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Linking host traits, interactions with competitors and disease: Mechanistic foundations for disease dilution

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Abstract

1. The size of disease epidemics remains difficult to predict, especially when parasites interact with multiple species. Traits of focal hosts like susceptibility could directly predict epidemic size, while other traits including competitive ability might shape it indirectly in communities with a "dilution effect."
2. In a dilution effect, diluter taxa can reduce disease by regulating (lowering) the density of focal hosts (i.e. through competition) or by reducing encounters between focal hosts and parasites. However, these dilution mechanisms are rarely grounded in focal host traits, and the relative importance of host regulation vs. encounter reduction remains understudied.
3. Here, we map focal host traits to disease—via these dilution mechanisms—in communities with diluters. We measured two traits (competitive ability and susceptibility) for eight genotypes of a focal host (*Daphnia*), tracked the densities of each genotype in experimental mesocosms (+/− *Ceriodaphnia* competitor/diluters) and monitored their infections with a virulent fungal parasite (*Metschnikowia*) over 6–8 host generations. We disentangled the impacts of both traits on the density of infected hosts and partitioned dilution mechanisms using path models.
4. Higher susceptibility directly fuelled larger epidemics. Simultaneously, weaker competitive ability indirectly suppressed epidemics by enabling higher densities of diluters. These higher densities of diluters reduced the density of infected hosts indirectly via host regulation. In contrast, encounter reduction was much weaker.
5. Our experiment strengthens the dilution effect paradigm with a predictable, traits-oriented framework. Similar traits—susceptibility, competitive ability and their covariance—could help predict epidemic severity in a variety of other systems. Partitioning the direct and indirect effects of diluters could also delineate how they impact disease. Such trait-based insights could help broadly predict the size of epidemics in diverse communities.

KEYWORDS

Daphnia, density of infected hosts, dilution effect, encounter reduction, host regulation, host traits, intraspecific variation, path analysis

1 | INTRODUCTION

What makes disease epidemics smaller or larger? Disease theory indicates that, among other factors, traits of hosts can directly influence epidemic size (Anderson & May, 1981; Dwyer & Elkinton, 1993; Strauss, Civitello, Cáceres, & Hall, 2015). One obvious trait is susceptibility: the rate at which susceptible hosts become infected upon contact with parasite propagules, vectors or infected hosts. More resistant hosts should experience smaller epidemics, while more susceptible hosts should experience larger ones (Dwyer & Elkinton, 1993; Strauss et al., 2015). However, species interactions, like competition and predation, can also influence epidemics (Keesing, Holt, & Ostfeld, 2006; Strauss et al., 2016). Other traits like competitive ability may modulate the strength of these interactions, and hence indirectly shape disease (e.g. Strauss et al., 2015). Thus, multiple traits can govern epidemics in a community context, though both direct and indirect pathways.

Mechanistic dilution effect theory could help predict these community-level impacts of host traits on epidemic size. Dilution effects arise broadly (Civitello et al., 2015) when resistant "diluter" taxa interfere with transmission among more competent focal hosts (Ostfeld & Keesing, 2000), frequently via one or two mechanisms. First, diluters can *regulate* the density of focal hosts via predation or competition (Keesing et al., 2006), thus inhibiting direct or environmental transmission (Anderson & May, 1981). These diluters indirectly shape disease by decreasing the density of focal hosts. Whether such indirect effects constitute a dilution effect in the strict sense seems beside the point (but see Begon, 2008). Second, diluters might *reduce encounters* between focal hosts and parasites by diverting vectors away from focal hosts (Ostfeld & Keesing, 2000), modifying focal host behaviour, or consuming free-living parasites (Johnson et al., 2010). Trait-based insights into either of these general mechanisms could help broadly predict when diluters should exert the strongest impacts on disease.

Presently, such predictive power remains limited because few experiments link gradients of focal host traits to dilution mechanisms. Intuitively, host regulation might matter more when predation (Rohr et al., 2015) or competition (Strauss et al., 2015) depresses focal host densities more strongly. Encounter reduction appears stronger when diluters remove parasites more rapidly and strongly resist infection (Venesky, Liu, Sauer, & Rohr, 2014; but see Wojdak, Edman, Wyderko, Zemmer, & Belden, 2014). Yet, intraspecific variation in susceptibility among focal hosts may counter either dilution mechanism by fuelling uncontrollably large or inconsequentially small epidemics (Strauss et al., 2015). Thus, traits of focal hosts matter as well. Furthermore, impacts of multiple focal host traits could easily become confounded. For example, when susceptibility directly fuels epidemics, it could obscure how traits like competitive ability—which frequently covary with susceptibility (Duncan, Fellous, & Kaltz, 2011)—modulate the impacts of diluters. Therefore, stronger mechanistic foundations for disease dilution require experiments that disentangle the impacts of covarying focal host traits.

