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Fatal diseases and parasitoids: from competition to facilitation in a shared host

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Diverse parasite taxa share hosts both at the population level and within individual hosts, and their interactions, ranging from competitive exclusion to facilitation, can drive community structure and dynamics. Emergent pathogens have the potential to greatly alter community interactions. We found that an emergent fungal entomopathogen dominated pre-existing lethal parasites in populations of the forest defoliating gypsy moth, *Lymantria dispar*. The parasite community was composed of the fungus and four parasitoid species that only develop successfully after they kill the host, and a virus that produces viable propagules before the host has died. A low-density site was sampled over 17 years and compared with 66 sites across a range of host densities, including outbreaks. The emergent fungal pathogen and competing parasitoids rarely co-infected host individuals because each taxa must kill its host. The virus was not present at low host densities, but successfully co-infected with all other parasite species. In fact, there was facilitation between the virus and one parasitoid species hosting a polydnavirus. This newly formed parasite community, altered by an emergent pathogen, is shaped both by parasite response to host density and relative abilities of parasites to co-inhabit the same host individuals.

1. Introduction

Emerging pathogens impact host communities [1,2], but they also impact communities of pre-existing natural enemies, potentially leading to unprecedented changes in both the host and parasite communities. Parasite communities can be both diverse and abundant [3,4] and, as such, must be recognized as important components of food webs, although they have generally not been included in food web analyses [5]. Multiple parasites in a community, ranging from microbial pathogens to metazoan parasites, often use the same host individual. The outcome of co-infections depends, in part, on life histories of the parasites, which can vary broadly based on characteristics such as modes of transmission, density dependence, whether death of the host is required to complete a life cycle [3], and the phenology and relative timing of infection/parasitism by different parasites [6]. Furthermore, co-infecting parasites interact in various ways, including modifying transmission efficiencies or virulence of co-inhabitants [7], or removing hosts from a shared resource pool [8], with resulting interactions ranging from exclusion to facilitation.

Emphasis in parasite and disease ecology has often focused on understanding the interactions in one host-one parasite systems. This has recently changed with studies focused on host biodiversity, establishing, for example, that dilution in parasitism can be associated with increasing diversity of host species [1,9]. However, the biodiversity of communities of microbial to metazoan parasites have received less attention than the biodiversity of hosts (but see [10]). Yet, multi-parasite occurrence and interactions are known to be important in systems such as malaria and helminths in humans, colony collapse disorder in honeybees, and protist and viral pathogens of frogs [11–13], and for many years have been recognized in communities of natural enemies attacking agricultural arthropod pests [14–16].

We investigated an emergent fungal pathogen, a viral pathogen, and a group of parasitoids that co-occur in eastern North America, attacking larvae of the gypsy moth, *Lymantria dispar*. All these natural enemies kill the host, however, their life strategies and speed of kill differ, as well as their relations with host density. In this relatively simplified system with an invasive host and exotic natural enemies, we hypothesized that host density and parasitoid/pathogen life strategy would influence the impact of the emergent fungal pathogen on the already-established virus and parasitoids. In particular, we addressed the competition between parasites by determining the extent of successful co-infection (greater than one parasite species successfully using a host caterpillar), given the prevalence of each species in the host populations. We hypothesized that successful co-infection involving the two strategists that kill the host when ready to exit and continue development as transmissive/parasitic forms (the fungus and parasitoids), would be rare, whereas successful co-infection involving the virus with either the fungus or parasitoids would be more common because the virus produces transmissive stages while the host is alive [17,18]. Further, we expected that these interactions among parasites within an individual host would vary with host density. The study is based on detailed records from rearing healthy, singly and multiply infected gypsy moth larvae collected in two large-scale studies. One study was conducted at a single low-density site in New York State, sampled over 17 years, and the second included 66 sample sites in the mid-Atlantic USA hosting a large range in gypsy moth densities, from outbreak to sparse, sampled over one year.

2. Material and methods

(a) Study system

The host, *L. dispar*, is native to Eurasia and north Africa but was introduced from France to the Boston area in North America in 1869, and has since spread so that it now occurs from Nova Scotia to Wisconsin and Ontario to Virginia [19]. Adult female gypsy moths usually lay egg masses on tree trunks and first instars actively disperse. Gypsy moth larvae are polyphagous forest defoliators and cyclical outbreaks are typical of this univoltine species in some areas [20]. At low population densities, predatory small mammals are thought to be the most important natural enemies, while crashes of outbreaks are generally caused by entomopathogens [21–23].

