not feeding. We paired eight replicates of non-viruliferous and viruliferous aphid preference assays across three temporal blocks for a total of two replicates.

The second, complementary experiment determined aphid preference for annual vs. perennial hosts. We germinated and grew four annual species: *Vulpia myuros*, *Lolium multiflorum*, *Taeniatherum caput-medusae* and *Bromus hordeaceus* and three perennial species: *Koeleria macrantha*, *Bromus carinatus* and

Elymus glaucus. We followed a dual-choice assay similar to the one described above, where we paired eight replicates examining preference of non-viruliferous and viruliferous aphids (from the colonies raised on *Avena sativa*) across three temporal blocks for a total of 24 replicates. Annual and perennial species were randomly paired for each replicate to remove any phylogenetic signal of host species, where 11 of the 12 possible species-pairs occurred and the most any species-pair occurred by chance was five times. We placed seven apterae aphids in the middle of a petri-dish with pre-weighed 4 cm clippings from an annual and a perennial at opposite sides of the petri-dish. We waited four hours to allow aphids to settle on plants, and afterwards recorded the number of aphids on annual tissue, perennial tissue, and living aphids not feeding on either.

Statistical analyses

We analysed the proportion of aphids that preferred infected vs. non-infected plant tissue and annual vs. perennial hosts using separate logistic regressions. In both models, we tested how aphid treatment (non-viruliferous vs. viruliferous) altered preference for infected vs. non-infected or annual vs. perennial host plants. We accounted for plant type, temporal block, leaf weight, and the interaction of plant type and leaf weight as fixed effects. Temporal block accounts for length of time the colonies were grown, tissue quality, and potential differences in ambient conditions, and was included as a fixed effect as there were only three blocks. We included leaf weight as the standardised difference in leaf weight between tissue clippings (infected minus non-infected tissue weight or perennial minus annual tissue weight), since aphids may prefer larger leaves with more surface area, or alternatively, smaller leaves that are easier to probe. We used backwards model selection using AIC to determine the best-fitting models. For the annual vs. perennial preference assay, we additionally accounted for any potential species-pairing driving our results by leave-one-out validation, where we reran our regressions while systematically leaving out each species pair. All analyses were carried out in R version 3.4.2 (R Core Team, 2013) using the lme4 library (Bates *et al.* 2007).

Single host preference model

We modelled the impacts of pathogen-induced changes in vector behaviour on pathogen spread over a single host-growing season by modifying the classical vectored-transmission model (Keeling & Rohani 2008). We assumed vector settlement depends on preference for host type (non-infected or infected) and the fraction of hosts of each type available, such that:

$$\alpha_{x,y} = \frac{\gamma_{x,y} P_y}{\sum_{z=1}^Z \gamma_{x,z} P_z} \quad (1)$$

where $\alpha_{x,y}$ is the proportion of vector type x (viruliferous or non-viruliferous) that settle on host type y (infected or non-infected), $\gamma_{x,y}$ is the preference of vector x for plant host y , and P_y is the abundance of host type y (Chesson 1983). In the denominator, z indexes all possible host scenarios: non-infected or infected hosts in this case. Explicitly incorporating this vector foraging behaviour into a model of disease

dynamics for non-infected (healthy) plant hosts (P_h), infected plant hosts (P_i), non-viruliferous (healthy) vectors (V_h), and viruliferous vectors (V_i) yields:

$$\underbrace{\frac{dP_h}{dt}}_{\text{non-infected plants}} = -\underbrace{\beta_{v,p} \frac{\gamma_{i,h} P_h}{\gamma_{i,h} P_h + \gamma_{i,i} P_i}}_{\text{host transmission}} V_i \quad (2)$$

$$\underbrace{\frac{dP_i}{dt}}_{\text{infected plants}} = \underbrace{\beta_{v,p} \frac{\gamma_{i,h} P_h}{\gamma_{i,h} P_h + \gamma_{i,i} P_i}}_{\text{host transmission}} V_i \quad (3)$$

$$\underbrace{\frac{dV_h}{dt}}_{\text{non-viruliferous vectors}} = \underbrace{r N_h \left(1 - \frac{N_h}{K P_h}\right)}_{\text{vector growth}} - \underbrace{\beta_{p,v} \frac{\gamma_{h,i} P_i}{\gamma_{h,i} P_i + \gamma_{h,h} P_h}}_{\text{vector transmission}} V_h \quad (4)$$

$$\underbrace{\frac{dV_i}{dt}}_{\text{viruliferous vectors}} = \underbrace{r N_i \left(1 - \frac{N_i}{K P_i}\right)}_{\text{vector growth}} + \underbrace{\beta_{p,v} \frac{\gamma_{h,i} P_i}{\gamma_{h,i} P_i + \gamma_{h,h} P_h}}_{\text{vector transmission}} V_h \quad (5)$$

where $\beta_{v,p}$ is the transmission coefficient from vector to plant host, $\beta_{p,v}$ is the transmission coefficient from plant to vector, K is the per-host carrying capacity of vectors, and r is the vector intrinsic growth rate (parameter values in Table 1). Note that the $\alpha_{x,y}$ terms are expanded in the above equations for clarity. Additionally, $N_h = \alpha_{h,h} V_h + \alpha_{i,h} V_i$ and $N_i = \alpha_{h,i} V_h + \alpha_{i,i} V_i$, such that we assume all vectors born on non-infected plants remain non-viruliferous and, correspondingly, all vectors born on infected plants become viruliferous before dispersing (Shaw *et al.* 2017).

