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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 fueled 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.

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

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 *et al.* 2015). One obvious trait is susceptibility, i.e., 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, Holt & Ostfeld 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 behavior, 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 *et al.* 2014; but see Wojdak *et al.* 2014). Yet intraspecific variation in susceptibility among focal hosts may counter either dilution mechanism by fueling 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., Ogden & Tsao 2009; Rohr *et al.* 2015; Dallas, Hall & Drake 2016; 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 *et al.* 2009), Lyme (Ogden & Tsao 2009), *Schistosoma* (Johnson et al. 2009), and parasites in intertidal (Thieltges *et al.* 2009), amphibian (Johnson *et al.* 2013), and plant communities (Mitchell, Tilman & Groth 2002; Lacroix *et al.* 2014). 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 multi-generational 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 a gradient 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 fueled larger epidemics. Simultaneously, stronger competitive ability indirectly allowed higher densities of

infected hosts, because the populations of diluters were constrained. Finally, the density of infected hosts was primarily reduced 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.

MATERIALS AND METHODS

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 *et al.* 2010b; Strauss *et al.* 2016). Focal hosts consume infectious fungal spores while foraging (Hall *et al.* 2007) but vary in their susceptibility to infection (Hall *et al.* 2010a). 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 (Hall *et al.* 2009; Strauss *et al.* 2015). Among a set of 28 Indiana lakes (see Strauss *et al.* 2016), these two taxa 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).

Trait Measurements

We quantified indices of two important traits, susceptibility and competitive ability, for eight different genotypes of the focal host (see Appendix S1 in Supporting Information for details). 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—fifteen individuals were exposed to each of three parasite concentrations, maintained individually, and later inspected for signs of infection. Susceptibility was fit (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 *et al.* 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.

Mesocosm Experiment

The mesocosm experiment crossed focal host genotype (8 levels) with presence/absence of competitor/diluters (2 levels). All combinations of treatments were replicated 4 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 Results). After the focal host and competitor/diluter populations grew for two weeks, we began sampling by mixing and sieving 1

L per tank per week (80 µm mesh). After one week of sampling, we added fungal spores (5,000 L^{-1}) and continued sampling for seven 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 (50X). Only 4 of 6,375 competitor/diluters examined were infected (0.06%), confirming their high resistance.

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 outcompeted by diluters.

Univariate linear models linked trait indices to mesocosm dynamics. Because several patterns exhibited pronounced heteroscedasticity (e.g., see Fig. 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 mean densities or prevalence. The first set tested whether susceptibility (β) directly predicted variation in epidemic size (i.e., mean density or prevalence of infected hosts). It also evaluated whether presence of competitor/diluters (denoted C) modulated these relationships (as β x 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 Results), we focus our path models on the density of infected hosts.

Statistics – Path Models

While the univariate models facilitated a close inspection of each relationship (see Figs. 1-3), 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 versus 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 parameter estimates.

RESULTS

Focal hosts varied in both traits (Fig. 1). Susceptibility, β, ranged 1.8–5.2 x10^{-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 $P = 0.13$). 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 (Fig. S1), G1, G3, and G4 (Fig. S2), and G5, G6, and G7 (Fig. S3). However, rather than focus on each genotype individually here, we summarize their mean responses along continuous gradients of their traits.

Linear Model Results

Variation in susceptibility shaped the size of epidemics (Fig. 2). Higher susceptibility fueled both higher mean densities of infected hosts (β effect, $P = 0.0046$; Fig. 2a) and higher infection prevalence (β effect: $P = 0.0008$; Fig. 2b). Mere presence of competitor/diluters did not effect either metric of epidemic size via main effect or interaction (all $P > 0.2$).

Competitive ability of focal hosts—the second trait—governed diluter densities and hence potential dilution mechanisms (Fig. 3). Strongly competing focal hosts constrained competitor/diluters to lower mean densities ($P < 0.0001$; Fig. 3a). In turn, higher densities of competitor/diluters regulated densities of focal hosts ($P = 0.0011$; Fig. 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 ($P = 0.0005$; Fig. 3c) and elevated by higher densities of focal hosts (*Hd* effect: $P = 0.0048$; Fig. 3d). A path model distills the causal structure underlying this result below. In contrast, infection prevalence was not significantly impacted by the density of competitor/diluters ($P = 0.27$; Fig. 3e) or focal hosts (*Hd* effect: $P = 0.58$; Fig. 3f). Presence of diluters (included as a covariate with focal host density) was not a significant predictor for either metric of disease (both $P > 0.9$). Analyses using the density of focal hosts from week 2 only (when spores were added) mirrored all of these results (see Fig. S4 in Appendix S1).