A baculovirus infecting gypsy moth larvae, LdMNPV, was accidentally introduced to New England before 1907 and subsequently was considered the most abundant and effective natural enemy in outbreaking gypsy moth populations, responsible for epizootics that resulted in population crashes [24]. LdMNPV grows intracellularly in hosts, is highly specific, and infects and kills all gypsy moth larval instars, usually infecting when occlusion bodies are eaten. LdMNPV produces occlusion bodies while the host is alive although these are only released after host death.

The entomopathogenic fungus *Entomophaga maimaiga* infecting gypsy moth larvae is an emergent pathogen that was first found in New England during the exceptionally rainy spring of 1989 [22]. It was accidentally introduced, probably from Japan [25] after 1971 [26]. *Entomophaga maimaiga* spread across the gypsy moth distribution and has taken over the role as the major pathogen causing epizootics ending gypsy moth outbreaks [27]. This host-specific fungus infects by penetrating through the larval cuticle. It then grows throughout the host as vegetative cells, kills the host, and produces spores only after host death. Laboratory studies estimate that *E. maimaiga* kills larvae at least twice as fast as LdMNPV ([28]; electronic supplementary material). For both LdMNPV [29] and *E. maimaiga* [30] higher doses are required to kill later instars and time to death is longer for larvae infected at later versus earlier instars.

The third group of natural enemies includes wasp (*Cotesia melanoscela* and *Phobocampe unicincta*) and fly (*Parasetigena sylvestris* and *Compsilura concinnata*) parasitoids that were introduced purposefully for classical biological control of gypsy moth in North America [31]. The flies and *C. melanoscela* also parasitize hosts other than the gypsy moth [21,31], presumably within the study area. Little is known about the host specificity of *P. unicincta* but, based on the high rate of encapsulation of eggs and larvae [32], it does not appear to be well adapted to gypsy moth. Both of the parasitic wasps mostly lay their eggs in early instar host larvae and emerge from middle instars. The flies attack later instar host larvae and emerge from later instars or pupae. All these parasitoids are koinobiont endoparasitoids, so after oviposition the host and parasitoid both develop until the parasitoid larva is ready to pupate, at which point the parasitoid emerges from the host, which kills or permanently disables the host. *Cotesia melanoscela* carries a polydnavirus that is injected into hosts during oviposition and disrupts the larval immune response, ensuring successful survival and growth of the parasitoid larva [33].

(b) Study sites and sampling

Lymantria dispar larvae were sampled in areas dominated by oaks, *Quercus* spp., in the mid-Atlantic and northeastern regions of North America. We sampled a stable low-density population in a 5.7 ha red oak (*Quercus rubra*) plantation in Yellow Barn State Forest, central New York State yearly, over 17 years (1996–2010, 2012–2013). Throughout the study, host densities at this site never increased significantly (in 2011, although larvae were not sampled, egg mass density was quantified) (table 1). By contrast, during 2009, we sampled 66 2.5–7 ha non-contiguous sites in forests within four states in the outbreak-prone mid-Atlantic area (Maryland, Pennsylvania, Virginia, and West Virginia), with 11–26 sites in each state. The mid-Atlantic sites hosted populations across a range of densities (table 1) with 19 of the 66 sites (29%) at outbreak densities of more than 5 000 egg masses/ha.

Before gypsy moth eggs hatched in spring, *L. dispar* egg mass densities were quantified when trees were leafless using standard methods for gypsy moth monitoring [34]. Egg masses were counted yearly at nine plots in the stable, low-density area and three to six plots at each of the 66 sites in the outbreak-prone area.

To quantify infection and parasitism, gypsy moth larvae were collected in the field and reared to determine causes of mortality and frequency of successful co-infection. Methods for sampling larvae were generally consistent across the different sites and years. At the stable, low-density site, approximately one week after egg hatch began in spring and each week until pupation seven to nine weeks later, as many larvae as could be found within 1 h of searching, but not more than 30 were collected. In the outbreak-prone mid-Atlantic area, gypsy moths that were predominantly 4–6th instars were collected. In central Pennsylvania (PA) (Centre and Huntingdon Counties), at nine *ca* 7 ha forested sites larvae were sampled every 4–7 days. At these sites, sampling generally began 5–9 June when fourth instars were first seen and ended 22–26 June when pupation had begun [35]. An attempt was made to collect at least 50 larvae per sample date although occasionally populations were too sparse, but at least 18 larvae were taken per visit. For sampling at the 57 other mid-Atlantic sites, a maximum of 30 larvae were collected each week or, when populations were less abundant, as many as could be found within 1 h. Owing to variability in population densities at the 57 sites, development rates and mortality occurring in the