Drivers of epidemics in multi-host communities become even harder to delineate when host regulation and encounter reduction operate simultaneously (e.g. Dallas, Hall, & Drake, 2016; Ogden & Tsao, 2009; Rohr et al., 2015; Strauss et al., 2016). Dilution theory rarely embraces this challenge; yet hosts and diluters that encounter the same parasites also frequently compete. We label this combination of encounter reduction and competitive host regulation "friendly competition" (Hall et al., 2009). Examples likely include the transmission of hantavirus (Clay, Lehmer, Jeor, & Dearing, 2009), Lyme (Ogden & Tsao, 2009), *Schistosoma* (Johnson, Lund, Hartson, & Yoshino, 2009), and parasites in intertidal (Thieltges, Reise, Prinz, & Jensen, 2009), amphibian (Johnson, Preston, Hoverman, & Richgels, 2013) and plant communities (Lacroix et al., 2014; Mitchell, Tilman, & Groth, 2002). In friendly competition, impacts of diluters—hereafter, competitor/diluters—likely depend on the competitive ability of focal hosts (Strauss et al., 2015). Competitor/diluters could become rare if focal hosts compete strongly, but remain numerous if focal hosts compete weakly. High densities of competitor/diluters could reduce disease via host regulation, encounter reduction or both. However, the relative strength of these dilution mechanisms remains understudied (but see Ogden & Tsao, 2009).

Here, we disentangle the impacts of covarying focal host traits and partition the dilution mechanisms operating in a multigenerational mesocosm experiment. A two-host planktonic example provides tractability and captures the natural history of our study system (see Strauss et al., 2016). First, we picked eight clonal genotypes of the focal host (*Daphnia dentifera*) to establish gradients of two correlated traits: susceptibility and competitive ability. Then, we created epidemics of a virulent fungus *Metschnikowia bicuspidata* in mesocosms with and without a key competitor/diluter (*Ceriodaphnia* sp.). Finally, we combined linear and path models to map host traits via dilution mechanisms to disease. Although we compare two metrics of epidemic size—the density of infected hosts and infection prevalence—we focus on the former since it responded more clearly to diluters. Higher susceptibility directly fuelled larger epidemics. Simultaneously, stronger competitive ability indirectly allowed higher densities of infected hosts, because the populations of diluters were constrained. Finally, diluters primarily reduced the density of infected hosts via host regulation. In other words, the indirect effects of competitor/diluters, via changes in focal host density, outweighed their direct effects on disease (i.e. via encounter reduction). This trait-based framework and tractable case study brings dilution theory closer to predicting the size of epidemics in multi-host communities.

2 | MATERIALS AND METHODS

2.1 | Natural history of the study system

The focal host in this study, the cladoceran *Daphnia dentifera*, dominates grazer communities in many North American freshwater lakes (Tessier & Woodruff, 2002). It frequently suffers autumnal epidemics caused by the virulent fungus *Metschnikowia bicuspidata* (Hall,

Smyth, et al., 2010; Strauss et al., 2016). Focal hosts consume infectious fungal spores while foraging (Hall et al., 2007) but vary in their susceptibility to infection (Hall, Becker, Duffy, & Cáceres, 2010). Infected hosts release spores after death. A second dominant cladoceran *Ceriodaphnia* sp., often competes (Tessier & Woodruff, 2002) and can reduce disease by regulating *Daphnia* density (Strauss et al., 2016). These competitor/diluters also consume fungal spores while foraging but strongly resist infection, hence reducing encounters between focal hosts and parasites (Strauss et al., 2015). Among a set of 28 Indiana lakes (see Strauss et al., 2016), these two competitors constitute 88% of cladoceran individuals. Although higher diversity correlated with lower disease across these lakes, this dilution effect was driven more specifically by higher frequencies of *Ceriodaphnia* in the more diverse lakes (rather than diversity *per se*). Competitive regulation appeared to reduce the density of infected hosts in these lakes, while encounter reduction lowered infection prevalence more strongly. The current experiment with two-host communities is inspired by these field patterns (Strauss et al., 2016).

2.2 | Trait measurements

We quantified indices of two important traits, susceptibility and competitive ability, for eight different genotypes of the focal host (see Appendix S1). These genotypes were selected from laboratory stocks, using limited prior information, in order to spread the range of both traits. In short, we estimated an index of susceptibility (the transmission coefficient, β) by fitting a mathematical model to infection assays (e.g. Hall et al., 2007). In these assays—replicated among genotypes—15 individuals were exposed to each of three parasite concentrations, maintained individually, and later inspected for signs of infection. Susceptibility was fit (with bootstrapped standard errors) with maximum likelihood using the BBMLE package in R (Bolker, 2008; R Core Team, 2017). This parameter (β) represents the probability of a focal host becoming infected in the absence of conspecifics or competitor/diluters, given its body length (*L*), density of infectious spores (*Z*) and the duration of spore exposure (*t*).

