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Parasite exposure and host susceptibility jointly drive the emergence of epidemics

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Abstract. Parasite transmission is thought to depend on both parasite exposure and host susceptibility to infection; however, the relative contribution of these two factors to epidemics remains unclear. We used interactions between an aquatic host and its fungal parasite to evaluate how parasite exposure and host susceptibility interact to drive epidemics. In six lakes, we tracked the following factors from pre-epidemic to epidemic emergence: (1) parasite exposure (measured observationally as fungal spores attacking wild-caught hosts), (2) host susceptibility (measured experimentally as the number of fungal spores required to produce terminal infection), (3) host susceptibility traits (barrier resistance and internal clearance, both quantified with experimental assays), and (4) parasite prevalence (measured observationally from wild-caught hosts). Tracking these factors over 6 months and in almost 7,000 wild-caught hosts provided key information on the drivers of epidemics. We found that epidemics depended critically on the interaction of exposure and susceptibility; epidemics only emerged when a host population's level of exposure exceeded its individuals' capacity for recovery. Additionally, we found that host internal clearance traits (the hemocyte response) were critical in regulating epidemics. Our study provides an empirical demonstration of how parasite exposure and host susceptibility interact to inhibit or drive disease in natural systems and demonstrates that epidemics can be delayed by asynchronicity in the two processes. Finally, our results highlight how individual host traits can scale up to influence broad epidemiological patterns.

Key words: Daphnia*; disease; epidemic; exposure; host trait; immune; infection stage;* Metschnikowia*; susceptibility; transmission.*

Introduction

Disease epidemics, parasite invasions, and emerging infections have long plagued humans and wildlife, and continue to cause illness and mortality today. These three ecological phenomena share a common theme: a parasite that was once rare or absent proliferates to infect many hosts. Disease epidemics, in particular, show rapid spread of infection to many individuals over a short period of time. But what ignites this rapid spread? When considering the origin of epidemics, ecologists often focus on the introduction of a novel parasite or on a shift in the health and susceptibility of a host population. In reality, both of these processes—changes in exposure and susceptibility—likely work in concert to promote transmission.

Theory predicts that disease emerges when two criteria are met: (1) infective propagules are present in the environment and (2) hosts can support and transmit infection (Combes 2001, Gilbert and Parker 2006). These criteria can be thought of as exposure and susceptibility filters that open or close the pathway to parasite transmission (Combes 2001; Fig. 1a). With an open exposure filter, hosts acquire parasite infective stages necessary for infection. Then, with an open susceptibility filter, hosts support infection and subsequent transmission. Although closure of the exposure filter precludes infection, closure of the susceptibility filter can produce a transmission bottleneck that terminates parasite spread (even with an open exposure filter). Accordingly, disease should fail to emerge when either filter is closed.

Broad-scale environmental changes are thought to impact both exposure and susceptibility for wildlife (and people), and therefore motivate tests of exposure–susceptibility theory. Parasite exposure is changing with global transportation networks, habitat modification, and shifts in species ranges (Tatem et al. 2006). Moreover, susceptibility of wildlife is predicted to increase with anthropogenic stressors, like climate change and environmental contamination (Martin et al. 2010). These changes can influence the emergence of disease (Harvell et al. 1999, Daszak et al. 2000, Lafferty 2009,

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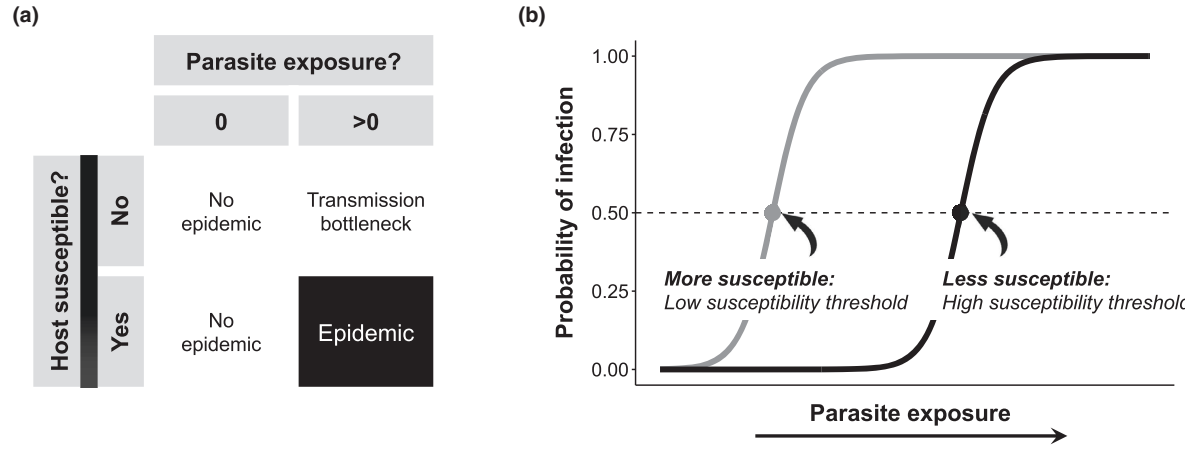

FIG. 1. Theoretical links between exposure, susceptibility, and epidemics. (a) Epidemic requirements: For an epidemic to emerge, a population must be exposed and susceptible to a parasite. That is, parasite exposure and host susceptibility filters must be open. Closure of either filter inhibits transmission. Epidemics cannot occur without exposure (parasite exposure = 0), and parasites meet a transmission bottleneck when the susceptibility filter is closed. But how is susceptibility quantified? (b) Interconnectedness of exposure and susceptibility: By evaluating probability of terminal infection as a function of parasite exposure, the shape of the exposure-response curve defines susceptibility. More susceptible hosts (gray curve) succumb to terminal infection at low exposure (low susceptibility threshold; where curve intersects 50% line). Less susceptible hosts (black curve) withstand high exposure before succumbing to terminal infection (high susceptibility threshold). Uniting epidemic requirements (a) with their interconnectedness (b), epidemics may become more likely as a population's exposure meets or exceeds its susceptibility threshold.

Altizer et al. 2013), including the frequency and size of epidemics. Despite these predictions, few empirical studies link exposure and susceptibility to the failure or success of epidemics. One reason for this gap is observation bias: wildlife disease is typically studied after epidemics start (i.e., when both exposure and susceptibility filters are open). Without pre-epidemic data, we cannot observe which filter(s) caused epidemic failures and determine why both filters opened to enable epidemic success (Harvell et al. 1999, Plowright et al. 2008). Another reason hinges on measurement of exposure and susceptibility. Commonly used proxies for exposure (like infection prevalence) can dramatically underestimate actual exposure. Conversely, susceptibility is typically neglected in wildlife disease research, because tools to measure it can be costly, complicated, and uninformative (Boughton et al. 2011). Hence, despite the pressing need to test key epidemic filters, much remains empirically underexamined.