Table 1. Gypsy moth egg mass densities, total gypsy moth larvae collected, and numbers of larvae killed by pathogens and parasitoids for study sites. Means ± standard error (s.e.) are across years for the stable area (New York) sites but across sites within states in the outbreak-prone area sampled in 2009.

stable area		outbreak-prone area					
sampling years	1996–2010, 2012–2013	sampling years	2009				
state	New York	state	Maryland	Pennsylvania	Virginia	West Virginia	overall
no. sites/no. years	1/17	no. sites/no. years	18/1	26/1	11/1	11/1	66/1
mean gypsy moth egg mass density/ha (min–max)	39.3 ± 23.4 (0–405)	mean gypsy moth egg mass density/ha (min–max)	2954.4 ± 646.9 (49–10 028)	5198.5 ± 995.8 (454–18 827)	8348.6 ± 2167.2 (1063–22 946)	504.0 ± 246.2 (0–2648)	4251.5 ± 1668.7 (0–22 946)
mean gypsy moth larvae collected/site	80.8 ± 12.1 (122.8 ± 16.8)[a]	mean gypsy moth larvae collected/site	38.4 ± 5.7	286.2 ± 68.8	74.4 ± 10.1	54.5 ± 8.2	113.3 ± 58.0
% *E. maimaiga*	26.5 ± 5.1 (24.2 ± 0.1)[a]	% *E. maimaiga*	49.1 ± 6.0	48.6 ± 6.0	57.3 ± 6.0	50.7 ± 8.3	51.4 ± 2.0
% LdMNPV	0[a]	% LdMNPV	2.7 ± 1.1	8.6 ± 2.1	9.6 ± 1.9	4.1 ± 1.8	6.3 ± 1.7
% total parasitoids	15.1 ± 3.4 (12.7 ± 2.9)[a]	% *Cotesia melanoscela*	7.8 ± 3.1	4.4 ± 1.2	1.7 ± 0.7	1.5 ± 0.7	3.9 ± 1.5
		% Other parasitoids	0.8 ± 0.5	3.2 ± 1.3	1.0 ± 0.3	2.1 ± 0.8	1.8 ± 0.6

[a]Numbers of *L. dispar* larvae collected each year beginning the week when fourth instars were first collected until pupation. This is equivalent to the larvae collections in the outbreak areas. For analyses of stable site populations, larval collections from all instars were used, which are in parentheses.

population, larvae were collected from 1 to 6 times/site (median approx. three times) [27].

After collection all larvae were reared individually at 18–23°C in 29 ml clear plasticcups containing a high wheat germ diet and were checked daily for mortality for at least 30 days (allowing for detection of parasitoids emerging during both the larval and pupal stages and for slow deaths due to LdMNPV). Cadavers were monitored daily for 3 days after host death to record production of conidia by *E. maimaiga*. Any larvae that died or pupae that did not eclose to adults were stored at 4°C until cause of death could be determined. Subsequently, parasitoids emerging from cadavers were identified to species. Larvae dying (including cadavers of larvae from which parasitoids had emerged) were examined microscopically (at 100–400×) for the presence of *E. maimaiga* resting spores and LdMNPV occlusion bodies. As an exception, cadavers of gypsy moth from New York from which parasitoids had emerged were not dissected and examined microscopically before 2007. Success for pathogens and parasitoids was measured as the ability to develop to the next stage: spore production by *E. maimaiga*, occlusion body production by LdMNPV, and emergence from host larvae by parasitoids.