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 (Fig. 4). The traits covaried positively but not significantly (P = 0.14). Nevertheless, each trait shaped disease through a unique pathway. Higher susceptibility directly elevated disease (P = 0.004). In contrast, higher competitive abilities indirectly increased disease by constraining the density of competitor/diluters (P = 0.015). In turn, higher densities of diluters reduced the density of infected hosts (P = 0.006). Thus, diluters impacted disease more strongly when focal hosts competed weakly, because diluters were more numerous.

The second path model partitioned host regulation vs. encounter reduction as drivers of the density of infected hosts (Fig. 5). Intraspecific variation in susceptibility still strongly impacted the size of epidemics (P = 0.004). Additionally, higher total densities of focal hosts led to higher densities of infections (P < 0.001). However, higher densities of competitor/diluters did not directly lead to a lower density of infected hosts (P = 0.37). This weak effect may seem surprising, since it appeared significant when tested univariately (see Fig. 3c). Instead, in this path model, higher densities of competitor/diluters suppressed densities of focal hosts (P = 0.002), 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.

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 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 fueled 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., LoGiudice *et al.* 2003; Johnson *et al.* 2013; Lacroix *et al.* 2014). 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 *et al.* 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, Hall & Drake 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 (Mitchell, Tilman & Groth 2002; Johnson *et al.* 2012a; Dallas, Hall & Drake 2016). 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 *inter*-specifically between hosts and diluters or amplifiers of disease. For example, susceptibility to trematodes and pace of life covary among amphibian taxa (Johnson *et al.* 2012b); 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, Fellous & Kaltz 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 (Mitchell, Tilman & Groth 2002; Johnson *et al.* 2012a; Dallas, Hall & Drake 2016) 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 *et al.* 2012a; 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.* 2011). More partitions in other systems should test these hypotheses and delineate when host regulation vs. encounter reduction matter more.

Our trait-centered framework for friendly competition could be readily expanded. First, parallel experiments could incorporate traits of diluters (Venesky *et al.* 2014) or impacts of predation. 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 (Ostfeld & Keesing 2000; Johnson *et al.* 2013). Maintenance of diluters in a metacommunity could depend less of local competitive ability and more on dispersal ability or risk of extinction (Joseph *et al.* 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 imposed 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 fueled 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.

AUTHORS' CONTRIBUTIONS

ATS, SRH, MAD, and CEC designed the study. ATS led trait measurement assays. AMB set up the mesocosm experiment with assistance from ATS and SRH. AMB led sampling. ATS wrote the first draft of the manuscript, and all authors contributed to revisions.

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DATA ACCESSIBILITY

All data and scripts have been archived on Dryad Digital Repository: doi:10.5061/dryad.1f7sk

REFERENCES

Anderson, R.M. & May, R.M. (1981) The population dynamics of micro-parasites and their invertebrate hosts. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences,* **291,** 451-524.

Begon, M. (2008) Effects of host diversity on disease dynamics. *Infectious disease ecology: Effects of ecosystems on disease and of disease on ecosystems* (eds R.S. Ostfeld, F. Keesing & V.T. Eviner), pp. 12-29. Princeton University Press, 41 William St, Princeton, NJ 08540 USA.

Bolker, B.M. (2008) *Ecological Models and Data in R*. Princeton University Press.

Civitello, D.J., Cohen, J., Fatima, H., Halstead, N.T., Liriano, J., McMahon, T.A., Ortega, C.N., Sauer, E.L., Sehgal, T., Young, S. & Rohr, J.R. (2015) Biodiversity inhibits parasites: Broad evidence for the dilution effect. *Proceedings of the National Academy of Sciences of the United States of America,* **112,** 8667-8671.