We also estimated an index of competitive ability, using growth rate assays with low food resources (e.g. Hall, Becker, Duffy, & Cáceres, 2012). Mass accrual of neonates during a 5–6 day juvenile period is directly proportional to fitness (Lampert & Trubetskova, 1996). In turn, competitive ability depends on fitness when resources are limiting (reviewed in Grover, 1997). Therefore, we provided hosts with low resources in our assay (0.15 mg mass/L *Ankistrodesmus falcatus* daily). We dried and weighed body mass of individuals at birth (mean $N = 9.8$ among genotypes) and other individuals 5–6 days later (mean $N = 14.5$). Then, we calculated growth rate as ln(mass accrual)/time. Thus, this index of competitive ability represents the growth rate of an individual consuming limited resources in the absence of conspecifics, infection or competitor/diluters. Although we use this index to predict interspecific competition here, it also predicts intraspecific competition (i.e. clonal selection and evolution) among *Daphnia* genotypes (Strauss et al., 2017).

These indices of susceptibility and competitive ability provided continuous gradients of two covarying focal host traits. Next, we used these trait gradients to predict outcomes among the same genotypes in a multi-generational mesocosm experiment.

2.3 | Mesocosm experiment

The mesocosm experiment crossed focal host genotype (8 levels) with the presence/absence of competitor/diluters (2 levels). All combinations of treatments were replicated four times in 75-L tanks. Details are presented in Appendix S1. Mesocosms began with focal hosts (15 L^{-1}), and in competition treatments, a single genotype of competitor/diluters (5 L^{-1}). Although competition treatments therefore began at slightly higher total densities (20 L^{-1}), the transient starting conditions impacted densities little over the following 6–8 generations. Instead, competitive ability structured the densities of focal hosts and diluters (see Section 3). After the focal host and competitor/diluter populations grew for 2 weeks, we began sampling by mixing and sieving 1 L per tank per week (80 μm mesh). After 1 week of sampling, we added fungal spores (5,000 L^{-1}) and continued sampling for 7 additional weeks (~7 host generations). Removal of infected individuals (via sampling only 1.7% of tank volume per week) likely did not impact epidemic sizes. We tracked changes in densities of focal hosts, competitor/diluters, and infected hosts using microscopes to count densities and diagnose infections (50×). Only 4 of 6,375 competitor/diluters examined were infected (0.06%), confirming their high resistance.

2.4 | Statistics—Linear models

For all models, we averaged time series for each tank over the 8-week (6–8 host generations) duration. Even if it obscured complex temporal signals of competition or disease transmission, this averaging enabled synthesis of traits, dilution mechanisms and disease metrics. Mean infection prevalence was calculated as the total number of infections summed across all weeks divided by the total number of hosts sampled during the experiment (rather than the temporal mean of prevalences calculated each week). This method reduced sampling error on prevalence due to extremely low host densities when focal hosts were out-competed by diluters.

Univariate linear models linked trait indices to mesocosm dynamics. Because several patterns exhibited pronounced heteroscedasticity (e.g. see Figure 3a), we fit the linear models with generalized least squares (GLS). With GLS, we included an additional parameter to allow variance to change with the independent variable, if it improved model fit via likelihood ratio test. These GLS models were implemented using the NLME package in R (Pinheiro & Bates, 2000). When focal host traits served as independent variables, we also fit complementary mixed models (also using NLME) that assigned random intercepts to each focal host genotype (see Appendix S1).

Two sets of linear models evaluated specific linkages between host traits and disease or mean densities. The first set tested whether susceptibility (β) directly predicted variation in epidemic

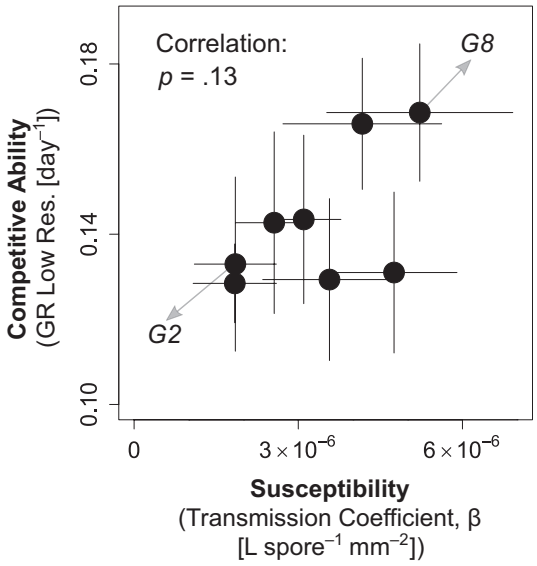

FIGURE 1 Two key traits covary among eight focal host genotypes. Susceptibility is indexed by a transmission coefficient (β; measured with infection assays). Growth rate of juveniles on low resources represents an index of competitive ability. The traits covary positively but non-significantly (p = .13). However, both traits and their covariation become foundations for linear (Figures 2 and 3) and path models (Figures 4 and 5). Genotypes are named according to variation in susceptibility (along x axis; Figures S1–S3 in Appendix S1 present each genotype's time series in the mesocosm experiment). Error bars are bootstrapped standard errors

size (i.e. mean density or prevalence of infected hosts). It also evaluated whether presence of competitor/diluters (denoted C) modulated these relationships (as $\beta \times C$ interactions). The second set of models mapped competitive ability of focal hosts to the density of competitor/diluters, linked densities of diluters and focal hosts, and evaluated how each density impacted each metric of disease. In other words, this second suite of models mapped the indirect effect of competitive ability on disease, mediated through potential dilution mechanisms.