(c) Statistical analysis

In this empirical study, it was not possible to know when two parasites initially attacked the same host and only one successfully reproduced, or in any co-infected hosts, which parasite attacked first. We only learned which parasites were able to develop to the next stage. Therefore, we used probability to discern whether levels of co-infection differed from expectation based on densities of each natural enemy. When attacks by two parasites occurred in which one parasite prevented successful host use by a second, this was detected as lower co-infection than expected by chance. To get a general picture of the observed rates of co-infection versus what we would see if there was no interference between natural enemy species co-infecting the same host individual, we estimated the expected number of co-infections given the sample sizes and numbers of caterpillars infected by each natural enemy, assuming that they do not interfere with one another, using random Monte Carlo simulations (10 000) in MATLAB. We then compared the mean expected number of co-infections from the simulations with the observed number of co-infections from the rearing records using a *t*-test. For the long-term stable dataset we analysed the co-infection rate of *E. maimaiga* and parasitoids for each year separately, leaving out the two years with no parasitoids. For the outbreak-prone areas we analysed co-infection between *E. maimaiga*, LdMNPV, *C. melanoscela*, and other parasitoids. These analyses revealed the general trends in the data, but did not take into account differences in both observed and expected co-infection due to year, locality, or host density.

To study the potential for co-infection in greater detail, we analysed the association of infection of individual larvae with infection by other natural enemies and host density using logistic regression. Parasitoid species were merged per year for analyses of the long-term stable site data due to low numbers. For the outbreak-prone sites *C. melanoscela* was separated for analysis and data from other parasitoids were merged. Statistical analyses were performed using the statistical package JMP. For each enemy a separate model was made, with infection status of an individual caterpillar (0/1) as the response variable, and host egg mass density (at the level of site), instar, and infection with each of the other natural enemies as explanatory variables. States (Maryland, Pennsylvania, Virginia, and West Virginia) and sample sizes were included in the analysis of the outbreak-prone regional dataset. Year was included in the analysis of the long-term stable site.

The stable long-term dataset followed a time series so we first analysed the temporal autocorrelation among years, with a lag of 1–5 years of host egg mass density, rate of infection by *E. maimaiga*, and prevalence of parasitoids. There was no significant autocorrelation between years for any of the traits so temporal autocorrelation was not included in further analyses. We did not analyse co-infection by more than two natural enemies because this was not detected during this study. Models were first built with all two-way interactions included. Then insignificant factors were eliminated if their inclusion did not contribute significantly to the model. The contribution of each factor to the statistical models were assessed by comparing Akaike information criterion (AIC) values.

3. Results

(a) Long-term stable site

Gypsy moth populations remained at sparse densities from 1996 through 2013 in the stable site in New York State (table 1). During this time 2 019 gypsy moth larvae were collected and reared. Each year infections by *E. maimaiga* were detected, and overall, the fungus was the most abundant natural enemy. Single LdMNPV infections were only found in two of the 16 years. For all but two of the study years, parasitoids were reared from gypsy moth larvae. *Parasetigena silvestris* was the most common parasitoid and *C. melanoscela* was rare. In these sparse host populations, *E. maimaiga* prevalence was positively associated with gypsy moth egg mass density ($p = 0.027$; electronic supplementary material, table S1*a*) and negatively associated with parasitoids ($p < 0.001$; electronic supplementary material, table S1*b*, figure 1*a*).

Co-infection of caterpillars with *E. maimaiga* and parasitoids was lower than would be expected to occur by chance, indicating that the two enemies cannot generally occupy the same host individual and both successfully continue development. This is illustrated when comparing the observed co-infection with the co-infection expected if there was no interference between parasites within hosts (figure 2). It is shown statistically, taking into account variation among years, for *E. maimaiga* ($p < 0.001$, electronic supplementary material, table S1*a*) and for parasitoids ($p < 0.001$, electronic supplementary material, table S1*b*). The few co-infections that were detected happened when host egg mass densities were low (for *E. maimaiga*: $p = 0.017$, electronic supplementary material, table S1*a*; for parasitoids: $p = 0.017$, electronic supplementary material, table S1*b*).

(b) Outbreak-prone areas

Gypsy moth populations at the mid-Atlantic sites began the 2009 field season at a range of densities, including outbreak populations at 29% of sites. In total, 4 092 gypsy moth larvae were collected and reared. *Entomophaga maimaiga* was the dominant natural enemy (table 1 and figure 1*b*), occurring at all sites except two with very low host densities. Although LdMNPV-infected larvae occurred in 44 of the 66 sites, levels of virus infection were virtually always low (table 1 and figure 1*b*). The per cent parasitism by parasitoids remained at levels similar to the virus infection. In contrast with the long-term stable site, *C. melanoscela* was the most common parasitoid species.