Fortunately, insight into these filters can come from the developmental trajectory of parasites inside hosts. Many parasites transition through within-host developmental stages that signal exposure and then susceptibility. For instance, trematode miracidia enter snails during exposure, develop into sporocysts and rediae (if the snail does not clear the infection), and ultimately produce infective cercariae (Esch and Fernandez 1994). The within-host temporal dynamics hold key information: earliest developmental stages manifest exposure, and final (terminal) stages reflect susceptibility. More mechanistically, these stages can be used to quantify exposure-response curves (Fig. 1b), which provide measurement and interpretation of both exposure and susceptibility. Exposure is measured as the number of early developmental stages a host contacts. Then, the probability of terminal infection is determined by measuring the successful development of parasites into terminal stages. From these axes, susceptibility emerges as the shape of the exposure-response curve, which tells us how susceptible a host may be to a particular level of exposure. Because exposure and susceptibility are intrinsically linked, epidemics should become predictable from both the magnitude of exposure a host population faces and the shape of the population's exposure-response curve.

The start of epidemics may also depend on variation in parasite exposure, that is, unequal distribution of exposure within a population. Parasites generally exhibit aggregated distributions, where a few hosts are infected with many parasites, and many hosts have few parasites or are parasite free (Crofton 1971, Poulin 2007). Exposure may be similarly aggregated, with consequences for transmission (Woolhouse et al. 1997, Lloyd-Smith et al. 2005, Martin et al. 2019). With strongly aggregated exposure, some individuals may face high levels of exposure from which they cannot recover, thereby producing the first generation of infections in an epidemic. Conversely, with homogeneous exposure, the per-individual exposure level may remain too low to result in infection and transmission (however, more uniform exposure could provide more opportunity for infection and subsequent epidemic emergence). One simple way to capture exposure heterogeneity is to quantify a population's average exposure and its maximum. These two values will become increasingly dissimilar as heterogeneity increases. Predicting epidemics with one metric or the other can therefore inform whether epidemics are more

likely to result from a few heavily exposed individuals or many, less exposed individuals.

Variation in susceptibility is a direct outcome of host traits (Stewart Merrill 2019, Stutz et al. 2019). Following exposure, hosts attempt to prevent parasite entry using physical, chemical or immunological barriers, or barrier resistance traits. If parasites successfully enter the host, hosts can use immune defense, or internal clearance traits, to eliminate an infection before it achieves its terminal state. Individual-level variation in these traits, owing to genetic or environmental factors, may produce variation among host populations and through time. Hence, measurement of barrier resistance and internal clearance traits may reveal how host defenses, by opening or closing the susceptibility filter, contribute to the inhibition of epidemics or their start.

In this study, we connect traits governing exposure and susceptibility to the inhibition and start of epidemics in an aquatic system. The zooplankton host, *Daphnia dentifera*, and its fungal parasite, *Metschnikowia bicuspidata*, offer tractability for two reasons. First, *Metschnikowia* produces annual epidemics, starting in summer or autumn, that replicate disease emergence among lakes (Cáceres et al. 2014). Therefore, in replicate populations we can track exposure and susceptibility before epidemics emerge, then evaluate subsequent failures and successes of epidemic emergence. Second, we can readily measure within-host developmental stages of the parasite, as well as barrier resistance and internal clearance traits that comprise susceptibility, in hosts collected from the wild (Stewart Merrill and Cáceres 2018; Stewart Merrill et al. 2019). Together, these elements provide a powerful system to test empirically how exposure and susceptibility filters influence the emergence of epidemics.

With this system, we examine the relative contributions of parasite exposure and host susceptibility to the emergence of fungal epidemics. We delineate among three possibilities: (1) if exposure regulates epidemics, epidemics will become more likely as fungal spores become more abundant; (2) if susceptibility regulates epidemics, epidemics will become more likely if/when hosts become more susceptible; (3) if exposure and susceptibility jointly regulate epidemics (i.e., both filters are open), then epidemics should emerge when host susceptibility matches the current level of exposure. This third possibility means that we could observe epidemic failures in the face of high exposure (but closed susceptibility filter) or high susceptibility (but closed exposure filter). To test these predictions, we tracked (1) population-level exposure, with prevalence of early developmental stage infections in field-collected hosts and abundance of naturally occurring fungal spores; (2) susceptibility, using prevalence of terminal infections in field-collected hosts, and experimental exposure-response curves; and (3) timing of the start of epidemics. Using this combination of field and laboratory data, we found that epidemic emergence depended on a critical interaction between parasite exposure and host susceptibility: epidemics only began when a population's level of exposure met or exceeded the population's susceptibility threshold (see Fig. 1b). We further unpacked the importance of susceptibility for epidemics by comparing host barrier resistance and internal clearance traits during transmission bottlenecks (susceptibility filter closed) and during epidemic emergence (both filters open). We observed consistent declines in *Daphnia* internal clearance traits (hemocyte response) from bottleneck to emergence, indicating that increases in exposure—paired with declines in the effectiveness of host defenses—lead to the emergence of epidemics.

Methods

Study system

Metschnikowia bicuspidata is an ascomycete fungal parasite of the freshwater zooplankter, *Daphnia dentifera*. *Daphnia* hosts are exposed to *Metschnikowia* spores while filter-feeding. Once consumed, spores must penetrate the *Daphnia* gut and enter the body cavity to initiate infection. Hence, the gut forms a resistant barrier (the first susceptibility trait). If *Metschnikowia* spores breach the gut, they progress through a series of within-host developmental stages (Stewart Merrill and Cáceres 2018). *Daphnia* can clear early developmental stages of *Metschnikowia* with hemocytes (the second susceptibility trait; Stewart Merrill et al. 2019). If *Metschnikowia* survives internal clearance and produces asci (infective spores), the fungus kills its host. Host death releases spores into the water to continue environmental transmission (Ebert 2005).

We sought to explain variation in timing of fungal epidemics using three assays on field-collected adult *Daphnia*. *Metschnikowia* epidemics tend to emerge in late August and early September, but there is variation in the timing of emergence (Cáceres et al. 2006, Shocket et al. 2018). To capture this variation, we sampled six *Daphnia* populations in central Indiana every 2 weeks over 6 months spanning the pre-epidemic and epidemic periods (June–December 2017). We examined 6,781 *Daphnia* hosts. Below, we sketch key methods; see Appendix S1 Section S1 for extended methods and a complete description of the sampling regime.