Clay, C.A., Lehmer, E.M., Jeor, S.S. & Dearing, M.D. (2009) Sin nombre virus and rodent species diversity: A test of the dilution and amplification hypotheses. *Plos One*, **4**.

Dallas, T., Hall, R.J. & Drake, J.M. (2016) Competition-mediated feedbacks in experimental multispecies epizootics. *Ecology,* **97,** 661-670.

Decaestecker, E., De Gersem, H., Michalakis, Y. & Raeymaekers, J.A.M. (2013) Damped long-term host-parasite Red Queen coevolutionary dynamics: a reflection of dilution effects? *Ecology Letters*, **16,** 1455-1462.

Duncan, A.B., Fellous, S. & Kaltz, O. (2011) Reverse evolution - Selection against costly resistance in disease-free microcosm poulations of *Paramecium caudatum*. *Evolution,* **65,** 3462-3474.

Dwyer, G. & Elkinton, J.S. (1993) Using simple models to predict virus epizootics in gypsy-moth populations. *Journal of Animal Ecology,* **62,** 1-11.

Grover, J.P. (1997) *Resource Competition*. Chapman & Hall.

Hall, S.R., Becker, C.R., Duffy, M.A. & Caceres, C.E. (2012) A power-efficiency trade-off in resource use alters epidemiological relationships. *Ecology,* **93,** 645-656.

Hall, S.R., Becker, C.R., Duffy, M.A. & Cáceres, C.E. (2010a) Variation in resource acquisition and use among host clones creates key epidemiological trade-offs. *American Naturalist,* **176,** 557-565.

Hall, S.R., Becker, C.R., Simonis, J.L., Duffy, M.A., Tessier, A.J. & Cáceres, C.E. (2009) Friendly competition: evidence for a dilution effect among competitors in a planktonic host-parasite system. *Ecology,* **90,** 791-801.

Hall, S.R., Sivars-Becker, L., Becker, C., Duffy, M.A., Tessier, A.J. & Cáceres, C.E. (2007) Eating yourself sick: transmission of disease as a function of foraging ecology. *Ecology Letters,* **10,** 207-218.

Hall, S.R., Smyth, R., Becker, C.R., Duffy, M.A., Knight, C.J., MacIntyre, S., Tessier, A.J. & Cáceres, C.E. (2010b) Why are *Daphnia* in some lakes sicker? Disease ecology, habitat structure, and the plankton. *Bioscience,* **60,** 363-375.

Johnson, P.T.J., Dobson, A., Lafferty, K.D., Marcogliese, D.J., Memmott, J., Orlofske, S.A., Poulin, R. & Thieltges, D.W. (2010) When parasites become prey: ecological and epidemiological significance of eating parasites. *Trends in Ecology and Evolution,* **25,** 362-371.

Johnson, P.T.J., Lund, P.J., Hartson, R.B. & Yoshino, T.P. (2009) Community diversity reduces *Schistosoma mansoni* transmission, host pathology and human infection risk. *Proceedings of the Royal Society B-Biological Sciences,* **276,** 1657-1663.

Johnson, P.T.J., Preston, D.L., Hoverman, J.T., Henderson, J.S., Paull, S.H., Richgels, K.L.D. & Redmond, M.D. (2012a) Species diversity reduces parasite infection through cross-generational effects on host abundance. *Ecology,* **93,** 56-64.

Johnson, P.T.J., Preston, D.L., Hoverman, J.T. & Richgels, K.L.D. (2013) Biodiversity decreases disease through predictable changes in host community competence. *Nature,* **494,** 230-233.

Johnson, P.T.J., Rohr, J.R., Hoverman, J.T., Kellermanns, E., Bowerman, J. & Lunde, K.B. (2012b) Living fast and dying of infection: host life history drives interspecific variation in infection and disease risk. *Ecology Letters,* **15,** 235-242.

Joseph, M.B., Mihaljevic, J.R., Orlofske, S.A. & Paull, S.H. (2013) Does life history mediate changing disease risk when communities disassemble? *Ecology Letters,* **16,** 1405-1412.

Keesing, F., Holt, R.D. & Ostfeld, R.S. (2006) Effects of species diversity on disease risk. *Ecology Letters,* **9,** 485-498.