The prevalence of infection by *E. maimaiga* increased with host egg mass density ($p = 0.003$, electronic supplementary material, table S2*a*; figure 1*b*) and this association was nearly significant for LdMNPV ($p = 0.058$, electronic supplementary material, table S2*b*). The overall rate of co-infection of larvae by both *E. maimaiga* and LdMNPV was as would be

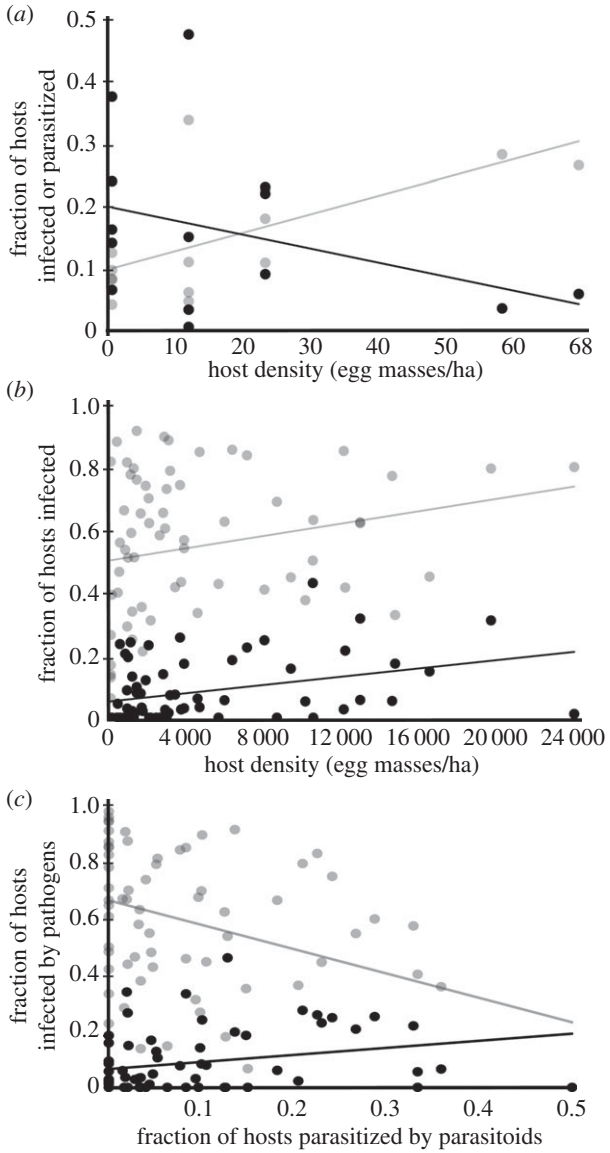

Figure 1. Relationship of gypsy moth egg mass density with (*a*) infection with *E. maimaiga* (grey, $p = 0.027$, electronic supplementary material, table S1*a*) and parasitoids (black, $p < 0.001$, electronic supplementary material, table S1*b*) in the long-term dataset for the stable site, (*b*) *E. maimaiga* (grey, $p = 0.003$, electronic supplementary material, table S2*a*) and LdMNPV (black, $p = 0.0058$, electronic supplementary material, table S2*b*) infections in the outbreak-prone region, and (*c*) the association of parasitism by parasitoids with infection by *E. maimaiga* (grey, $p = 0.001$, electronic supplementary material, table S2*a*) and LdMNPV (black, $p = 0.001$, electronic supplementary material, table S2*b*) in the outbreak-prone region. The positive association of LdMNPV with parasitism is due primarily to *C. melanoscela*.

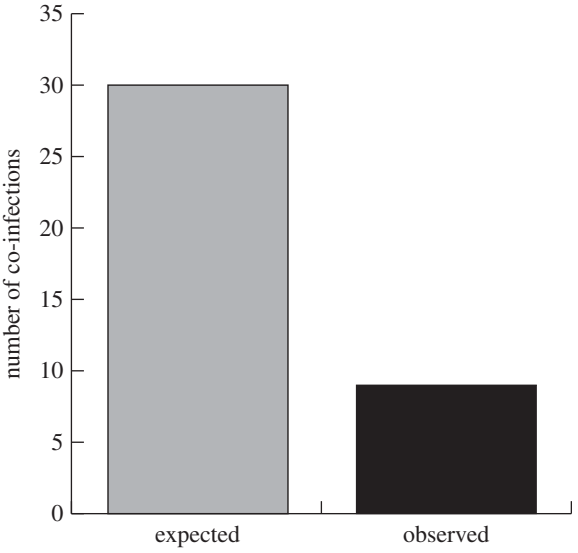

Figure 2. The sums of the expected (grey) and observed (black) numbers of hosts successfully both infected with *E. maimaiga* and parasitized by a parasitoid in the long-term stable dataset. There are significantly fewer co-infections than expected based on random association using Monte Carlo simulations ($t = 3.002$ $p = 0.004$, $N = 15$ years (missing two years in which no parasitoids occurred)).