All significant relationships between traits, mean densities and metrics of disease then became the scaffolding for path models. Because we detected strong impacts competitor/diluters on the density but not prevalence of infections (see Section 3.1), we focus our path models on the density of infected hosts.

2.5 | Statistics—Path models

While the univariate models facilitated a close inspection of each relationship (see Figures 2 and 3), they also raised two specific questions better suited for path analysis. First, susceptibility and competitive ability covaried, and univariate models suggested that both traits might shape the density of infected hosts. Were both traits actually important, or was one relationship merely a correlational shadow, masked by the other? Path analysis accounted for the covariation between traits and disentangled their simultaneous impacts on disease. Second, did diluters shape disease more strongly through host regulation or encounter reduction? Path analysis partitioned these dilution mechanisms by evaluating the direct vs. indirect pathways between the densities of competitor/diluters and infected hosts. We interpreted host regulation as the indirect effects of diluters on infected hosts, mediated by changes in the density of focal hosts (i.e. via competition). In contrast, we interpreted encounter reduction as the direct effects of diluters on infected hosts (not mediated by the density of focal hosts).

We fit hierarchical path models using the LAVAAN package in R (Rosseel, 2012) and a maximum likelihood estimator (MLM) that was robust to non-normal standard errors. Mesocosm tank served as the unit of replication (n = 64). However, the trait measurements were replicated by focal host genotype (n = 8). Therefore, we specified a two-level hierarchical structure with the LAVAAN survey package (Oberski, 2014). Unfortunately, collinearity among parameters prevented the fit of a comprehensive model that included both traits, density of focal hosts and density of diluters. This undesirable collinearity likely arose due to the covariation among traits and the "small" sample size at the genotype level of replication (n = 8). Given this constraint, we fit two complementary hierarchical models. The first model (which excluded the density of focal hosts) disentangled the impacts of each trait on disease. The second model included only one trait (susceptibility) but partitioned the strength of indirect host regulation vs. direct encounter reduction. Tables S2–S4 in Appendix S1 present model fit statistics and all parameter estimates.

3 | RESULTS

Focal hosts varied in both traits (Figure 1). Susceptibility, β, ranged $1.8–5.2 \times 10^{-6}$ (L spore^{-1} mm^{-2}) among the eight genotypes. Hereafter, we rank genotypes by this trait (i.e. the genotype with lowest susceptibility becomes "G1"). The second trait, juvenile growth rate on low resources (the index of competitive ability), ranged 0.13–0.17 (day^{-1}). These traits covaried positively but non-significantly (Pearson's P = .13). Nevertheless, this covariance became an essential link in the path models. Focal host genotypes also drove divergent outcomes in mesocosms. Appendix S1 presents time series for each genotype: G2 and G8 as illustrative examples (Figure S1), G1, G3 and G4 (Figure S2), and G5, G6 and G7 (Figure S3). However, rather than focus on each genotype individually here, we summarize their mean responses along continuous gradients of their traits.

3.1 | Linear model results

Variation in susceptibility shaped the size of epidemics (Figure 2). Higher susceptibility fuelled both higher mean densities of infected hosts (β effect, p = .0046; Figure 2a) and higher infection prevalence (β effect: p = .0008; Figure 2b). The mere presence of competitor/diluters did not effect either metric of epidemic size via main effect or interaction (all p > .2).