Assay 1: Infection states of field-collected animals

With field-collected *Daphnia*, we identified natural infection patterns of *Metschnikowia*. Fifty *Daphnia* per lake and time point were examined for presence of *Metschnikowia* within the body. If *Metschnikowia* was absent, the host was recorded as unexposed. If present, we categorized hosts by the most advanced developmental stage possessed. These stages progress from spore I, spore II, hypha, sporocyst, conidium, to ascus (Stewart Merrill and Cáceres 2018). In the spore I stage, hosts

have fungal spores which failed to penetrate the gut. The subsequent developmental stages are those occurring inside the host's body cavity; spore II are spores that successfully entered the body cavity, which can then produce fungal hyphae, before developing sequentially into sporocysts, conidia, and asci. Based on presence of the most advanced stage possessed, *Daphnia* were classified into infection states (Table 1: Assay 1). Hosts in the early interaction state had only those developmental stages from which the host could recover using barrier resistance or internal clearance (spore I–sporocyst). Hosts with within-host infections had developmental stages inside the body cavity (spore II–ascus). Finally, hosts with terminal infections included hosts full of conidia or asci leading to host death (i.e., internal clearance was no longer possible; see analyses for further explanation).

Assay 2: Mechanistic exposure and susceptibility

We measured each population's exposure by counting fungal spores in field-collected *Daphnia*. For each *Daphnia* ($N = 50$ per lake and time point), we counted the number of spores embedded in the gut barrier (spore I stage) as well as those that successfully crossed into the body cavity (spore II stage). We label the sum of these categories as attacking spores; they represent a measure of exposure at the level of the individual. Using attacking spore counts, we calculated three summary metrics for *Metschnikowia* exposure (Table 1: Assay 2). Exposure risk is the average number of attacking spores among all sampled *Daphnia*, both exposed and unexposed (analogous to parasite abundance; Bush et al. 1997). Exposure intensity is the average number of attacking spores among only exposed *Daphnia* (analogous to parasite intensity; Bush et al. 1997). Exposure maximum is the highest count of attacking spores observed among individuals. Deviations between these metrics provide an indication of how exposure is distributed. When all hosts experience equal exposure, the three metrics converge, but as exposure becomes more aggregated (unequal), the three metrics deviate.

We measured each population's susceptibility with experimental infections and exposure-response curves of field-collected *Daphnia* (Table 1: Assay 2). On a monthly basis (every second sampling event) we inoculated *Daphnia* ($N = 25$ per lake) with a standard spore dose and counted their attacking spores 24 h later to estimate

Table 1. A comprehensive data set allows connections between parasite exposure, host susceptibility, and epidemics. Infection states of field-collected animals (Assay 1) contains parasitological data, in which we classified *Daphnia* by their *Metschnikowia* developmental stage. Stages, in progressing order, are spore I (SpI), spore II (SpII), hypha (H), sporocyst (SC), conidium (C), and ascus (A) (columns). Presence of any stage (× symbols) determined whether hosts were unexposed or exposed, and whether hosts had early interactions, within-host infections, and/or terminal infections. The distinction between these latter three categories is whether and how *Daphnia* recover. Mechanistic exposure and susceptibility (Assay 2) contains exposure, measured as number of attacking spores (spore I and II) in field-collected *Daphnia* (abundance, intensity, and maximum values), and susceptibility, experimentally quantified using susceptibility thresholds from exposure-response curves (with terminal infections or hyphae). Host traits that influence the probability of infection (Assay 3) were also measured during experimental infections.

Variable	Description	SpI	SpII	H	SC	C	A
Assay 1: Infection states of field-collected animals							
Unexposed	Absence of any *Metschnikowia* developmental stage within a *Daphnia* host						
Exposed	Presence of any *Metschnikowia* developmental stage within a *Daphnia* host	X	X	X	X	X	X
Early interaction	Early phase of the host–parasite interaction. *Daphnia* can recover by resisting attack at the gut barrier and/or by clearing a within-host infection	X	X	X	X		
Within-host infection	Fungus has successfully crossed host resistance barriers. *Daphnia* can only recover by clearing a within-host infection		X	X	X	X	X
Terminal infection	Late phase of the host–parasite interaction. *Daphnia* are full of conidia or asci and have reached the point of no recovery (i.e. infection is lethal)					X	X
Assay 2: Mechanistic exposure and susceptibility							
Exposure risk	Average number of attacking spores (spore I and II) per *Daphnia* (denominator includes exposed and unexposed hosts)						
Exposure intensity	Average number of attacking spores (spore I and II) per exposed *Daphnia* (denominator includes only exposed hosts)						
Exposure maximum	Maximum number of attacking spores (spore I and II) in all examined *Daphnia*						
Susceptibility threshold	Average number of attacking spores (spore I and II) required to produce a 50% probability of terminal infection						
Assay 3: Host traits that influence the probability of infection							
Exposure trait	Spore consumption, or the number of spores a *Daphnia* consumes following inoculation						
Barrier resistance trait	Gut resistance, or likelihood that attacking spores will be blocked by the gut barrier						
Internal clearance trait	Hemocyte defense, or likelihood that hemocytes will defend against spores infecting the body cavity (stage spore II)						

exposure. Inoculated hosts were then held for 9 d to determine their infection fate (i.e., whether they recovered or succumbed to terminal infection). Using attacking spores and infection fate, we constructed exposure-response curves (binomial distribution, logit-link) and extracted their susceptibility thresholds, or the number of attacking spores producing 50% probability of terminal infection (Fig. 1b). Higher susceptibility thresholds indicate lower susceptibility, as more attacking spores are required to produce terminal infection.

Our monthly assessment of infection fates meant that *Daphnia* susceptibility was assessed over a coarser timescale than *Metschnikowia* exposure (assessed twice monthly). Additionally, exposure-response curves were only constructed using a subset of experimental *Daphnia* (we had 50 total experimental *Daphnia* for each lake and time point that were measured for susceptibility traits). To capitalize on the larger sample of experimental *Daphnia*, we used presence of fungal hyphae to calculate susceptibility thresholds. Growth of hyphae is a good early indicator of future terminal infection: one hypha consistently results in a >50% probability of terminal infection. So, we could also estimate susceptibility thresholds by determining the number of attacking spores necessary to produce one hypha. Thresholds measured with hyphae were measured twice monthly and had greater sample sizes ($N = 50$ per lake).

Assay 3: Host traits that influence the probability of infection

We used experimental infections of field-collected *Daphnia* to measure three traits that mediate infection. Following inoculation with a standard spore dose, we measured *Daphnia* exposure, barrier resistance, and internal clearance traits ($N = 50$ per lake and time point; Table 1: Assay 3). The exposure trait represents a snapshot of spore consumption and was quantified as the number of inoculated spores observed inside the host gut lumen (spores passing through the gut with food). The barrier resistance trait was assessed as the proportion of attacking spores that failed to penetrate the gut barrier (spore I/attacking). Gut resistance ranged from 0 (no resistance) to 1 (complete barrier resistance). Finally, the internal clearance trait was assessed as the proportion of spores in the body cavity (spore II) that were defended against by hemocytes (0 = no hemocytes, 1 = hemocytes recruited to all spores). Lower barrier resistance and internal clearance values indicate higher susceptibility.