To quantify the effect of vector preference on pathogen spread, we compared four scenarios. In each, we altered $\gamma_{x,y}$ while holding all other parameters constant. In the baseline scenario, (no preference), $\gamma_{x,y} = 0.5$ for all x, y combinations and pathogens do not alter vector preference (e.g. Mauck *et al.* 2010; Cornet *et al.* 2013). In comparison, we parameterise the ‘observed scenario’ such that $\gamma_{x,y}$ is the mean preference observed experimentally: $\gamma_{h,i} = 0.63$, $\gamma_{h,h} = 0.37$, $\gamma_{i,i} = 0.49$, and $\gamma_{i,h} = 0.51$. We examined two additional scenarios: one where the pathogen induces strong behavioural changes consistent with the vector manipulation hypothesis (‘opposite preference’ $\gamma_{h,i} = 0.85$, $\gamma_{h,h} = 0.15$, $\gamma_{i,i} = 0.15$, $\gamma_{i,h} = 0.85$), and one with strong preference for the ‘same’ vector-host status (‘same preference’ i.e. non-viruliferous vectors prefer non-infected plant tissue and viruliferous vectors prefer infected plant tissue; $\gamma_{h,i} = 0.15$, $\gamma_{h,h} = 0.85$, $\gamma_{i,i} = 0.85$, $\gamma_{i,h} = 0.15$). Although preference for the same status runs counter the vector manipulation hypothesis, such pathogen induced changes have been observed empirically (Fereses *et al.* 2016).

Multi-host extension

We extended our model to a multi-host framework, generalised for any number of host species, S . Here, vector settlement depends on preference for host type (non-infected or infected), the fraction of hosts available, and the host species such that:

Table 1 Single and multi-host model parameters and descriptions. Vector growth rate is averaged from Jiménez-Martínez *et al.* (2004) and transmission rates are estimated from multiple transmission experiments (unpublished data). While model parameters are derived from BYDV-aphid experiments, the model is general for any vectored disease system.

Model	Parameter	Description	Units	Value
Single host	$\beta_{p,v}$	Transmission coefficient from plant host to aphid vector	day ⁻¹	0.05
Single host	$\beta_{v,p}$	Transmission coefficient from aphid vector to plant host	host vector ⁻¹ day ⁻¹	0.05
Single host	$\gamma_{x,y}$	Preference of vector x for host type y	None	Varies
Single and Multi-host	r	Vector growth rate	day ⁻¹	0.2245
Single and Multi-host	K	Carrying capacity of an individual host	vectors host ⁻¹	5
Multi-host	$\beta_{s,v}$	Transmission rate from plant host s to aphid vector v	day ⁻¹	0.05
Multi-host	$\beta_{v,s}$	Transmission rate from aphid vector v to plant host s	host vector ⁻¹ day ⁻¹	0.05
Multi-host	γ_{x,y_s}	Preference of vector x for host type y of species s	None	Varies

$$\alpha_{x,y_s} = \frac{\gamma_{x,y_s} P_{y_s}}{\sum_{s=1}^S \sum_{z=1}^Z \gamma_{x,z_s} P_{z_s}} \quad (6)$$

where γ_{x,y_s} is the preference of vector type x (non-viruliferous or viruliferous) for host species s of status y (non-infected or infected). P_{y_s} is the total number of individuals of plant species s of status y , and $s = 1, \dots, S$ indexes all host species, while $z = 1, \dots, Z$ indexes all host statuses (non-infected or infected). Incorporating preference in a multi-host context yields:

$$\frac{dP_{hs}}{dt} = \underbrace{-\beta_{v,s} \alpha_{i,hs} V_i}_{\text{species } s \text{ host transmission}} \quad (7)$$

species s non-infected plants

$$\frac{dP_{is}}{dt} = \underbrace{\beta_{v,s} \alpha_{i,hs} V_i}_{\text{species } s \text{ host transmission}} \quad (8)$$

species s infected plants

$$\frac{dV_h}{dt} = \underbrace{\sum_{s=1}^S r N_{hs} \left(1 - \frac{N_{hs}}{K P_{hs}}\right)}_{\text{vector growth on species } s} - \underbrace{\sum_{s=1}^S \beta_{s,v} \alpha_{h,is} V_h}_{\text{vector transmission}} \quad (9)$$

non-viruliferous vectors

$$\frac{dV_i}{dt} = \underbrace{\sum_{s=1}^S r N_{is} \left(1 - \frac{N_{is}}{K P_{is}}\right)}_{\text{vector growth on species } s} + \underbrace{\sum_{s=1}^S \beta_{s,v} \alpha_{h,is} V_h}_{\text{vector transmission}} \quad (10)$$

viruliferous vectors

where $N_{hs} = \alpha_{h,hs} V_h + \alpha_{i,hs} V_i$ and $N_{is} = \alpha_{h,is} V_h + \alpha_{i,is} V_i$. Parameters are the same as for the single species model where now $\beta_{v,s}$ is the transmission coefficient from the vector to host species s , and $\beta_{s,v}$ is the transmission coefficient from hosts of species s to vectors (Table 1).