Lacroix, C., Jolles, A., Seabloom, E.W., Power, A.G., Mitchell, C.E. & Borer, E.T. (2014) Non-random biodiversity loss underlies predictable increases in viral disease prevalence. *Journal of the Royal Society Interface,* **11,** 10.

Lampert, W. & Trubetskova, I. (1996) Juvenile growth rate as a measure of fitness in *Daphnia*. *Functional Ecology,* **10,** 631-635.

LoGiudice, K., Ostfeld, R.S., Schmidt, K.A. & Keesing, F. (2003) The ecology of infectious disease: Effects of host diversity and community composition on Lyme disease risk.

Proceedings of the National Academy of Sciences of the United States of America, **100,** 567-571.

Mitchell, C.E., Tilman, D. & Groth, J.V. (2002) Effects of grassland plant species diversity, abundance, and composition on foliar fungal disease. *Ecology,* **83,** 1713-1726.

Ogden, N.H. & Tsao, J.I. (2009) Biodiversity and Lyme disease: Dilution or amplification? *Epidemics,* **1,** 196-206.

Ostfeld, R. & Keesing, F. (2000) The function of biodiversity in the ecology of vector-borne zoonotic diseases. *Canadian Journal of Zoology-Revue Canadienne De Zoologie,* **78,** 2061-2078.

Penczykowski, R.M., Forde, S.E. & Duffy, M.A. (2011) Rapid evolution as a possible constraint on emerging infectious diseases. *Freshwater Biology,* **56,** 689-704.

Pinheiro, J. & Bates, D. (2000) *Mixed-Effects Models in S and S-PLUS*. Springer New York.

Pulkkinen, K. (2007) Microparasite transmission to Daphnia magna decreases in the presence of conspecifics. *Oecologia,* **154,** 45-53.

R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Randolph, S.E. & Dobson, A.D.M. (2012) Pangloss revisited: a critique of the dilution effect and the biodiversity-buffers-disease paradigm. *Parasitology,* **139,** 847-863.

Rohr, J.R., Civitello, D.J., Crumrine, P.W., Halstead, N.T., Miller, A.D., Schotthoefer, A.M., Stenoien, C., Johnson, L.B. & Beasley, V.R. (2015) Predator diversity, intraguild predation, and indirect effects drive parasite transmission. *Proceedings of the National Academy of Sciences of the United States of America,* **112,** 3008-3013.

Strauss, A.T., Civitello, D.J., Cáceres, C.E. & Hall, S.R. (2015) Success, failure and ambiguity of the dilution effect among competitors. *Ecology Letters,* **18,** 916-926.

Strauss, A.T., Hite, J.L., Shocket, M.S., Cáceres, C.E., Duffy, M.A. & Hall, S.R. (2017) Rapid evolution rescues hosts from competition and disease but—despite a dilution effect—increases the density of infected hosts. *Proceedings of the Royal Society B: Biological Sciences,* **284**.

Strauss, A.T., Shocket, M.S., Civitello, D.J., Hite, J.L., Penczykowski, R.M., Duffy, M.A., Cáceres, C.E. & Hall, S.R. (2016) Habitat, predators, and hosts regulate disease in *Daphnia* through direct and indirect pathways. *Ecological Monographs,* **86,** 393-411.

Tessier, A.J. & Woodruff, P. (2002) Cryptic trophic cascade along a gradient of lake size. *Ecology*, **83,** 1263-1270.

Thieltges, D.W., Reise, K., Prinz, K. & Jensen, K.T. (2009) Invaders interfere with native parasite-host interactions. *Biological Invasions,* **11,** 1421-1429.

Venesky, M.D., Kerby, J.L., Storfer, A. & Parris, M.J. (2011) Can Differences in Host Behavior Drive Patterns of Disease Prevalence in Tadpoles? *Plos One*, **6**.

Venesky, M.D., Liu, X., Sauer, E.L. & Rohr, J.R. (2014) Linking manipulative experiments to field data to test the dilution effect. *Journal of Animal Ecology*, **83,** 557-565.

Wojdak, J.M., Edman, R.M., Wyderko, J.A., Zemmer, S.A. & Belden, L.K. (2014) Host density and competency determine the effects of host diversity on trematode parasite infection. *PloS one*, **9,** e105059.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Appendix S1. Supplementary methods and results.