Pinpointing epidemic emergence

We characterized epidemics by evaluating prevalence of terminal infections in field-collected hosts (Table 1: Assay 1). With these data, epidemic emergence date was estimated when terminal infection prevalence exceeded background rates and continually increased toward its maximum (vertical dashed lines in Fig. 3).

Analyses

Testing drivers of epidemics with infection states.—We examined exposure and susceptibility as drivers of epidemic emergence by decomposing exposed hosts into nonoverlapping early interaction and terminal infection states (Table 1: Assay 1). During the early interaction (SpI–SC), *Daphnia* can recover with barrier resistance or internal clearance. In a terminal infection, *Daphnia* contain conidia and asci (C and A) from which they cannot recover. If epidemics are driven solely by exposure, they should emerge whenever early interactions are present (because exposure >0; Fig. 1a). However, when we observe exposure (early interactions), but not terminal infections, low *Daphnia* susceptibility may be inhibiting epidemics (transmission bottleneck; Fig. 1a). We compared prevalence of early interactions and terminal infections through time to test these predictions qualitatively.

Testing drivers of epidemics with mechanistic exposure and susceptibility.—We tested the mechanistic measures of exposure and susceptibility (Table 1: Assay 2) as drivers of epidemic emergence using an information theoretic approach (Burnham and Anderson 2002). For each lake and time point, we coded epidemic emergence as 0 or 1, then fit generalized linear models (binomial distribution, logit-link) testing the effects of different predictors on epidemic emergence. We competed four model types: (1) null; (2) exposure only; (3) susceptibility only; and (4) exposure and susceptibility. The null model estimated only an intercept. The exposure-only models contained any of the exposure metrics as predictors (exposure risk, intensity, or maximum). Competing these exposure models allowed us to ask whether a few heavily exposed individuals (indicated by exposure maximum and to a lesser extent, exposure intensity) better explained epidemics than many, less exposed individuals (exposure risk). The susceptibility-only models contained the susceptibility threshold as a predictor (quantified monthly with terminal infections or twice monthly with hyphae). Finally, the exposure and susceptibility models used the difference (Δ) of each exposure metric and the susceptibility threshold (which share common units of spores). When $\Delta < 0$, exposure falls below the susceptibility threshold, and epidemics should fail. Conversely, when $\Delta > 0$, exposure exceeds the susceptibility threshold, (potentially) permitting epidemic emergence.

For each model, we calculated Akaike's information criterion (AIC) values and ranked models from lowest to highest AIC. The lowest AIC value represents the most likely model given the data. We compared model fits based on performance relative to the best-ranked model (ΔAIC), where ΔAIC of two or greater represents

substantially better fit. Finally, we compared models based on model weights (w_i), which represent the probability that a model fits best, given the suite of models considered (Burnham and Anderson 2002). We initially included lake as a random effect in all models. However, lake did not alter AIC rankings or qualitative results, so it was removed to simplify model structure.

Identifying transmission bottlenecks.—We used infection states to identify transmission bottlenecks. With observational data from the field (Table 1; Assay 1), we asked: at what point in the infection process does exposure lead to a transmission bottleneck? To make this determination, we plotted prevalence of hosts in the exposed, within-host infection, and terminal infection states against each population's exposure risk (average number of attacking spores per host). Then, we fit saturating rectangular hyperbolae to each infection state and compared half-saturation constants (K_m). If the susceptibility filter is open, all three curves should converge on the same shape (similar K_m): positively saturating with exposure up to a maximum prevalence. If host barrier resistance produces a bottleneck, then the within-host infection and terminal infection curves should deviate from the exposed curve; that is, their prevalence should grow more slowly. If host internal clearance produces a bottleneck, the terminal infection curve should deviate from the exposed and within-host infection curves, growing even more slowly to its maximum prevalence.

Connecting Daphnia traits to transmission bottlenecks.—We connected host traits to transmission bottlenecks by comparing them among two time periods. We evaluated experimentally measured traits for exposure, barrier resistance, and internal clearance (Table 1: Assay 3) when lakes experienced a bottleneck (Fig. 3; purple points) and when epidemics emerged (Fig. 3; vertical lines). We identified bottlenecks as times when parasite exposure was high, but terminal infections were low at the subsequent sampling event (indicated in Appendix S1: Table S2). Host traits were compared using general linear models. All analyses were conducted in R version 3.3.3.

Results

Testing drivers of epidemics with infection states

Terminal infections are the tip of the iceberg for *Metschnikowia* infection prevalence. Before epidemics, 10% of *Daphnia* were exposed (on average), while during epidemics, prevalence of exposure averaged approximately 75% and could achieve 100%. Prevalence of the latest, conspicuous stage of infection (ascus [A]; Fig. 2a)