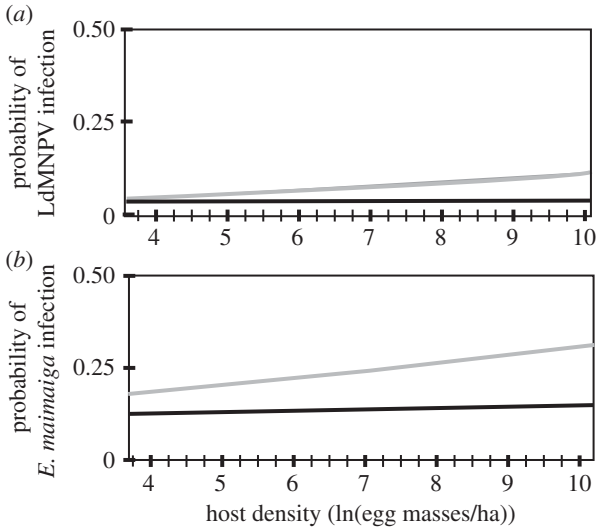

Figure 3. Logistic regression fit of the association of infection (0/1) with host egg mass density for (*a*) LdMNPV in caterpillars infected (black line, $\chi^2 = 793.01$, $p < 0.001$) and uninfected with *E. maimaiga* (grey line, $\chi^2 = 98.69$, $p < 0.001$), and (*b*) *E. maimaiga* in caterpillars infected (black line, $\chi^2 = 361.01$, $p < 0.001$) and uninfected with LdMNPV (grey line, $\chi^2 = 516.93$, $p < 0.001$). Only sites in which both LdMNPV and *E. maimaiga* were detected are included in the analyses, and the observations are weighted by the site sample size.

expected by chance (for *E. maimaiga*: $p = 0.430$, electronic supplementary material, table S2*a*; for LdMNPV: $p = 0.190$, electronic supplementary material, table S2*b*). However, the prevalence of co-infection depended on host density. For both pathogens, the positive association with host egg mass density was strongly associated only with larvae that were not infected with the other pathogen (for *E. maimaiga*: $p < 0.001$, electronic supplementary material, table S2*a*; LdMNPV: $p = 0.006$, electronic supplementary material, table S2*b*; figure 3*a*,*b*). That is, prevalence of infection increased with host egg mass density, but prevalence of co-infection did not.

In contrast with the pathogens, parasitoid occurrence was not directly associated with host egg mass density (*C. melanoscela*: $p = 0.544$, electronic supplementary material, table S2*c*; other parasitoids: $p = 0.484$, electronic supplementary material, table S2*d*). Co-infection of *E. maimaiga* with either *C. melanoscela* or other parasitoid species was rare ($p < 0.001$, figure 1*c*; electronic supplementary material, table S2*c*,*d*), whereas co-infection of LdMNPV and *C. melanoscela* occurred more than expected ($p < 0.001$, figure 1*c*; electronic supplementary material, table S2*c*), and co-infection of LdMNPV and other parasitoids was as expected ($p = 0.152$, electronic supplementary material, table S2*d*; figure 4). Although overall parasitism by parasitoids was not associated with host egg mass density,

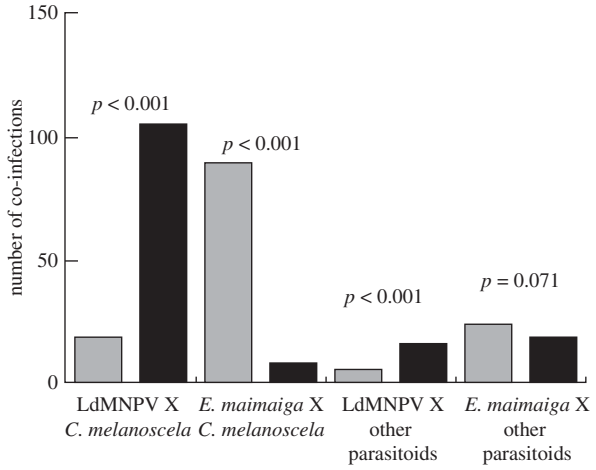

Figure 4. The expected (grey) and observed (black) number of pathogen–parasitoid co-infections in the outbreak-prone regions. The *p*-values represent the probability that the observed and expected number of co-infections are equal based on *t*-tests after Monte Carlo simulation.

the co-infection of *C. melanoscela* with both pathogens decreased with increasing host egg mass density (egg mass density × LdMNPV: $p < 0.001$; egg mass density × *E. maimaiga:* $p = 0.001$, electronic supplementary material, table S2*c*).