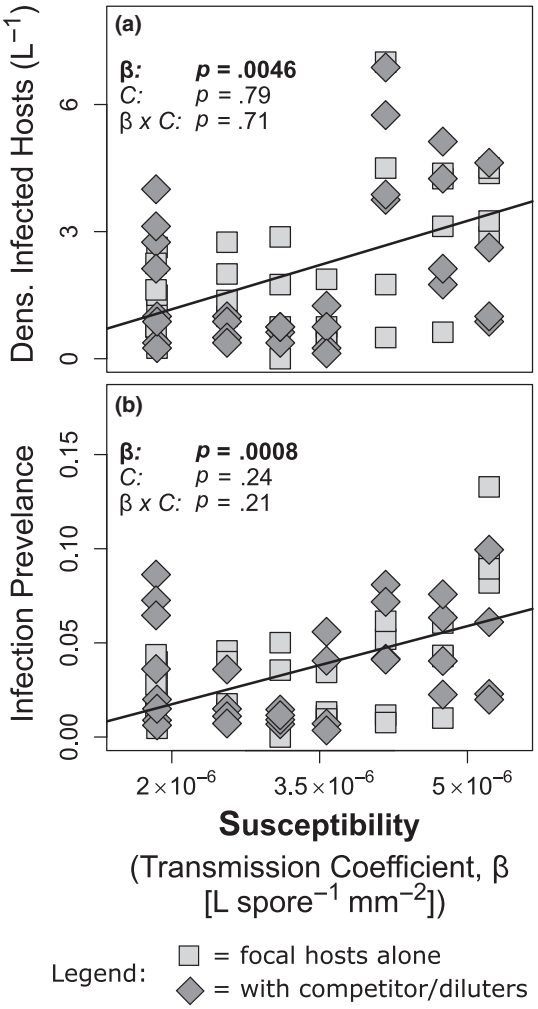

FIGURE 2 Variation in susceptibility predicts the size of epidemics. Points are temporal averages for each mesocosm tank. Higher susceptibility fuels both (a) higher mean densities of infected hosts and (b) higher mean infection prevalence (β effects; solid lines). Neither metric of epidemic size is effected by the mere presence of competitor/diluters (C), or its interaction with susceptibility (β × C). P values are fits of linear models. Key: squares = focal hosts alone; diamonds = with competitor/diluters

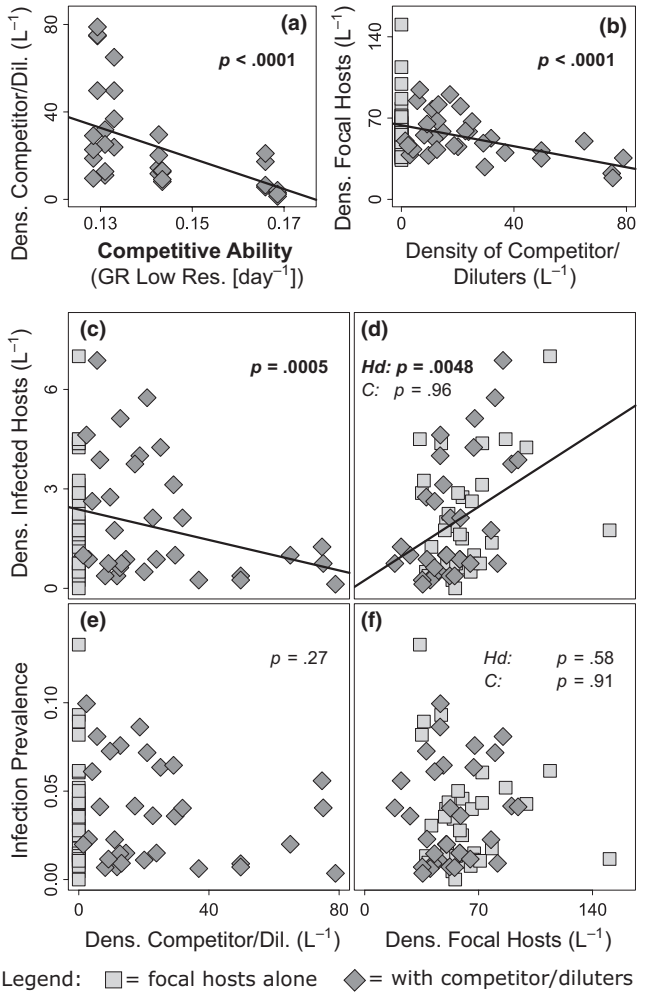

FIGURE 3 Variation in competitive ability structures the densities of diluters and focal hosts, and both correlate with the density of infected hosts. (a) Genotypes of focal hosts with higher competitive abilities constrain competitor/diluters to lower densities. (b) Higher densities of diluters reduce the density of focal hosts. In turn, the density of infected hosts is both c) lowered by higher densities of competitor/diluters and (d) elevated by higher densities of focal hosts. In contrast, infection prevalence is sensitive to neither densities of (e) competitor/diluters nor (f) focal hosts. *p* values are fits of linear models. Key: *C* = presence of competitor/diluters; *Hd* = density of focal hosts; squares = focal hosts alone; diamonds = with competitor/diluters

Competitive ability of focal hosts—the second trait—governed diluter densities and hence potential dilution mechanisms (Figure 3). Strongly competing focal hosts constrained competitor/diluters to lower mean densities ($p < .0001$; Figure 3a). In turn, higher densities of competitor/diluters regulated densities of focal hosts ($p = .0011$; Figure 3b; this test includes tanks without any diluters). However, densities of focal hosts and competitor/diluters only significantly impacted one metric of disease. The mean density of infected hosts appeared to be reduced by higher densities of competitor/diluters ($p = .0005$; Figure 3c) and elevated by higher densities of focal hosts (*Hd* effect: $p = .0048$; Figure 3d). A path model distils the causal structure underlying this result below. In contrast, infection prevalence was not significantly impacted by the density of competitor/diluters ($p = .27$; Figure 3e) or focal hosts (*Hd* effect: $p = .58$; Figure 3f). The presence of diluters (included as a covariate with focal host density) was not a significant predictor for either metric of disease (both $p > .9$). Analyses using the density of focal hosts from week 2 only (when spores were added) mirrored all of these results (see Figure S4 in Appendix S1).