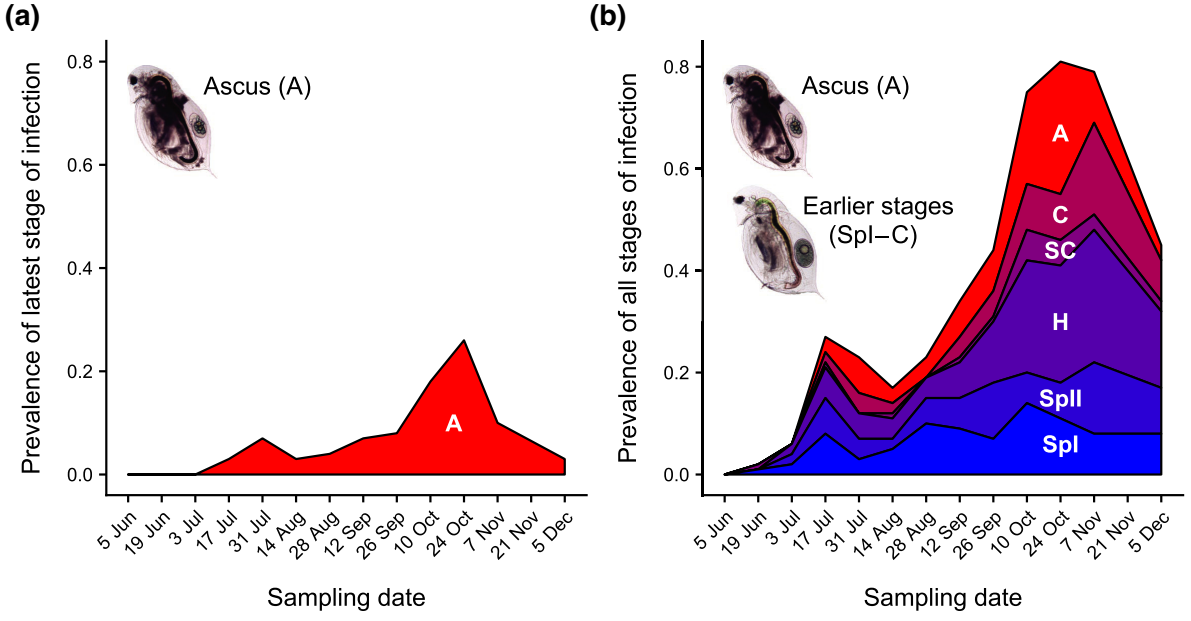

Fig. 2. Disease is just the tip of the iceberg. In late October, 26% of *Daphnia* had spore-producing *Metschnikowia* infections, and 55% hosted earlier developmental stages. These patterns convey that final stages of infection (ascus, A) dramatically underestimate actual parasite prevalence. (a) Average prevalence of ascus infections (white A) across six lakes in central Indiana from June to December 2017 (see image for readily detected ascus infection). (b) Average prevalence of all developmental stages of *Metschnikowia* in the same six lakes (these stages are not detectable without high magnification, as shown in "Earlier stages" image). Each shaded region represents a developmental stage, from the earliest (bottom) to the latest (top). Stages: SpI = spore I (not penetrating body cavity), SpII = spore II (infecting body cavity), H = hypha, SC = sporocyst, C = conidium, A = ascus (see also Table 1: Assay 1 for information about hosts having these developmental stages).

was five times lower, on average, than prevalence of more encompassing exposure (spore I [SpI] through ascus [A] stages; Fig. 2b). Exposure alone could not explain epidemic emergence. An index of exposure—prevalence of early interactions—consistently preceded epidemics and never resulted in epidemic emergence in one lake (Beaver Dam; Fig. 3).

Testing drivers of epidemics with mechanistic exposure and susceptibility

Epidemics emerged when *Metschnikowia* exposure matched or surpassed a population's susceptibility threshold. From the monthly susceptibility data (thresholds estimated using terminal infections), the best model included the difference of exposure maximum and *Daphnia* susceptibility ($w_i = 0.58$; Table S5). As this difference (Δ) approached zero, epidemic emergence became more likely (Fig. 4a). Additionally, all three of the Δ models were most highly ranked (cumulative w_i of 0.86; the best susceptibility-only model [Fig. 4b] had $w_i = 0.14$ and the best exposure-only model was worse [Fig. 4c]; Table S5). With hyphae-estimated susceptibility thresholds (measured twice monthly), the exposure and susceptibility (Δ) model still best predicted epidemics (Appendix S1: Table S5; Fig. 4d). Once again, the three Δ models outperformed all others (cumulative w_i of 0.64), and the model containing exposure maximum was highest ranked. However, in this set, exposure maximum was less competitive with the other exposure metrics (all ΔAIC < 2). In fact, all of the models were generally more competitive with one another, including the susceptibility-only (Fig. 4e) and exposure-only models (Fig. 4f; see Appendix S1: Table S3 for details). Across all models, exposure maximum generally outperformed exposure intensity and always outperformed exposure risk, indicating that the exposure level of heavily exposed individuals better predicted epidemics than the average level of exposure among all individuals.

Identifying transmission bottlenecks

Attacking spores in the field encountered a transmission bottleneck inside of their *Daphnia* hosts because of internal clearance (Fig. 5a–d provides predictions, with empirical data in e). Prevalence of the exposed state was well predicted by exposure risk (K_m [half-saturation constant] = 1.04, SE = 0.08, $P < 0.001$; Fig. 5e, blue), as was prevalence of within-host infections ($K_m = 1.76$, SE = 0.18, $P < 0.001$; Fig. 5e, purple). The exposed and within-host infection curves shared similar shapes and half-saturation constants, suggesting that host barrier resistance did not produce a transmission bottleneck. In contrast, the terminal infection curve sat below the exposed and within-host infection curves ($K_m = 6.12$, SE = 1.86, $P = 0.002$; Fig. 5e, red). Due to this decoupling of terminal infections from exposure risk, we infer that *Metschnikowia* meets a transmission bottleneck during its within-host development.

Connecting Daphnia *traits to transmission bottlenecks*

The experimentally measured internal clearance trait proved most important for creating transmission bottlenecks. First, we can rule out one possibility: epidemics did not start because hosts began consuming more spores (elevating exposure). The exposure trait (spore consumption) did not increase between greatest bottleneck and epidemic emergence ($F = 1.35$, $P = 0.246$; Appendix S1: Fig. S1). Spore consumption varied by lake ($F = 5.111$, $P < 0.001$) and by a lake–time interaction ($F = 5.80$, $P < 0.001$; Appendix S1: Fig. S1). Ruling out exposure, one or both of the susceptibility traits must have ended the transmission bottlenecks. The barrier resistance trait (gut resistance) represents penetrability of the gut to attacking spores; low values mean higher susceptibility and declines in gut resistance may therefore lead to epidemics. However, gut resistance was not uniformly lower when epidemics emerged ($F = 1.27$, $P = 0.261$; Appendix S1: Fig. S1), and was in some cases higher at epidemic emergence. Therefore, resistance was almost certainly not creating the bottleneck. Gut resistance varied by lake ($F = 11.02$, $P < 0.001$), and by a lake–time interaction ($F = 3.00$, $P = 0.019$). The internal clearance trait appeared to be the most likely trait regulating the change from bottleneck to epidemic. This trait was indexed as the proportion of infecting spores that were defended against by host hemocytes; lower values signal higher susceptibility. Hemocyte defense declined overall as epidemics emerged ($F = 19.76$, $P < 0.001$; Appendix S1: Fig. S1). Hemocyte defense varied by lake ($F = 11.32$, $P < 0.001$) with no lake–time interaction ($F = 1.50$, $P = 0.203$). The drop in hemocytes with epidemic emergence provides evidence that internal clearance traits created the transmission bottleneck.

Discussion

Our study demonstrates that parasite exposure and host susceptibility, together, play critical roles in epidemic emergence. We found that exposure to fungal parasites (*Metschnikowia*) was common among populations of a zooplankton host (*Daphnia*). However, epidemics often started long after exposure occurred. In support of general epidemiological theory (Gilbert and Parker 2006; Combes 2001), epidemic timing depended on the appropriate alignment of exposure and susceptibility: epidemics emerged when a population's exposure to spores matched or exceeded its susceptibility threshold. We further evaluated transmission bottlenecks, or those instances when the exposure filter was open, but the susceptibility filter was closed. By decomposing *Daphnia*–*Metschnikowia* interactions into three stages (early interaction, within-host infection, and terminal