We first considered only the effect of preference for host species on pathogen spread. Here $\gamma_{x,y_s} = \gamma_{x,y} \gamma_{x,s}$ where we fix $\gamma_{x,y} = 0.5$ to remove any preference effect for non-infected vs. infected hosts. We examined three community compositions holding total hosts constant at 100: non-preferred host dominated (80% annual; 20% perennial), even composition (50% annual; 50% perennial), and preferred host dominated (20% annual; 80% perennial). In our two baseline scenarios, we compared pathogen spread through time for a generalist vector (for all combinations of x : non-viruliferous and viruliferous and s : annual [a] and perennial [p], $\gamma_{x,s} = 0.5$) and for a specialist vector ($\gamma_{x,p} = 0.85$ for non-viruliferous and viruliferous vectors on

preferred hosts and $\gamma_{x,a} = 0.15$ for non-viruliferous and viruliferous vectors on non-preferred hosts). Pathogen-modification of vector behaviour may change the generalist vector to become a specialist ($\gamma_{h,a} = 0.5$, $\gamma_{h,p} = 0.5$, $\gamma_{i,a} = 0.15$, $\gamma_{i,p} = 0.85$), or correspondingly the specialist vector to behave as a generalist ($\gamma_{h,a} = 0.15$, $\gamma_{h,p} = 0.85$, $\gamma_{i,a} = 0.5$, $\gamma_{i,p} = 0.5$). We compared these scenarios to the multi-host preference experiment ('observed' $\gamma_{h,a} = 0.57$, $\gamma_{h,p} = 0.43$, $\gamma_{i,a} = 0.21$, $\gamma_{i,p} = 0.79$).

Combining preferences for infection status and host type

Finally, we explored the combined effects of pathogen-induced changes in preference for infection status and host species. Across the three community compositions described above, we compared pathogen spread in four scenarios: (1) no vector preference ($\gamma_{x,y_s} = 0.5$ for all combinations of x , y , and s), (2) observed preference for infection status (vectors prefer non-infected or infected hosts but do not distinguish between annual and perennial; $\gamma_{h,i} = 0.63$, $\gamma_{h,h} = 0.37$, $\gamma_{i,i} = 0.49$, $\gamma_{i,h} = 0.51$, and $\gamma_{x,s} = 0.5$ for all combinations of x and s), (3) observed preference for host type (vectors prefer annual or perennial hosts but do not distinguish based on infection status; $\gamma_{x,y} = 0.5$ for all combinations of x and y , $\gamma_{h,a} = 0.57$, $\gamma_{h,p} = 0.43$, $\gamma_{i,a} = 0.21$, $\gamma_{i,p} = 0.79$), and (4) the combined effect of both observed preferences ($\gamma_{h,i} = 0.63$, $\gamma_{h,h} = 0.37$, $\gamma_{i,i} = 0.49$, $\gamma_{i,h} = 0.51$, $\gamma_{h,a} = 0.57$, $\gamma_{h,p} = 0.43$, $\gamma_{i,a} = 0.21$, and $\gamma_{i,p} = 0.79$). We quantified pathogen spread in each host population, the community, and the vector population.

We additionally examined the effect of host community composition, calculating time until 75% infection of hosts and vectors across communities ranging from 10% to 90% non-preferred hosts. We compared (1) no preference to the experimentally observed preference for (2) infection status, (3) host type, and (4) their combined effects across community composition. All simulations began with 50 non-viruliferous vectors and 10% of the host community infected chosen uniformly at random. Model code is available at <https://github.com/lash1937/PathogenVectorModel>.

RESULTS

Preference experiment

Vector infection status significantly affected aphid preference for non-infected vs. infected hosts (Fig. 1a), where non-

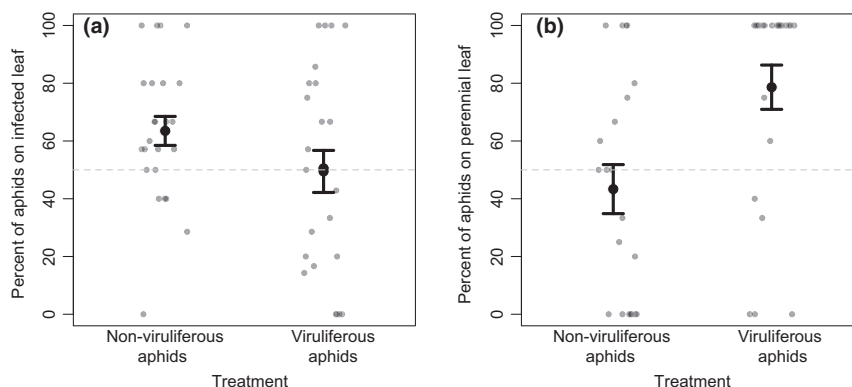


Figure 1 Non-viruliferous and viruliferous aphid preference. (a) Vector preference for infected vs. healthy plant tissue. (b) Vector preference for perennial vs. annual plant tissue. Mean \pm standard error is shown in black and individual replicates are shown in grey ($n = 24$).