Figure S1. Mesocosm dynamics of two illustrative focal host genotypes varying in key traits.

Figures S2 & S3. Mesocosm dynamics of all other focal host genotypes.

Table S1. Comparisons between GLS and mixed models.

Figure S4. Density of focal hosts during week 2 (when parasites were added).

Table S2. Test statistics and cutoff criteria for path models.

Table S3. Parameter estimates for the first path model (Fig. 4).

Table S4. Parameter estimates for the second path model (Fig. 5).

FIGURES & CAPTIONS

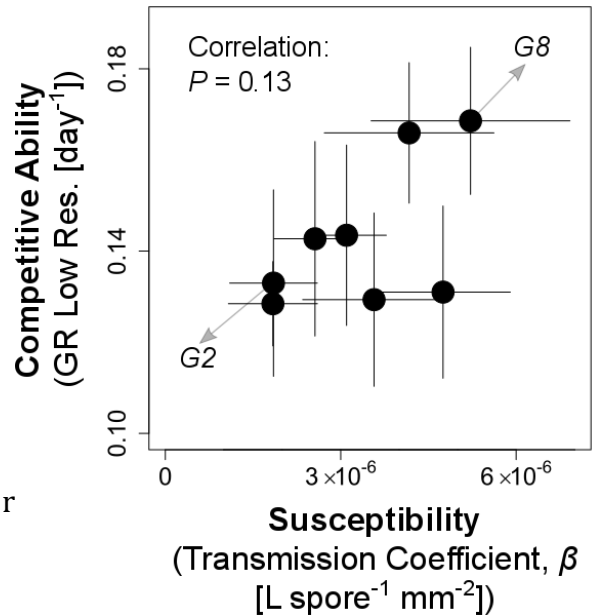
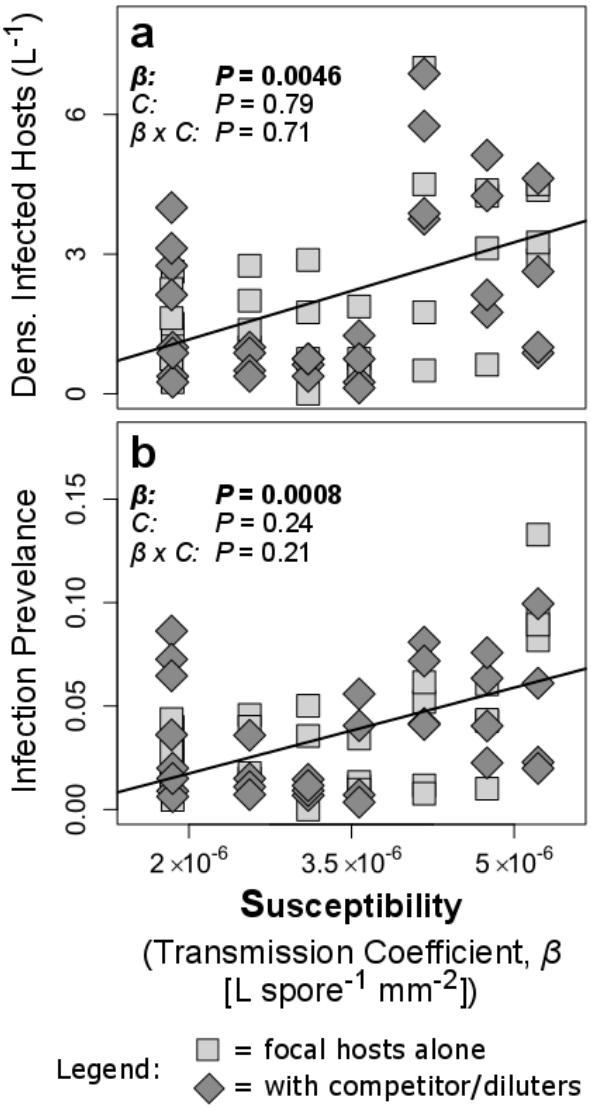


Figure 1. *Two key traits covary among eight*

focal host genotypes. Susceptibility is indexed as 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 ($P = 0.13$). However, both traits and their covariation become foundations for linear (Figs. 2–3) and path models (Figs. 4–5). Genotypes are named according to variation in susceptibility (along x axis; Figs. S1-S3 in Appendix S1 present each genotype's time series in the mesocosm experiment). Error bars are bootstrapped standard errors.