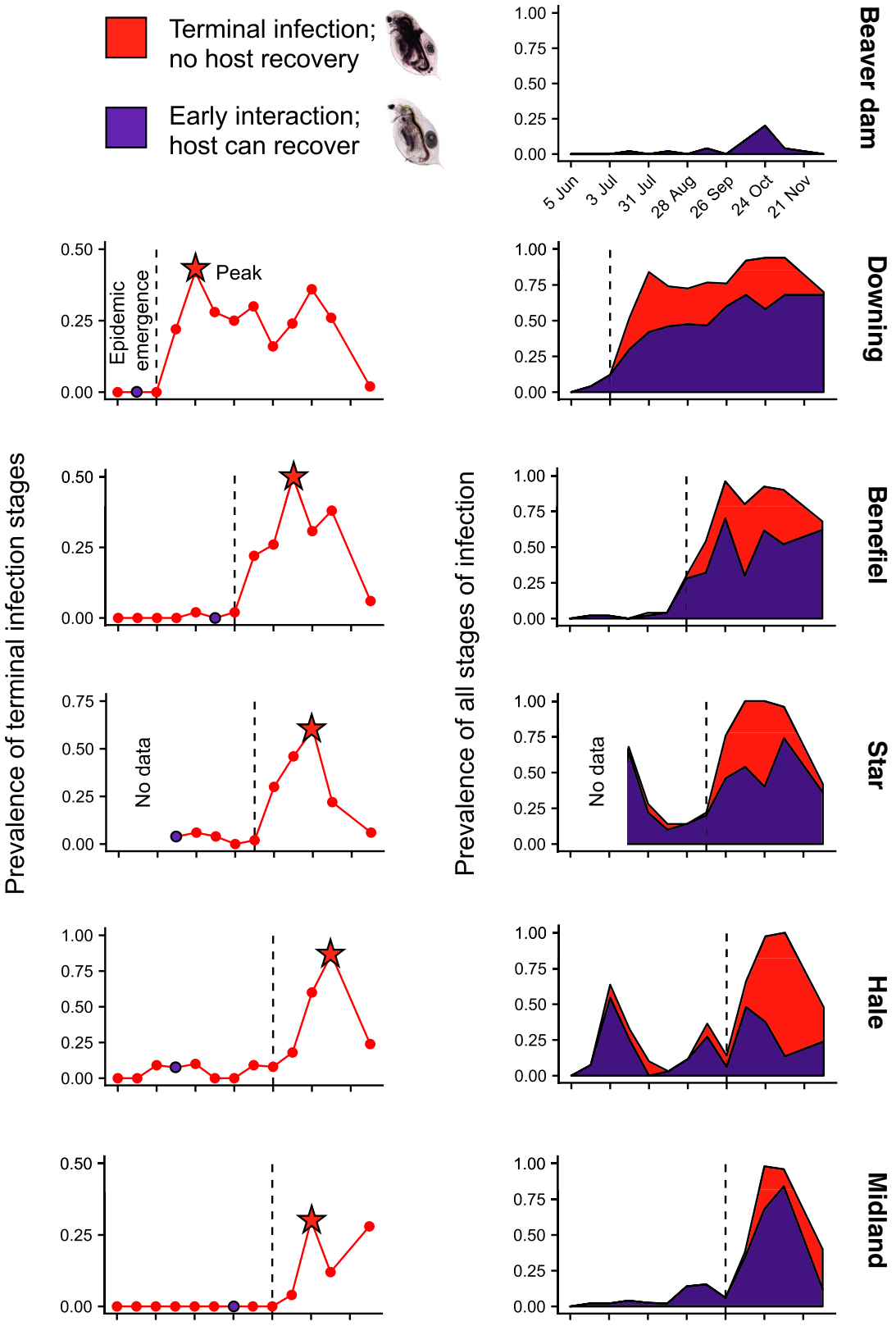
Terminal infection; no host recovery
Early interaction; host can recover
Prevalence of terminal infection stages
Prevalence of all stages of infection
Epidemic emergence
Peak
No data
No data
5 Jun
3 Jul
31 Jul
28 Aug
26 Sep
24 Oct
21 Nov
Beaver dam
Downing
Benefiel
Star
Hale
Midland

FIG. 3. Testing drivers of epidemics with infection states. Qualitative evidence that parasite exposure alone does not explain epidemic emergence in the *Daphnia–Metschnikowia* system. In the left column: epidemic emergence (vertical dashed line) happens when terminal infection prevalence (red line) begins to increase to its peak (maximal) value, while exceeding background rates. Purple points represent bottlenecks for each lake (see Appendix S1: Table S2). The right column shows prevalence of early interactions from which hosts can recover (purple; spore I through sporocyst stages [SpI–SC]; Table 1, Fig. 2) and terminal infections from which hosts cannot recover (red; conidium and ascus [C and A]; Table 1, Fig. 2). Early interactions were often present without terminal infections, suggesting low susceptibility. *Metschnikowia* exposure clearly preceded emergence of epidemics (vertical dashed lines) in all lake populations (rows; note: Beaver Dam experienced exposure at low levels, but not an epidemic).