Entomophaga maimaiga infection increased with larval instar, meaning that there were more infections in larvae collected in later instars (likelihood ratio χ^2 $p < 0.001$, electronic supplementary material, table S2*a*). By contrast, prevalence of LdMNPV (likelihood ratio χ^2 $p = 0.003$, electronic supplementary material, table S2*b*) and *C. melanoscela* (likelihood ratio χ^2 $p < 0.001$, electronic supplementary material, table S2*c*) were negatively associated with larval instar, meaning that they were more abundant in larvae collected at earlier instars. The prevalence of all natural enemies decreased with increasing sample size and also differed among states (electronic supplementary material, table S2), with the highest *E. maimaiga* infection in Virginia, LdMNPV infection in West Virginia and Maryland, and parasitoids in Pennsylvania (electronic supplementary material, table S2).

4. Discussion

(a) Co-infection of gypsy moth larvae by multiple natural enemies

Animal and plant species are typically hosts to communities of pathogens and parasites [36] and these interacting natural enemies can partition the host as a resource by attacking different tissues within hosts, different host individuals, or different life stages. However, there are many cases in which enemies use the same host individual at the same time and multiple natural enemies, even lethal ones, can successfully develop within a single host. The inability of one parasite to exclude another leads to coexistence in increasingly complex communities. The parasites that we studied differed in life strategies: the virus could produce transmissive propagules while the infected host was alive while the fungus and parasitoids only developed to the next stage when ready to kill or permanently disable the host. The faster obligate killer, the fungus, dominated the community of natural enemies. As predicted based on these strategies, successful co-infections of *E. maimaiga* with parasitoids rarely occurred; we hypothesize that *E. maimaiga* usually excluded parasitoids by developing faster and killing the host and sporulating before the slower developing parasitoids were ready to emerge. In agreement, low levels of parasitoids in areas where *E. maimaiga* is the dominant natural enemy attacking gypsy moth larvae have been reported in other studies [27,37,38].

Conversely, LdMNPV produces transmissive stages before hosts die, so although on its own it kills more slowly than the fungus (see electronic supplementary material), the virus can reproduce to some extent when co-infecting with either *E. maimaiga* or parasitoids. Thus, as predicted by Malakar *et al.* [39], co-infection between LdMNPV and *E. maimaiga* occurred at the frequency that would be expected based on the prevalence of each in the host population. Although we did not quantify occlusion body, conidia, or resting spore production, it is likely that because both pathogens reproduced using only nutrients derived from the shared host individual they were not as productive as if they had been the only enemy using that host, leading to a cost to co-infection for both parasite species.

While overall co-infection of LdMNPV and *E. maimaiga* was common, it decreased with increasing host density. At high host densities there were fewer co-infections than expected, whereas at lower host densities the rate of co-infection was as expected due to chance. In general, resistance to infection can change with ecological context, including host density. Unfortunately, it is not known whether resistance of *L. dispar* to *E. maimaiga* changes at different host densities. However, resistance of *L. dispar* to LdMNPV decreases at high host density, causing infected larvae to die more quickly [40]. This could affect *E. maimaiga* because the faster mortality of virus-infected larvae in high-density populations would also decrease the opportunity for *E. maimaiga* to successfully infect and reproduce within hosts already infected by the virus before host death. Alternatively, co-infections could have been lower in the high-density populations simply due to inoculum from the faster-killing fungus causing more infections than the virus.