viruliferous aphids preferred infected hosts ($63\% \pm 5\%$ standard error), while viruliferous aphids showed no preference for either infection status ($49\% \pm 7\%$, $\beta = 0.6$; $P = 0.02$). These results closely match those of experiments with entire plants (Ingwell *et al.* 2012), and provide support for pathogen manipulation of vector behaviour. We found a significant effect of leaf weight on aphid preference ($\beta = -0.27$; $P = 0.04$), with aphids preferring lighter plant tissue. There was no significant interaction between aphid infection status and differences in leaf weight ($P = 0.81$) nor effect of temporal block ($P = 0.77$).

Pathogen manipulation of vector foraging behaviour occurred more strongly in a multi-host than single-host context. Non-viruliferous vectors exhibited generalist tendencies (Fig. 1b), showing no preference for annual vs. perennial hosts ($43\% \pm 8\%$ on perennial hosts), while viruliferous aphids exhibited a strong preference for the perennial tissue

($79\% \pm 8\%$). As in the infection status experiment, both aphid infection status ($\beta = -1.66$; $p = 0.0004$) and leaf weight ($\beta = -0.85$; $p = 0.003$) were significant, with aphids again preferring lighter plant tissue. Our results were not driven by any specific species-pairing, as the leave-one-out validation produced similar results across all species pairs ($-2.00 \leq \beta \leq -1.40$ and $0.0002 \leq p \leq 0.004$).

Single host preference model

When vectors exhibit a preference for hosts with the opposite infection status (Fig. 2 dashed orange), the pathogen spreads more rapidly in both the host and vector populations than in the baseline scenario where vectors exhibit no preference (solid gray). When vectors exhibit preference for hosts of the same status (Fig. 2 dashed green), the pathogen spreads more slowly in the host and vector populations. There is a non-

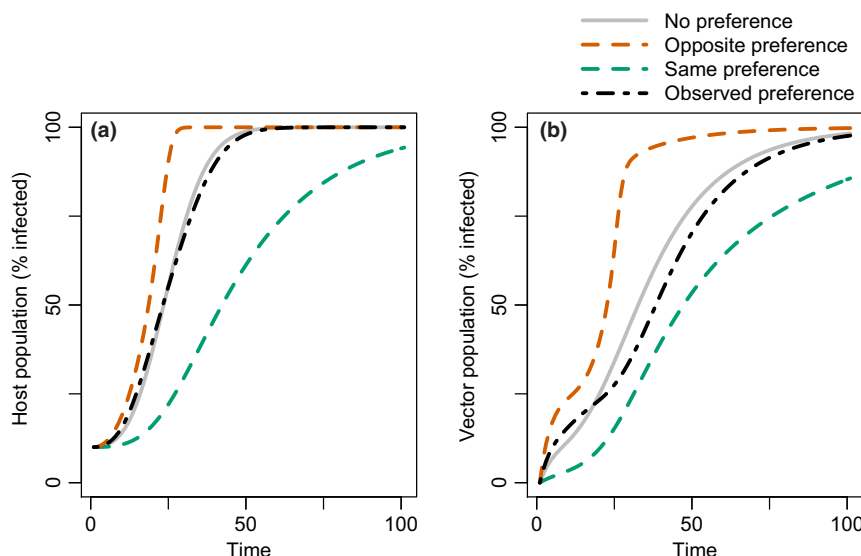


Figure 2 Projected pathogen spread through time in a single-host system for the (a) host population and (b) the vector population with pathogen-induced changes in preference. The baseline no preference scenario is shown in solid gray while preference for the opposite status (non-viruliferous vectors prefer infected hosts and viruliferous vectors prefer non-infected hosts) is shown in orange, preference for the same status (non-viruliferous vectors prefer non-infected hosts and viruliferous vectors prefer infected hosts) is shown in green, and black dashed lines show experimentally measured preference.