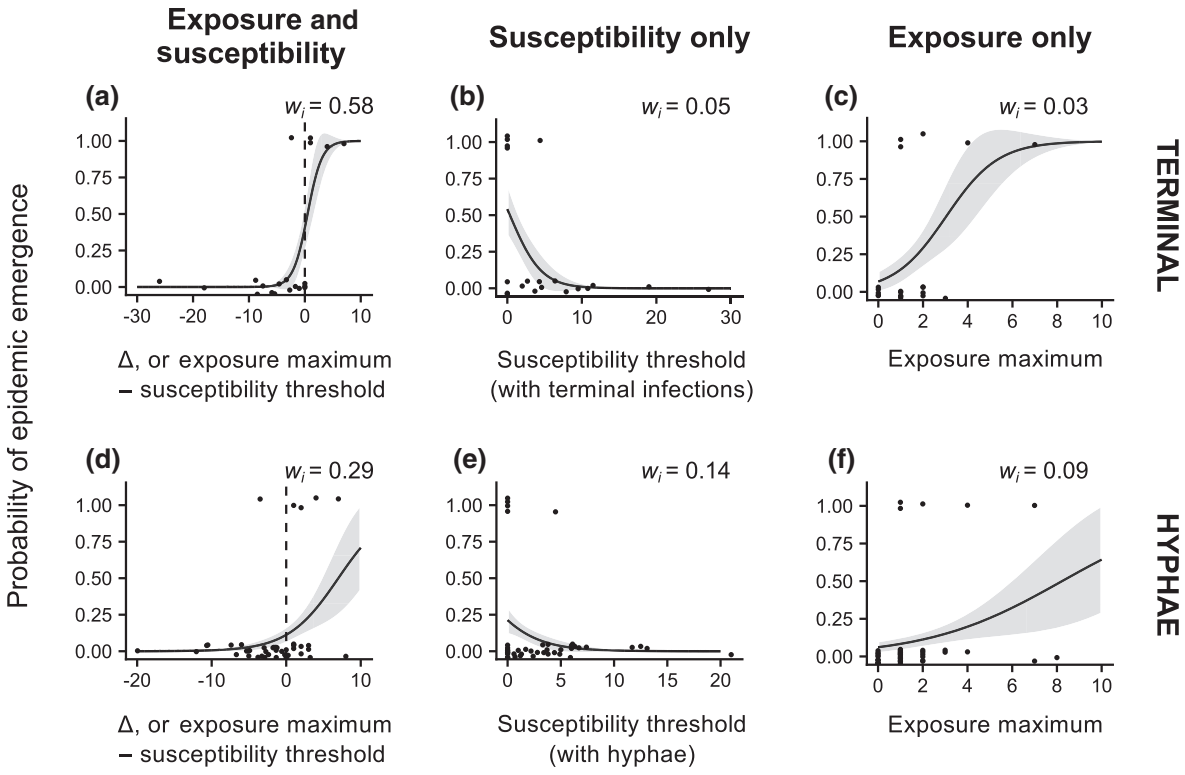

FIG. 4. Testing drivers of epidemics with mechanistic exposure and susceptibility. Plots contain raw data (points), predictions (curves), and AIC weights (w_i) from a subset of models (Appendix S1: Table S3). Panels (a)–(c) show susceptibility data estimated with terminal infections (on a monthly basis), and panels (d)–(f) show susceptibility data estimated with hyphae (twice-monthly basis). In both data sets, the combination of *Metschnikowia* exposure and *Daphnia* susceptibility (exposure maximum − susceptibility threshold) best predicted epidemics [(a) and (d)]. Positive Δ means exposure exceeded susceptibility thresholds. Higher susceptibility thresholds (spores required to produce infection) decreased the likelihood of epidemics but was a relatively weak predictor (b) and (e). Higher exposure (maximum count of attacking spores) increased likelihood of epidemics but was also a weak predictor (c) and (f). Each point represents a unique lake-by-date sample from pre-epidemic to epidemic emergence (vertical lines in Fig. 3). Vertical jitter added for visualization. Gray shading represents standard error.

infection), we determined that bottlenecks occur inside the host, likely because of internal clearance by hemocytes. Therefore, changes in susceptibility can create or remove transmission bottlenecks, governing the success and timing of epidemics.

Disease (the pathogenic manifestation of an infection) was just the tip of the iceberg in terms of actual infection. By observing early, less conspicuous infections, we documented higher prevalences and earlier appearances of *Metschnikowia* than past estimates (Cáceres et al. 2006, Penczykowski et al. 2014). Moreover, we found that every host could be infected during epidemics, highlighting how observable disease can vastly underestimate parasite abundance. Similar underestimates have been suspected in systems that target only patent stages of infection (Stewart et al. 2018). In invertebrate vectors, for instance, prevalence of infective stages is typically lower than expected based on biting rates (Sloan and Ligoxygakis 2017). Likewise, exposure–disease regressions in invertebrate–helminth systems commonly possess considerable unexplained variation (Smith 2007, Thieltges 2007). Our results suggest that these

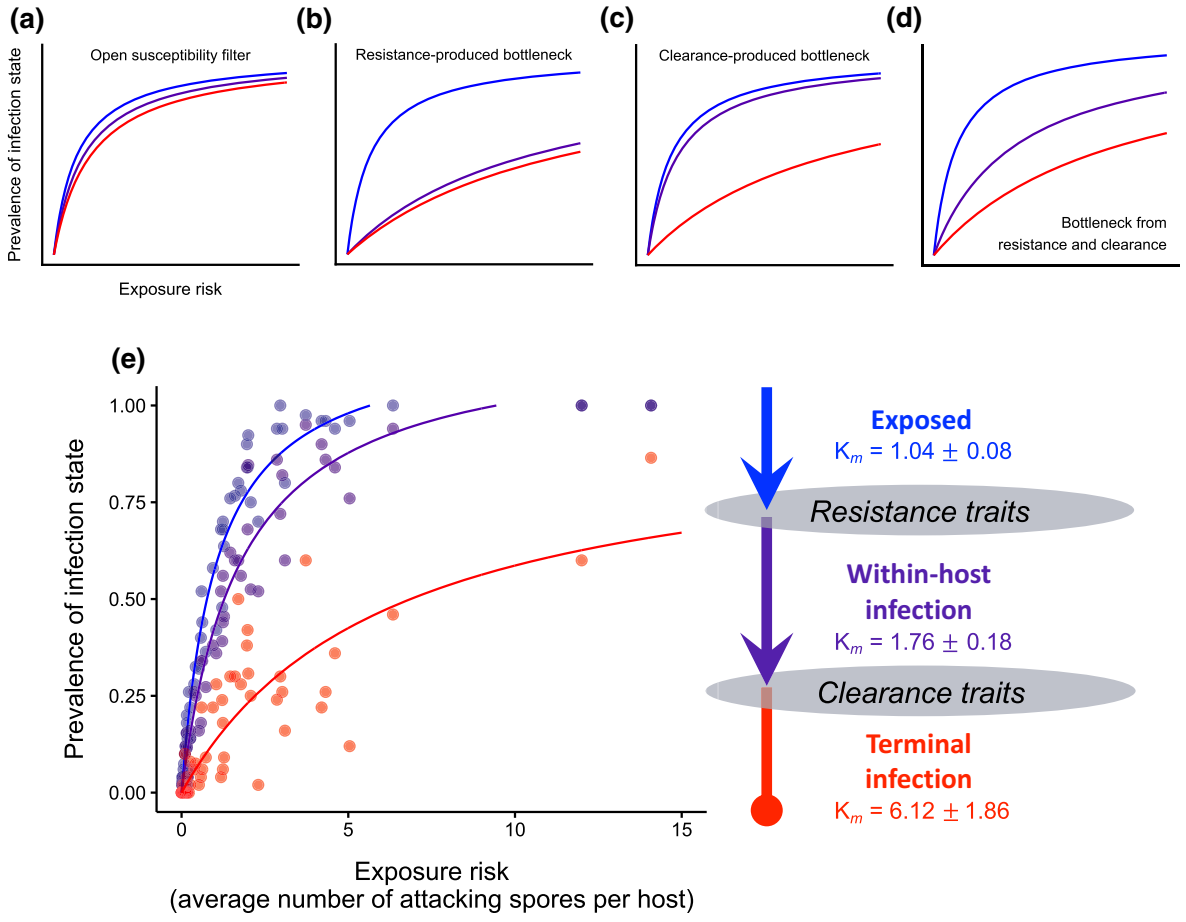

FIG. 5. Identifying transmission bottlenecks. Evaluating how exposure risk (average attacking spores per host) relates to prevalence of progressing infection stages reveals the step of infection at which the susceptibility filter is closed. Theoretical predictions are plotted for (a) whether the susceptibility filter is open or (b) whether a transmission bottleneck is produced by barrier resistance, (c) internal clearance, or (d) both. In (e) empirical data, each point represents a unique lake-by-date sample. Blue–purple: Exposure risk results in similar prevalences of exposure (blue; Table 1) and within-host infection (purple; Table 1). Similarity of these curves suggests that host barrier resistance does not produce a bottleneck. Purple–red: A bottleneck occurs as individuals move from within-host infections to terminal infections (red; Table 1). The large gap between these curves indicates that the same levels of exposure result in far fewer terminal infections than within-host infections. Hence, host internal clearance may be closing the susceptibility filter.

underestimates may stem from undetected early infections or transmission bottlenecks and reinforce that disease prevalence is not indicative of parasite exposure. Early infections are often underappreciated in field studies but have much to offer in disease ecology. Early developmental stages represent a marker for host exposure (a notoriously difficult factor to measure; McCallum et al. 2017) and can be leveraged to detect transmission bottlenecks. By comparing prevalence of exposure to prevalence of terminal infections, we identified points in time at which the susceptibility filter appeared closed, which then served as targets for narrowing in on host traits that regulate parasites.