3.2 | Path model results

Both path models fit well (see Appendix S1 for diagnostic statistics and parameter estimates). The first model disentangled the impacts of susceptibility and competitive ability on the density of infected hosts (Figure 4). The traits covaried positively but not significantly ($p = .14$). Nevertheless, each trait shaped disease through a unique

pathway. Higher susceptibility directly elevated disease (p = .004). In contrast, higher competitive abilities indirectly increased disease by constraining the density of competitor/diluters (p = .015). In turn, higher densities of diluters reduced the density of infected hosts (p = .006). Thus, diluters impacted disease more strongly when focal hosts competed weakly, because diluters were more numerous.

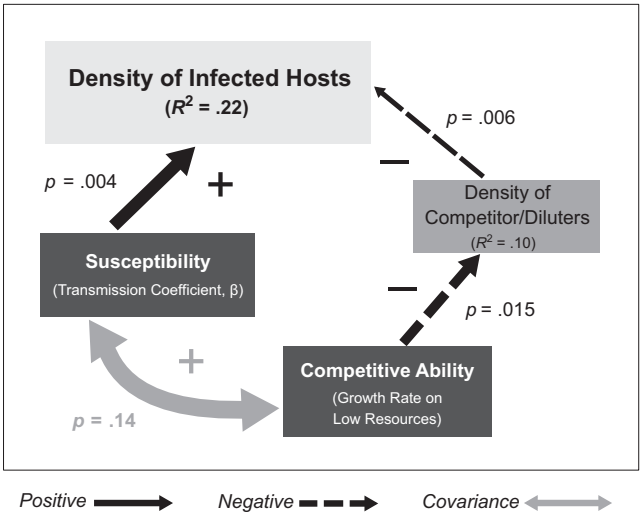

FIGURE 4 Both covarying focal host traits simultaneously govern the density of infected hosts. Higher susceptibility fuels larger epidemics directly (see Figure 2a). In contrast, stronger competitive ability enables epidemics indirectly by limiting the density of diluters (see Figure 3a). In turn, higher densities of diluters reduce the density of infected hosts. These impacts of diluters could be due to host regulation, encounter reduction, or both (partitioned in Figure 5). Key: solid = positive coefficients; dashed = negative coefficients; two-headed arrow = covariance between traits; arrow weights = standardized effect sizes

The second path model partitioned host regulation vs. encounter reduction for the dilution of the density of infected hosts (Figure 5). Intraspecific variation in susceptibility still strongly impacted the size of epidemics (p = .004). Additionally, higher total densities of focal hosts led to higher densities of infections (p < .001). However, higher densities of competitor/diluters did not directly lead to a lower density of infected hosts (p = .37). This weak effect may seem surprising, since it appeared significant when tested univariately (see Figure 3c). Instead, in this path model, higher densities of competitor/diluters suppressed densities of focal hosts (p = .002), which in turn lowered disease. This causal pathway defines host regulation. Using standardized effect sizes, this indirect effect accounted for 71% of the total effect of diluters on disease. In contrast, the direct effect, i.e. encounter reduction, accounted for only 29%. In other words, the impacts of diluters consuming shared resources (i.e. competition) proved much stronger than the impacts of diluters consuming parasites.

4 | DISCUSSION

Predicting the size of epidemics remains a central challenge in disease ecology. Host traits like susceptibility can directly fuel epidemics. However, other traits—including competitive ability—may govern epidemic size when other "diluter" taxa can reduce disease. Here, we evaluated a mechanistic, trait-based framework for "friendly

