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Virulence-driven trade-offs in disease transmission: A meta-analysis*

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The virulence–transmission trade-off hypothesis proposed more than 30 years ago is the cornerstone in the study of host–parasite co-evolution. This hypothesis rests on the premise that virulence is an unavoidable and increasing cost because the parasite uses host resources to replicate. This cost associated with replication ultimately results in a deceleration in transmission rate because increasing within-host replication increases host mortality. Empirical tests of predictions of the hypothesis have found mixed support, which cast doubt about its overall generalizability. To quantitatively address this issue, we conducted a meta-analysis of 29 empirical studies, after reviewing over 6000 published papers, addressing the four core relationships between (1) virulence and recovery rate, (2) within-host replication rate and virulence, (3) within-host replication and transmission rate, and (4) virulence and transmission rate. We found strong support for an increasing relationship between replication and virulence, and replication and transmission. Yet, it is still uncertain if these relationships generally decelerate due to high within-study variability. There was insufficient data to quantitatively test the other two core relationships predicted by the theory. Overall, the results suggest that the current empirical evidence provides partial support for the trade-off hypothesis, but more work remains to be done.

KEY WORDS: Host–parasite, host–pathogen, IPD meta-analysis, replication, trade-off hypothesis, transmission, virulence.

For over 30 years the virulence–transmission trade-off hypothesis has been the cornerstone for how we study and think about the relationship between host and pathogen fitness (Anderson and May 1982; Ewald 1983). This hypothesis still inspires much theoretical and empirical work in ecology and evolution (Alizon et al. 2009). In short, the trade-off hypothesis states that virulence—the harm that a pathogen inflicts on its host through a decrease in host fitness—is an unavoidable cost to the pathogen for using the host resources for replication (Bull 1994; Ewald 1994). This cost of increasing within–host replication results in a deceleration in transmission rate because increasing within-host replication rate increases mortality rates, which ultimately translates into a shorter infectious period at the population level. Thus, overall transmission should be the greatest at intermediate levels of virulence, which balances the costs of within-host replication and infectious period length (Anderson and May 1982; Leggett et al. 2013).

The virulence trade-off hypothesis requires competition among strains with varying degrees of virulence to the host. The fitness of these strains can be described by the basic reproduction number R_0, which was described by Anderson and May (1982) as a function of the pathogen's transmission rate (β), the host's population size (N), the host's natural mortality rate (μ), the host's pathogen-induced mortality rate (i.e., virulence, α) and the recovery rate ν such that,

$$R_0 = \frac{\beta N}{\mu + \alpha + \nu}. \quad (1)$$

R_0 would increase with increasing transmission rate (β) or with decreasing disease-induced mortality rate (α)—assuming that each parameter evolves independently with constant rates of recovery (ν) and natural mortality (μ). Following this logic, we would expect that R_0 would be maximized as virulence is minimized or $\alpha \to 0$. This is the foundation of the avirulence hypothesis, which proposes that pathogens evolve toward lower virulence to maximize the exploitation of host resources (Smith 1904). Evidence for this hypothesis would include high virulence for

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emergent pathogens that decreases with time. However, some studies document a transient increase in virulence for emergent pathogens (Anderson and May 1982; Read 1994; Bolker et al. 2010), evidence for a decrease in virulence with time is scarce (but see Knell 2004). Given the general lack of support, the avirulence hypothesis has fallen out of favor.

In contrast, the trade-off hypothesis suggests that recovery (ν) and transmission (β) rates are not independent, but related through a trade-off with virulence (α), such that R_0 can be redefined as

$$R_0 = \frac{\beta(\alpha)N}{\mu + \alpha + \nu(\alpha)}, \quad (2)$$

where transmission and recovery rates are functions of virulence (notation from Frank 1996).

Under the trade-off hypothesis, hosts may recover more quickly from less virulent strains than from highly virulent ones, making the recovery rate a negative function of virulence, $\nu(\alpha)$. There is some empirical evidence supporting this relationship. A decrease in the recovery rate with increasing virulence has been observed in *Myxoma* virus in Australian rabbits (Anderson and May 1982) and *Mycoplasma gallisepticum* infections in North American house finches (Williams et al. 2014). Under this scenario, decreasing virulence (α) will not necessarily increase pathogen's fitness (R_0), as predicted by the avirulence hypothesis, because a decrease in virulence may be coupled with an increase in the recovery rate (ν).

The relationship between transmission rate and virulence, $\beta(\alpha)$, is the cornerstone of the virulence trade-off hypothesis. If transmission and recovery rates are linked with virulence, the hypothesis predicts an increase in transmission rate with increasing virulence (often measured as host mortality rate) until it reaches a point after which the cost of increasing virulence is too high causing the relationship to decelerate (Fig. 1; Anderson and May 1982). Here, we focus on the predicted relationship between virulence and transmission rates. The relationship between virulence and lifetime transmission may result in a quadratic relationship instead of deceleration (Leggett et al. 2013). There are empirical examples supporting this decelerating relationship including HIV (Fraser et al. 2007) and rodent malaria (Mackinnon and Read 1999). However, other examples contradict this result (e.g., Chapuis et al. 2012).