LdMNPV and parasitoids were able to reproduce within shared host individuals. In fact, co-infection of *C. melanoscela* and LdMNPV occurred more than expected given each of their prevalences. This may be due to facilitation, which occurs when infection with one pathogen or parasitoid increases the vulnerability of the host to another pathogen or parasitoid. Competition for host resources due to co-infection may reduce the host immune response [41,42] and parasitoids are known to suppress the immune response of a host, which has been shown to facilitate infection by pathogens, including viruses [43,44]. Thus, *C. melanoscela* may facilitate LdMNPV by dampening the immune response of the host with the aid of a polydnavirus (though see [45]). Facilitation between LdMNPV and *C. melanoscela* could also result from the virus being vectored by this parasitoid species [16].

Prevalence of *E. maimaiga* increased with instar, although, as with the virus, later instars are more resistant [29,30]. Before the arrival of *E. maimaiga* to North America LdMNPV infection peaked in later *L. dispar* instars during epizootics [21] and tachinid parasitoids, which attack later instars, were more abundant [46]. Therefore, we hypothesize that the low prevalence of LdMNPV and parasitoids in later instars was principally caused by the presence of fewer available hosts, due to high levels of *E. maimaiga* infection.

We found that with *E. maimaiga* as part of the community, prevalences of both LdMNPV and parasitoids were low. By

contrast, studies conducted before *E. maimaiga* was established reported higher levels of both parasitoids and virus [46,47], including high co-infection of LdMNPV with tachinid flies during a viral epizootic [48].

(b) Associations with host density

In many ecological systems, the prevalences of pathogens and parasitoids are related to host density. It is common for the association to be positive for pathogens because at high host density transmission rates increase, although not always linearly [49,50]. Parasitoids are also known to increase with increasing host density due, in the short term, to aggregation of parasitoids to high-host density, and in the longer term to increases in parasitoid population size with increased resource availability [51]. However, this is by no means always the case for either pathogens or parasitoids. For instance, the performance of a pathogen may be strongly related to abiotic conditions or high infection levels of some pathogens can be due to high densities of propagules able to persist for long periods [52], and parasitism may be independent of host density if an oligophagous or polyphagous parasitoid has other host species in the environment [53,54]. In this study, we found that infections by the fungus increased with host density in the stable site where yearly densities varied from 0 to only 405 egg masses/ha. In the outbreak-prone area host density varied much more widely (from 0 to 22 956 egg masses/ha among sites) and was positively associated with both *E. maimaiga* and LdMNPV. This positive relation to density is what we would expect for LdMNPV [55], and has also been found for *E. maimaiga* in some studies [27,56], but is not always the case (e.g. [35]), probably because environmental conditions also impact *E. maimaiga* infection [57] and can restrict density-related responses.

We did not find a positive relationship between parasitism by parasitoids and host density. In the outbreak-prone region the fraction of hosts parasitized was generally low (2.7–8.6%, table 1). This is most probably because, although all the parasitoids in this study were introduced for the biological control of gypsy moth, none are known to parasitize gypsy moth at a high rate [21,31]. Additionally, all but *P. unicincta* are known to have other hosts, and the host range of *P. unicincta* is not known. Thus, the gypsy moth might not be the primary host of these parasitoids, so parasitoid abundances could then be related to the abundances other forest Lepidoptera species.

5. Conclusion

The emergent fungal pathogen *E. maimaiga* is now the dominant natural enemy among the parasites and pathogens in the system of introduced species we studied. Historically, before the fungus arrived in the USA, the virus caused epizootics ending host outbreaks [57] and the community of introduced parasitoids caused higher mortality than we found [46,47]. The fungal pathogen now outcompetes parasitoids within gypsy moth caterpillars at a range of host densities. These two natural enemies that each need to kill their host in order to develop further were rarely found successfully using the same host individual. Because the fungus develops more quickly, the parasitoid is excluded. Where the virus is present, it successfully co-infects with both fungal-infected and parasitoid-occupied hosts because it produces transmissive stages while hosts are alive, though the fitness of each may be lower than if it had been alone. Because successful co-infection occurs, the fungus does not preclude successful infection by the virus directly. However, because the virus is strongly density dependent, the decrease in host density due to the fungus must suppress the virus at the population level. As the virus and the parasitoids can co-infect they also do not interfere with one another. In fact, we detected facilitation between the virus and the parasitoid *C. melanoscela*, known to carry a polydnavirus that decreases the host immune response.

Data accessibility. Data are available at http://dx.doi.org/10.5061/dryad.3tn08.

Authors' contributions. A.E.H. designed and coordinated field studies and participated in data analysis; S.v.N. carried out the modelling and statistical analyses. Both authors designed the paper, drafted the manuscript, and gave final approval for publication.

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