The repeated emergence of pandemics in humans and wildlife is a stark reminder that it is only a matter of time before a novel pathogen will spread. Our results support the idea that epidemics may be largely inevitable (as we observed them in 5 of 6 lakes) but can be significantly delayed by asynchronous exposure and susceptibility filters. With our mechanistic assays, we observed several instances where *Metschnikowia* exposure was high or where *Daphnia* susceptibility was high, but epidemics failed to emerge. In other words, the asynchronous openings and closures of both exposure and susceptibility filters routinely inhibited epidemics. Only in a small subset of time points, when both processes were matched (indicated with Δ), could an epidemic take hold. Key to interpreting this finding is the linking of exposure and susceptibility through the exposure-response curve. One particular level of exposure did not necessarily produce consistent outcomes; it could trigger an epidemic in one lake, but not in another. The determining factor was then each lake's

susceptibility threshold, which established whether the level of exposure could result in infection and transmission.

The mutual dependence of epidemics on exposure and susceptibility supports Combes' (2001) conceptual paradigm, as well as assumptions of epidemiological models, in which transmission (β) is a function of an exposure parameter (f) and susceptibility parameter (μ; Bertram et al. 2013). But although conceptual and theoretical models make clear the importance of exposure and susceptibility for disease, these two processes are rarely investigated jointly. Recent and notable exceptions include Gibson et al. (2016), which used exposure and susceptibility of *Potamopyrgus* snails to *Microphallus* trematodes to explain spatial variation in trematode prevalence. In mesocosms with controlled exposure, Strauss et al. (2018) demonstrated that *Daphnia* susceptibility directly fuels large *Metschnikowia* epidemics. Finally, by tracking chytrid zoospores and amphibian susceptibility to chytrid, Voyles et al. (2018) demonstrated how increased host resistance enabled the rebound of Central American frog populations. Our study adds to this growing body of empirical research and reaffirms that parasites and the disease they cause are regulated by both environmental and within-host processes.

Disentangling epidemiological mechanisms to reveal their relative strengths is a new frontier in disease ecology (Luis et al. 2018, Rohr et al. 2019). Although our study has demonstrated that both exposure and susceptibility are required for epidemics, future work could go beyond their simple pairing and explore the strength of each process across diverse host–parasite systems. In some systems, one process alone might more strongly regulate epidemics if it is in flux, and the other process remains stable and homogeneous. For instance, if susceptibility is fixed and constant, but exposure changes through time, then exposure may be the critical factor that determines whether or not disease emerges. We should therefore ask: in what proportion of systems is parasite exposure stable versus fluctuating? And do host species more generally exhibit fixed or shifting levels of susceptibility? Developing creative metrics for exposure and susceptibility is key. Fortunately, there is a growing menu of empirical methods that can be leveraged to quantify exposure (e.g., antibody tracking, eDNA, and radio frequency identification; Huver et al. 2015, Manlove et al. 2017), as well as new computational methods that can estimate susceptibility traits and additional epidemiological parameters from host data (Borremans et al. 2016, Plowright et al. 2016, Stewart Merrill and Johnson 2020). These mechanistic tools will be powerful for extending the exposure–susceptibility framework to systems less tractable than *Daphnia*.

Exposure varied among lakes and through time, but also among host individuals. The exposure maximum was, on average, two spores greater than exposure risk before epidemics started, indicating uneven distribution of exposure within populations (range of difference: 0.85–7.82 spores [Appendix S1: Table S2]). Theoretical models predict that exposure heterogeneity can have strong consequences for parasite transmission (Woolhouse et al. 1997). Specifically, populations with highly aggregated exposure are predicted to experience rarer, but more explosive, outbreaks (Lloyd-Smith et al. 2005). Although degree of aggregation was beyond the scope of our analyses, we did find differences in predictive power among exposure metrics. Models that incorporated the exposure maximum were generally more competitive in predicting epidemics than those with exposure intensity (average among exposed individuals) or exposure risk (average among all individuals, exposed and unexposed). The consistent higher ranking of exposure maximum suggests that a few heavily exposed individuals can trigger epidemics, by pushing their level of exposure beyond a population's corresponding susceptibility threshold.

Looking into the within-host environment, we found strong connections between host susceptibility traits and epidemic onset. Susceptibility traits involve those that inhibit or promote an individual infection, and recent work in ecological immunology has suggested that such traits may contribute to parasite dynamics (Hawley and Altizer 2011, Becker et al. 2019). In support of this hypothesis, Halliday et al. (2018) provided evidence that plant defenses (immune signaling hormones) regulate transmission of an aggressive fungal pathogen. In prior work, we found that *Daphnia* barrier resistance and internal clearance traits acted in concert to explain individual infections (Stewart Merrill et al. 2019). We wondered: do these same traits scale up to influence epidemics? In the field, prevalence of terminal infections was greatly decoupled from attacking spores, suggesting that within-host processes were inhibiting *Metschnikowia* development and transmission. In accordance with this finding, our *Daphnia* internal clearance trait (hemocytes) was high during transmission bottlenecks (when *Metschnikowia* was not spreading) but declined at epidemic emergence (when *Metschnikowia* was spreading rapidly). Taken together, these findings suggest that host immunological defenses are responsible for the observed shifts in population-level susceptibility. The internal clearance trait we measured, defense by hemocytes, encompasses the recognition, location, and attack of spores infecting the body cavity. Variation in this trait could therefore emerge from differences in a suite of immunological factors, including surveillance proteins, upstream regulators of hemocyte production, and the hemocytes themselves (Loker et al. 2004). Further research into the basis and extent of this variation should illuminate the environmental contexts under which we expect susceptibility to increase.

Our results provide compelling empirical evidence that disease represents only a subset of parasite invasion attempts and that it is the combination of host susceptibility and parasite exposure that together dictate epidemic emergence. Additional study of whether changes

to exposure and susceptibility are predictable or stochastic may allow us to identify the environmental conditions under which these key processes align.

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Supporting Information

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.3245/suppinfo

Data Availability Statement

Data are available from the Dryad Digital Repository https://doi.org/10.5061/dryad.v15dv41ts (Stewart Merrill et al. 2021).