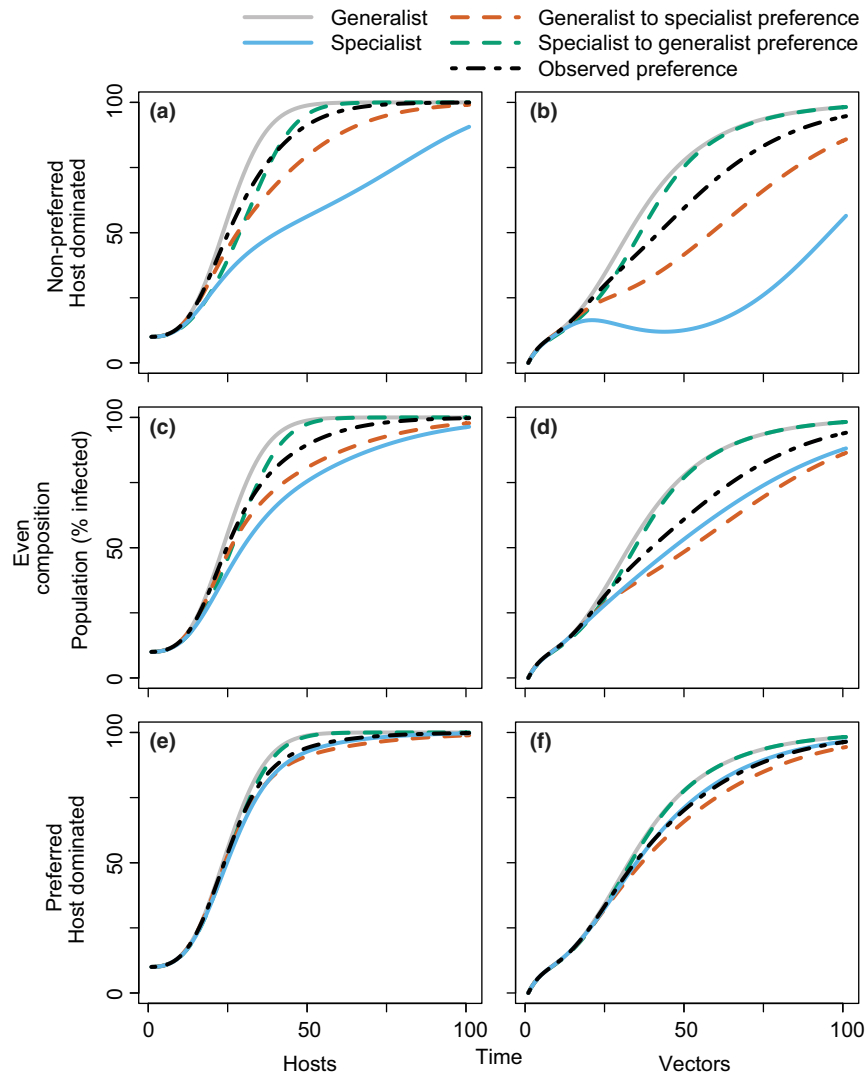


Figure 3 Projected pathogen spread through time in a two-host system with pathogen-induced behavioural changes. Pathogen spread is shown for hosts (left column) and vectors (right column) for communities dominated by the non-preferred host (panels a and b), even community composition (panels c and d), and for communities dominated by the preferred host (panels e and f). The baseline cases with no-pathogen induced behavioural changes are shown for a generalist (grey) and specialist (blue) pathogen. Pathogen-induced changes from generalist to specialist (dashed orange), specialist to generalist (dashed green), and the experimentally observed preference changes (dashed black) are shown with dashed lines.

symmetrical effect – preference for the same status decreases pathogen spread more strongly than preference for the opposite status increases pathogen spread.

While our preference experiments showed significant pathogen-modification (Fig. 1a), incorporating this into the single-host model yields minimal effect on pathogen spread, as the observed changes in preference are not strong enough to affect dynamics. In the host population, this change in preference yields pathogen spread that closely matches the no preference scenario (Fig. 2a dashed black). For the vector population, viral-induced behaviour changes initially lead to increased pathogen spread as intuitively expected (from time 1 to 18), as non-viruliferous vectors disperse to infected hosts (Fig. 2b dashed black). For the remainder of the simulation, however, pathogen spread is reduced compared to the no preference scenario. These effects are relatively minor, with

only 7% less of the vector population and < 1% less of the host population infected after 50 days compared to the no preference scenario.

Multi-host model extension

Without pathogen manipulation, vectors can behave as either generalists or specialists. These represent our two baseline cases for comparisons (Fig. 3 solid grey and blue lines). Regardless of the relative host community composition, pathogen spread occurs faster when vectors are generalists without a preference for either host species. When a pathogen induces generalists to behave as specialists, the rate of pathogen spread decreases in both host and vector populations. This effect is strongest in vector populations and more moderate in host populations (Fig. 3 grey vs. orange lines). In