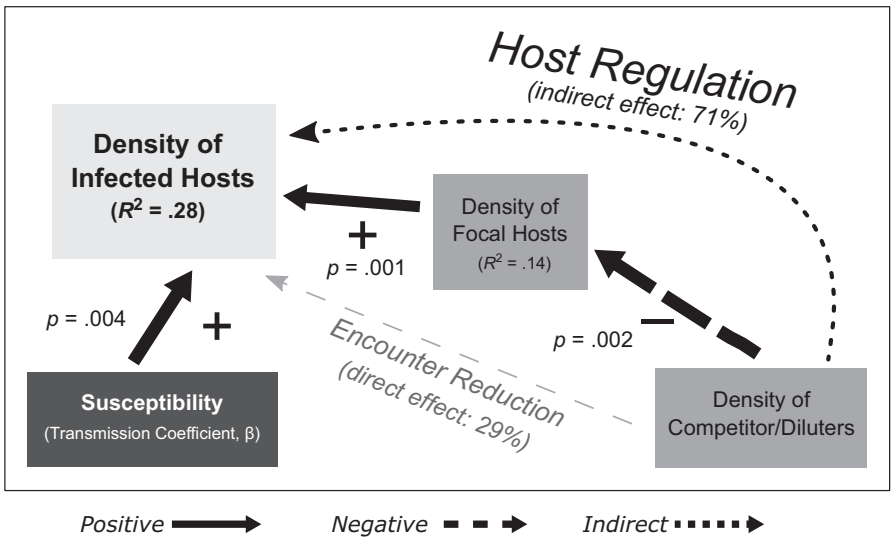

FIGURE 5 Partitioning two dilution mechanisms: Does host regulation or encounter reduction reduce the density of infected hosts? Higher total densities of focal hosts lead to a higher density of infected hosts (plotted in Figure 3d). However, higher densities of competitor/diluters did not directly lead to a lower density of infected hosts (despite the apparent relationship in Figure 3c). This direct effect (encounter reduction) explained a relatively small proportion (29%) of the net effect of diluters on disease. Instead, higher densities of competitor/diluters suppressed densities of focal hosts, which in turn lowered disease. This indirect effect (host regulation) explained the majority (71%) of the impact of diluters on disease. In addition to this dilution effect, variation in susceptibility remained an important driver of epidemic size. Key: solid = positive coefficients; dashed = negative coefficients; dotted = indirect effect; arrow weights = standardized effect sizes

competition," a form of local disease dilution combining competitive host regulation and encounter reduction. We measured susceptibility and competitive ability for eight focal host genotypes. Then we challenged each genotype with experimental epidemics, with and without diluters, in multi-generational mesocosms. Finally, we disentangled the impacts of covarying traits and partitioned host regulation vs. encounter reduction using path models. Higher susceptibility directly fuelled larger epidemics, both in terms of the density and prevalence of infections. Infection prevalence did not respond significantly to diluters. However, higher densities of diluters strongly reduced the density of infected hosts. Competitive ability—the second trait—indirectly shaped this metric of disease by governing the density of diluters. Finally, diluters reduced the density of infected hosts primarily via host regulation. In other words, their indirect effects on disease (mediated by changes in focal host density) outweighed their direct effects. This traits-based framework strengthens mechanistic foundations for dilution effects and brings us closer to predicting the size of epidemics in diverse communities.

Intraspecific variation in susceptibility strongly shaped epidemic size—both the density and prevalence of infections. Though seemingly obvious, few empirical examples link individually measured traits like susceptibility to epidemic size at the population-level (but see Dwyer & Elkinton, 1993; Strauss et al., 2015). In this plankton system, clonal variation in susceptibility of the focal host enabled such a test. Infection prevalence responded clearly to variation in susceptibility, but not the density of diluters. In contrast, the density of infected hosts responded to both. Yet in the final path model, susceptibility exerted a larger standardized effect on the density of infected host than the net effect of competitor/diluters. Thus, variation in susceptibility of focal hosts remained essential for predicting the size of epidemics, even in communities with diluters. Previous trait-based frameworks for disease dilution have focused almost exclusively on inter- (rather than intra-) specific variation in susceptibility (but see Pulkkinen, 2007; Strauss et al., 2015). Such interspecific differences are essential for identifying key diluter taxa (e.g. Johnson et al., 2013; Lacroix et al., 2014; LoGiudice, Ostfeld, Schmidt, & Keesing, 2003). However, as illustrated here, intraspecific variation in susceptibility can exert even stronger impacts on disease than presence of key diluters. Furthermore, traits like susceptibility frequently evolve during epidemics (Penczykowski, Forde, & Duffy, 2011). Thus, future theory should further explore the impacts of intraspecific variation on the community ecology of disease, especially when relevant host traits evolve (Decaestecker, De Gersem, Michalakis, & Raeymaekers, 2013; Strauss et al., 2017).

The second trait—competitive ability—directly governed host density and indirectly governed disease via host regulation. Both of these impacts manifested along a continuous trait gradient and 6-8 generations of multi-species feedbacks. Specifically, competitor/diluters constrained the density of weakly competing focal hosts, thereby indirectly lowering the density of infections (see Begon, 2008). However, these weakly competing focal hosts were driven extinct in some tanks. From the perspective of the focal host, this risk of extinction emphasizes a darker side of competition during epidemics (see also Dallas et al., 2016). Moreover, because diluters impacted disease primarily through host regulation (rather than encounter reduction), the dilution effect here was tightly linked to the density cost of competition. Both consequences of competition—disease dilution and risk of extinction—may frequently remain undetected in shorter experiments. However, among experiments that last multiple generations, competitive host regulation frequently becomes a dominant driver of disease (Dallas et al., 2016; Johnson, Preston, et al., 2012; Mitchell et al., 2002). Thus, long-term, trait-based perspectives on competition in other systems might also anticipate dilution via host regulation and the potential density cost suffered by focal hosts.