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 x C). P values are fits of linear models. Key: squares = focal hosts alone; diamonds = with competitor/diluters.



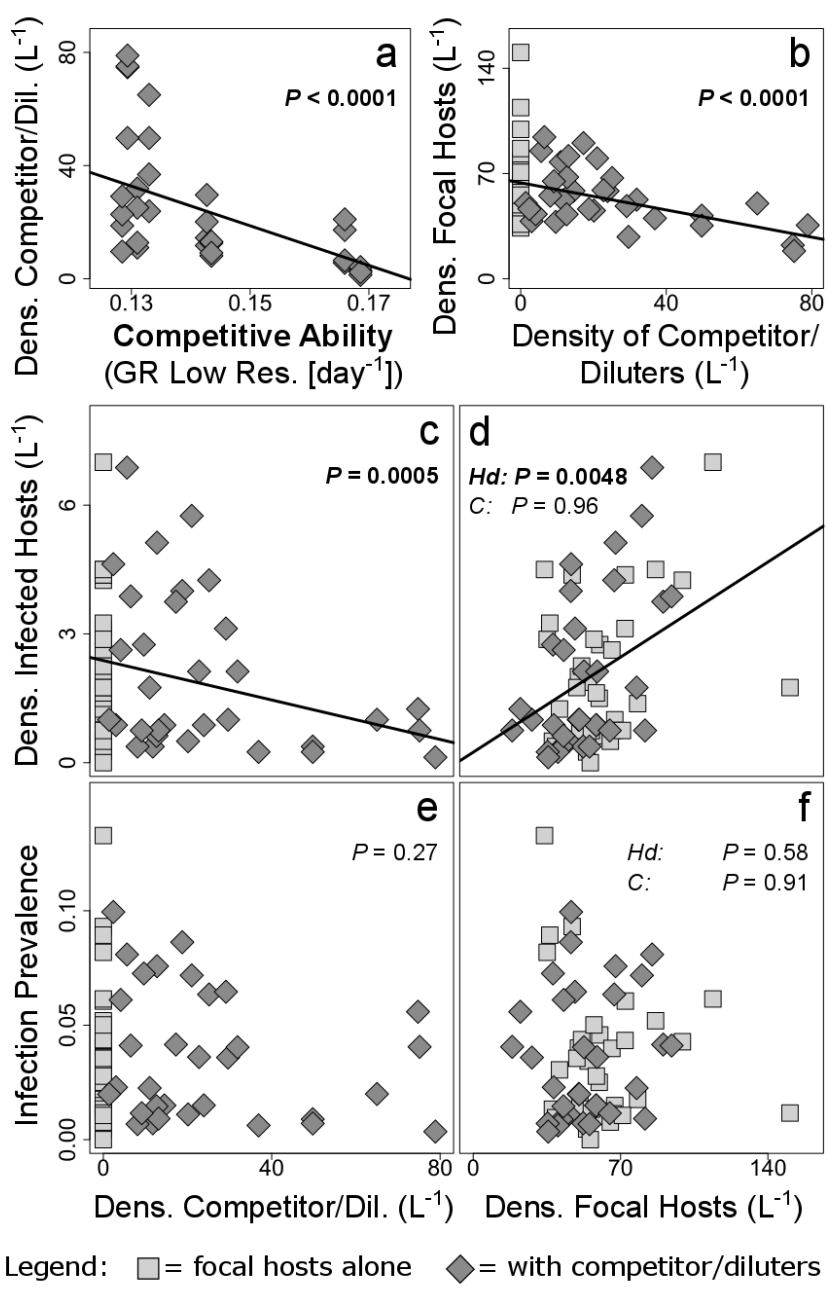


Figure 3. *Variation in competitive ability structures the densities of diluters and focal hosts, and both correlate with the density of infected hosts.* **a)** Genotypes of focal hosts with higher competitive abilities constrain competitor/diluters to lower densities. **b)** Higher densities of diluters 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.