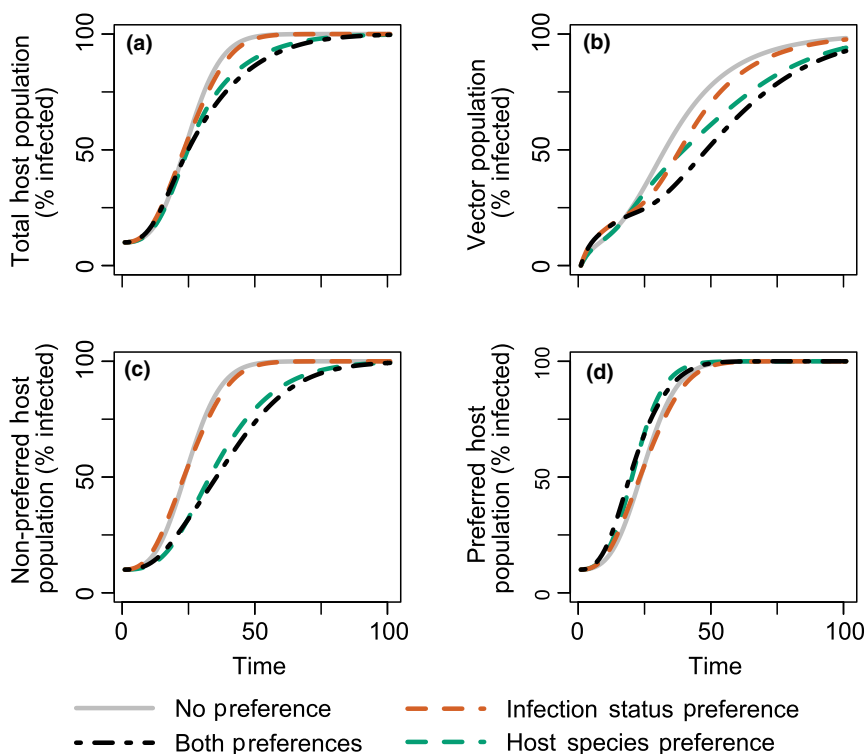


Figure 4 The combined effect of multiple pathogen-induced preference changes on pathogen spread through time. All results are shown for a community composed of 50% preferred and 50% non-preferred hosts (see Supplement S2 for different community compositions). The baseline case with no-pathogen induced behavioural changes is shown in grey, with pathogen preference for host infection status (non-infected vs. infected hosts) shown in dashed orange, pathogen preference for host species shown in dashed green, and the combined effect of both preferences shown in dashed black. Pathogen spread through time is shown for the entire host community (a), the vector population (b), the non-preferred host species (c), and the preferred host (d).

contrast, when a pathogen induces specialist vectors to behave as generalists, the pathogen spread increases (Fig. 3 blue vs. green lines). In both cases the effect is most prominent in communities dominated by the non-preferred host.

In our multi-host preference experiments, we observed that non-viruliferous aphid vectors act as generalists, with no significant preference for annuals over perennials. Once viruliferous, aphids then become specialised (with perennials preferred; Fig. 1b). Parameterizing the multi-host model with observed preferences, we find that pathogen-induced changes for multi-host preferences decrease pathogen spread in both the host and vector populations compared to when vectors behave as generalists (Fig. 3 dashed black vs. grey lines). This effect qualitatively matches our simulations when pathogens manipulate generalist vectors to behave as specialists (dashed orange) and is mediated by host community composition. The effect is less severe than in the simulated scenario, as the empirically observed pathogen-induced specialist preference is weaker (79% compared to 85%).

Combining preferences

Counter-intuitively, our model yields a net decrease in pathogen spread when incorporating pathogen manipulation of preference for both host infection status and life-history (Fig. 4). This decrease is more pronounced than when considering pathogen manipulation of preference for only host

infection status or life-history in isolation. The general pattern is robust across both the host and vector populations (Fig. 4a,b) regardless of the underlying community composition (Fig. S3) and potential for varying vector fecundity on annuals and perennials (Fig. S4).

The net decrease in pathogen spread across the host population is driven by the asymmetrical effects observed in the non-preferred (annuals) vs. preferred host populations (perennials). Pathogen spread decreases dramatically in the non-preferred host population, which is driven primarily by host-species preference rather than preference for infection status (Fig. 4c). However, pathogen spread in the preferred host population increases compared to the no preference baseline, as non-viruliferous vectors behave as generalists, while viruliferous vectors exhibit a strong preference for the preferred host (Fig. 4d). This strong preference of viruliferous vectors causes aggregation on the preferred hosts and an increase in pathogen spread. Importantly, these two effects are asymmetrical between non-preferred and preferred hosts, with a stronger decrease in pathogen spread in the non-preferred hosts than the increase in preferred hosts. The net effect is an overall decrease in pathogen spread in the host community.

Regardless of host community composition, pathogen manipulation of vector preference for host infection status and life-history decreases pathogen spread in a growing season. This effect is stronger with manipulation of both preferences compared to the effect of either preference alone