Despite their correlation, both susceptibility and competitive ability of focal hosts influenced epidemic size independently. This biological outcome—and the statistical power of path analysis which revealed it—matter because correlated traits present a general challenge for mechanistic community-disease theory. Multiple traits frequently differ interspecifically between hosts and diluters or amplifiers of disease. For example, susceptibility to trematodes and pace of life covary among amphibian taxa (Johnson, Rohr, et al., 2012); competence for Lyme and production of tick vectors covary among mammals (Randolph & Dobson, 2012); susceptibility to virus and production of aphid vectors covary among grasses (Lacroix et al., 2014); and susceptibility and encounter rates with chytrid spores covary among tadpoles (Venesky et al., 2014). When traits that promote disease correlate positively (e.g. competitive abilty and susceptibility as here; reviewed in Duncan et al., 2011), they can mask each others' potential impacts. Here, we addressed this challenge by partitioning impacts of both traits with path analysis. If important traits correlate negatively, their net impacts also challenge simple prediction, because they can pull epidemic size in opposite directions (see Randolph & Dobson, 2012). In both scenarios, community theory for disease must continue to grapple with covariation among key traits—both within and among species.

The statistical partition of variation in the second path model showed that the strength of host regulation exceeded encounter reduction. How general is this result? Here, it likely reflects the length of our experiment, metric of disease considered, and traits of diluters. As noted above, host regulation became more important than encounter reduction during other multi-generational experiments (Dallas et al., 2016; Johnson, Rohr, et al., 2012; Mitchell et al., 2002) and models (Ogden & Tsao, 2009). In contrast, shorter experiments might only allow effects of encounter reduction to manifest. Interestingly, host regulation sometimes reduces the density but not prevalence of infections (Johnson, Rohr, et al., 2012; Strauss et al., 2016). This can occur when host density correlates strongly with the density but not prevalence of infections (as it did here). In contrast, infection prevalence (which was unrelated to diluters in this experiment) can remain sensitive to encounter reduction, even when it is decoupled from host density (Strauss et al., 2016). Thus, the partition of dilution mechanisms can also depend on how strongly the chosen metric of disease scales with host density. Finally, it seems likely that certain traits

of diluters could increase the strength of encounter reduction relative to host regulation. Here, we focused on traits of focal hosts. However, the partition of dilution mechanisms could also depend on whether diluters reduce host density (Rohr et al., 2015), or how rapidly they remove parasites (Venesky et al., 2014). More partitions in other systems should test these hypotheses and delineate when host regulation vs. encounter reduction matter more.

Our trait-centred framework for friendly competition could be readily expanded. First, parallel experiments could incorporate traits of diluters (Venesky et al., 2014) or impacts of predators. Should diluters that consume parasites faster always reduce disease, or only when susceptibility of focal host falls within a certain range (Strauss et al., 2015)? When size-selective predators mediate competition between focal hosts and diluters (Strauss et al., 2016), do traits like body size become more important than "competitive ability" as measured here? Yet other traits might matter at the metacommunity scale, where much dilution effect research focuses (Johnson et al., 2013; Ostfeld & Keesing, 2000). Maintenance of diluters in a metacommunity could depend less of local competitive ability and more on dispersal ability or risk of extinction (Joseph, Mihaljevic, Orlofske, & Paull, 2013). Thus, expanding a traits-based framework for friendly competition to a metacommunity scale might predict the sizes of local epidemics and the emergence of a dilution effect across sites. Finally, eco-evolutionary perspectives could grapple with feedbacks between trait diversity in the focal host population (Decaestecker et al., 2013), trait-driven impacts on disease and dilution, and rapid evolution driven by competitor/diluters or parasites (Strauss et al., 2017). All of these expansions promise exciting frontiers.

In summary, intraspecific variation among focal host traits helped predict epidemic size through direct and indirect, dilution-mediated pathways. Using path models, we disentangled how variation in two general, correlated traits—susceptibility and competitive ability—shaped epidemics. Higher susceptibility directly fuelled larger epidemics, while stronger competitive ability constrained diluters and indirectly allowed higher densities of infections. The reduction of the density of infected hosts by diluters was driven primarily by competitive host regulation. The second dilution mechanism—encounter reduction—was relatively weak. This empirically evaluated framework provides mechanistic trait-based foundations for dilution effect theory. Such theory brings disease ecologists closer to predicting the size of epidemics in diverse communities.

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AUTHORS' CONTRIBUTIONS

A.T.S., S.R.H., M.A.D. and C.E.C. designed the study. A.T.S. led trait measurement assays. A.M.B. set up the mesocosm experiment with assistance from A.T.S. and S.R.H. A.M.B. led sampling. A.T.S. wrote the first draft of the manuscript, and all authors contributed to revisions.

DATA ACCESSIBILITY

All data and scripts have been archived on Dryad Digital Repository: https://doi.org/10.5061/dryad.1f7sk (Strauss, Bowling, Duffy, Cáceres, & Hall, 2018).

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SUPPORTING INFORMATION

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