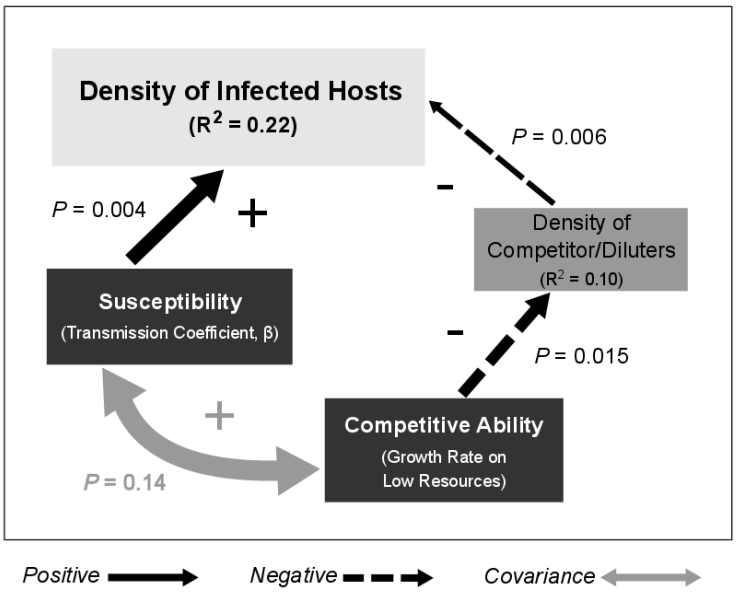


Figure 4. *Both covarying focal host traits simultaneously govern the density of infected hosts.* Higher susceptibility directly fuels larger epidemics directly (see Fig. 2a). In contrast, stronger competitive ability enables epidemics indirectly by limiting the density of diluters (see Fig. 3a). In 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 Fig. 5). Key: solid = positive coefficients; dashed = negative coefficients; two-headed arrow = covariance between traits; arrow weights = standardized effect sizes.

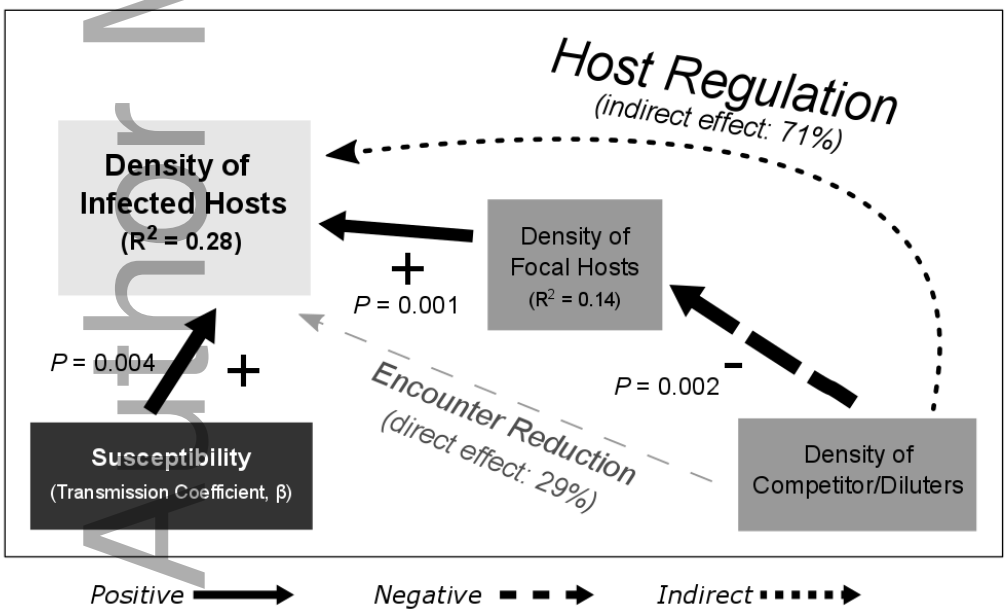


Figure 5. *Partitioning two dilution mechanisms: Does host regulation or encounter reduction*

reduce the density of infected hosts? Higher total densities of focal hosts lead to a higher density of infected hosts (plotted in Fig. 3d). However, higher densities of competitor/diluters did not directly lead to a lower density of infected hosts (despite the apparent relationship in Fig. 3c). This direct effect, i.e., 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, i.e., 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.