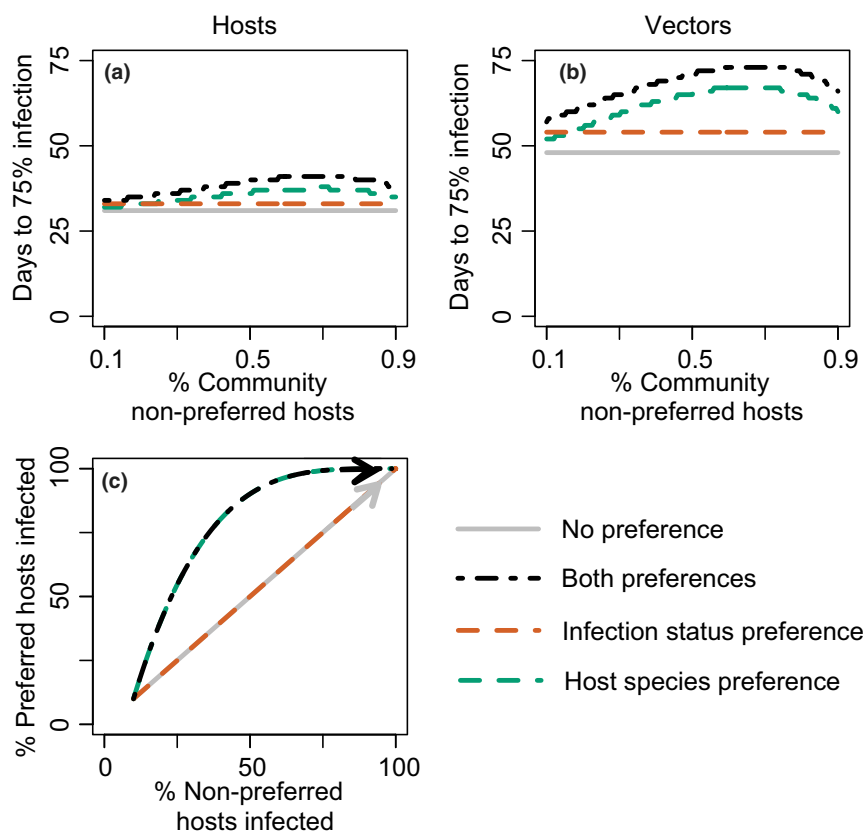


Figure 5 Examining the general effects of multiple pathogen-induced preference changes across community composition; effects on hosts shown in (a) and on vectors shown in (b). Trajectories of pathogen spread—regardless of community composition—is shown for hosts (c). The baseline case with no-pathogen induced behavioural changes is shown in grey, with pathogen preference for host infection status (non-infected vs. infected hosts) shown in dashed orange, pathogen preference for host species shown in dashed green, and the combined effect of both preferences shown in dashed black.

(Fig. 5a,b). Community composition has a unimodal effect on pathogen spread when incorporating vector manipulation, where it takes 34–41 days for 75% of the host community to be infected depending on the underlying community composition. The strongest decrease in pathogen spread from vector preference occurs with 50–75% of the community composed of the non-preferred host. A similar effect of community composition occurs in vector populations, where the number of days to 75% infection ranges from 57 to 73.

Although underlying community composition alters how fast hosts become infected, the trajectory of pathogen spread through a community is surprisingly consistent (Fig. 5c). When vectors exhibit no preference or only preference for infection status, an equal percent of non-preferred and preferred hosts are infected (dashed orange and solid grey lines). However, when vectors exhibit the observed preference for host type, preferred hosts become preferentially infected earlier in the growing season, whereas non-preferred hosts become infected later (Fig. 5c, dashed black line). The same trajectory occurs (although at a different rate), with pathogen manipulation of vector preference for host type (dashed green line).

DISCUSSION

Most disease vectors are foraging animals, exhibiting preferences and behaviours that help meet their energetic demands

while minimising costs and avoiding predation. Natural selection will favour foraging behaviour that optimizes fitness, as proposed in optimal foraging theory (Pyke 1984). However, the optimal foraging strategy for vectors may not be the same for pathogens, leading to the hypothesised evolution of pathogens manipulating vector behaviour to increase pathogen fitness (Gandon 2018). Here, we provide empirical evidence for pathogen manipulation of vector preference across host infection status and species. We show that, while non-viruliferous aphids behave as generalists with regards to host species, aphids carrying B/CYDV strongly prefer long-lived perennial hosts. These results suggest that vector behaviour and pathogen manipulation of preference may play an important role in disease spread. Surprisingly, even though we find that viruliferous vectors prefer healthy hosts compared to non-viruliferous vectors, these changes in vector preference minimally alter disease spread in monocultures. However, in more diverse host communities, pathogen manipulation of vector preference causes aggregation of viruliferous vectors on the preferred hosts – perennial plants in this case – and faster disease spread across the preferred hosts consistent with an amplification effect. A net decrease in disease spread occurs across the host community, mirroring patterns observed with the dilution hypothesis and suggesting that vector preference could be an additional underlying mechanism behind observed diversity-disease dynamics across scales (Johnson & Thielges 2010; Venesky *et al.* 2014; Strauss *et al.* 2018).

Widespread evidence for pathogen manipulation of vector behaviour in a single-host context comes from a variety of pathogen, vector, and host systems (Evans 1982; Stafford *et al.* 2011; Cator *et al.* 2012; Ingwell *et al.* 2012; Martini *et al.* 2015). Previous work by Ingwell *et al.* (2012) demonstrated vector manipulation of BYDV in a single host species. We build on this previous work by finding generality of vector manipulation across species and methodological differences (cuttings vs whole plants), exemplifying the robustness of observed preferences. However, we find a stronger effect of manipulation of vector preference in a multi-host than single host context, with viruliferous aphids preferring perennials and no preference for non-viruliferous aphids. Our results for non-viruliferous aphids show a similar but dampened effect compared to those of Borer *et al.* (2009), which found a strong preference of non-viruliferous aphids for annuals. These differences suggest that aphid density, changes in chemical cues between tissue cuttings and whole plants, or differences in plant architecture may alter preference across host species, causing observed preferences to be heightened. Critically, our model is general in that these observed variations in preferences can be incorporated and compared through different model parameterisations. We encourage future work to compare non-viruliferous and viruliferous vector preferences using whole plants and incorporating differences in plant architecture.

From an evolutionary standpoint, manipulating vector preference for annual vs. perennial hosts may be highly advantageous to the pathogen, as perennial hosts maintain the virus across growing seasons (Malmstrom *et al.* 2005). Then again, proliferation of the vector population on annuals could also benefit virus fitness (Borer *et al.* 2009), leading to a potential trade-off between maximising persistence or transmission. Indeed, the evolution of manipulation of host choice can have dramatic epidemiological consequences, such as altering the basic reproductive ratio R_0 (Gandon 2018). While current literature focuses on single host communities, similar logic would suggest that manipulation of preference in diverse host communities would likely have similar or even stronger evolutionary advantages.

The ecological implications of vector behaviour manipulation depend strongly on the underlying host community composition. Observed manipulation of vector behaviour in host monocultures has surprisingly minimal impacts, yet has been suggested to increase the rate of pathogen spread in verbal models. While simulations show that stronger manipulation of vector behaviour could increase disease spread, the required rates are stronger than most reported values in the literature (reviewed in Gandon 2018). In comparison, observed manipulation of preference can have significant implications on dynamics in more diverse communities. The aggregation of vectors onto a preferred host species decreases spread, paralleling the effect of vector aggregation on individual hosts within a host species (Shaw *et al.* 2019). Our experiment and model represent a snapshot in time, but seasonality of preference could occur, where pathogen fitness may be heightened by an early season preference for annuals, causing a higher vector growth rate, and a late season preference for perennials for overwintering. While our model focuses within a growing season, the interplay of vector

behaviour and population dynamics could strongly alter dynamics across multiple growing seasons, suggesting future work examine ecological impacts across multiple host generations, including implications for invasion dynamics (Borer *et al.* 2007; Strauss *et al.* 2012; Clark *et al.* 2015) and conservation (Borer *et al.* 2007).

Vector foraging behaviour and pathogen manipulation in multi-host systems have important implications for understanding the dynamics of vectored pathogens, likely for both animal and plant hosts. Many of these focal pathogens such as B/CYDV and *Plasmodium* malaria occur in communities with a diversity of hosts (Borer *et al.* 2010; Nah *et al.* 2010), while widespread evidence for pathogen-induced changes in behaviour currently focus on preference within single-host systems. Additional empirical preference studies, including experiments from the field and with high host diversity, are necessary to determine the frequency of pathogen-manipulation of vector preference in multi-host systems, potential interactive effects between host species and infection status, and how vector preference may create patterns consistent with amplification or dilution effects, depending on scale. Future work tracking demographics of both hosts and vectors could highlight important feedbacks between pathogen induced changes in vector behaviour and changes in vector population and host community composition (though we find minimal effects within a single growing season; Fig. S4). Pathogen-manipulation of vectors may additionally have wide-spread consequences for managing future epidemics, especially with increased interactions between wildlife, livestock, and human populations (Power & Mitchell 2004).

CONCLUSIONS

Vectors are critical links between pathogens and hosts, often exhibiting more complex behaviours than can be represented via probabilistic transmission. Importantly, vector foraging behaviour can change pathogen spread, with the potential to alter the spatial structure and aggregation of vectors on hosts of a given phenotype or infection status. Here, we find evidence for pathogen manipulation of vector preference for host infection status, and stronger manipulation of preference for host life-history. These preferences of viruliferous and non-viruliferous vectors overall decrease pathogen spread through a diverse host community. They aggregate vectors on preferred host species, increasing pathogen spread in some host types, while decreasing spread in the non-preferred host and the overall community. Given the breadth of literature examining the role of host diversity on pathogen spread, it is critical for cross-system comparisons and management of future epidemics to consider mechanisms – such as vector preferences and pathogen manipulation of behaviour – that determine how host diversity alters expected pathogen spread (Schmidt & Ostfeld 2001; Lacroix *et al.* 2014). Here we show that effects of pathogen manipulation of vector behaviour are determined by the diversity of the host community. More generally, this work demonstrates the need for a closer integration of behavioural, community, and disease ecology.

ACKNOWLEDGEMENTS

We thank the Shaw and Borer-Seabloom laboratory groups, three anonymous reviewers, the editor, and the theory group at the University of Minnesota for feedback which greatly improved the manuscript. Support for this work was funded by NSF IOS-1556674 and the James S. McDonnell Foundation grant 220020513.

AUTHORSHIP

LGS, ATS, ETB, EWS and AKS came up with the conceptual framing of the manuscript, LGS, EH and APK performed the experiments, LGS, CPWL and AKS created the model, LGS wrote the manuscript, and all authors contributed substantially to revisions.

DATA ACCESSIBILITY STATEMENT

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.lnv0351>. Model code is available on GitHub (<https://github.com/lash1937/PathogenVectorModel>).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Editor, Jessiaca Metcalf

Manuscript received 19 November 2019

First decision made 5 January 2019

Second decision made 8 March 2019

Manuscript accepted 24 March 2019