The relationship between transmission rates and virulence, $\beta(\alpha)$, is mediated by the pathogen's ability to use the host's resources for replication. Theoretical studies predict a similar decelerating functional relationship between within-host replication (r) and transmission rates, $\beta(r)$. There is some empirical support for this relationship including *Ophryocystis elektroscirrha* infections in monarch butterflies (De Roode et al. 2008) and in HIV infections (Fraser et al. 2007). Yet, some studies have found evidence for other various types of functional relationships (e.g., Magalon et al. 2010). A positive decelerating relationship between within-host replication rate and virulence also has contradicting empirical support. Some studies support a positive decelerating relationship such as *Mycoplasma gallisepticum* in North American house finches (Hawley et al. 2013). Others show different functional forms like bacterial infections in *Daphnia magna*, which shows a positive relationship (Jensen et al. 2006). Thus, qualitative support for this theoretical relationship is not clear given the literature.

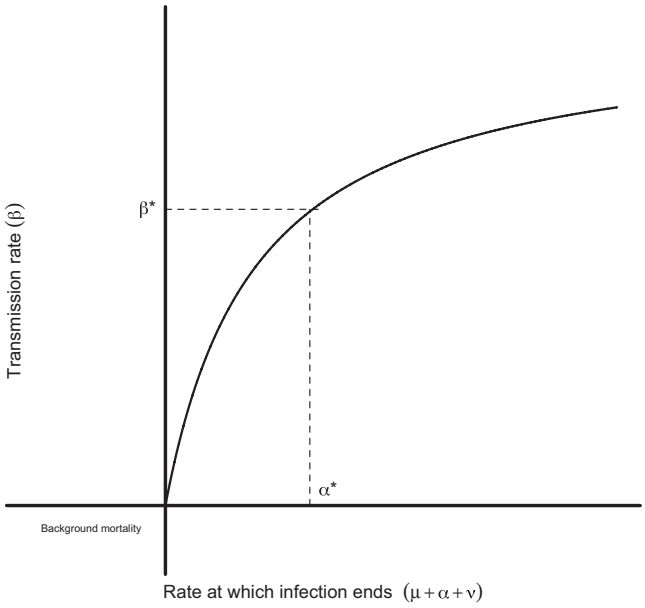

Figure 1. The trade–off hypothesis suggests a decelerating relationship between transmission rate (β) and pathogen-induced mortality (α). When natural host mortality rate (μ) and the recovery rate (ν) are assumed constant, β* and α* represent the transmission rate and virulence that will result in optimal R_0.

The beauty of the trade-off hypothesis lies in its simplicity, which has also served as a source of a major critique regarding its utility (Ebert and Bull 2003). The simple models described in equations 1 and 2 stem from an analysis of a susceptible-infected-recovered (SIR) model. This model makes multiple simplifying assumptions about disease dynamics such as horizontal contact transmission and homogeneous mixing. Some studies argue that the simple predictions from the theory can accurately describe virulence evolution in a small subset of systems with simple life cycles, but prove less useful for diseases with more complicated dynamics or transmission processes (Froissart et al. 2010; Alizon and Michalakis 2015). Yet, this theoretical approach has inspired empirical work in a wide variety of host–pathogen systems that do not necessarily meet the simplifying assumptions.

Recent narrative reviews suggest that supporting evidence for the virulence–transmission trade-off hypothesis is becoming more common and we are close to being able to make generalizing conclusions supporting the hypothesis (Leggett et al. 2013).

Yet, other empirical studies still find contradicting evidence (see review in Ebert and Bull 2003, and Alizon et al. 2009). Thus, qualitatively, the results are equivocal. These qualitative assessments do not explicitly and quantitatively test the relationships described above. In this study, we ask: Is there enough empirical support to generalize the four characteristic relationships of the virulence trade-off hypothesis: recovery rate and virulence, replication rate and virulence, replication rate and transmission rate, or virulence and transmission rate? Given the support or lack of support, which relationships should future studies emphasize, examine, and possibly refine? To answer these questions, we conducted a systematic review and meta-analysis of empirical studies.

Methods

LITERATURE SEARCH

We conducted a comprehensive search of the peer-reviewed literature using the Web of Knowledge (http://thomsonreuters.com/web-of-knowledge/) on December 2016 and December 2018. We searched for the following keywords: `(recovery-rate OR recovery rate) AND virulence AND (disease OR parasite)`, `replication AND transmission AND (disease OR parasite)`, `replication AND virulence AND(disease OR parasite)`, `virulence AND transmission AND (disease OR parasite)`. Because some studies may quantify recovery rate as clearance, we also performed a search for `clearance AND virulence AND (disease OR parasite)`. This search resulted in over 6800 studies. To limit the number of studies from categories that are unlikely to have relevant data (e.g., physics, pediatrics, analytical chemistry, and so forth), we refined the search to the following subjects: parasitology, ecology, evolutionary biology, infectious diseases, zoology, biology, microbiology, virology, entomology, mycology, immunology, and tropical medicine. We also searched studies included in a review by Alizon et al. (2009) and a recent comprehensive review by Leggett et al. (2013) in addition to studies that cite them to include any study missed by our Web of Knowledge search. In the end, our initial review resulted in a potential pool >6000 studies.

We examined each of the remaining studies to determine if they met the following criteria to be included in the meta-analysis: (i) the study empirically tested the relationship between recovery rate and virulence, replication and virulence, replication and transmission rate, or virulence and transmission rate, (ii) these relationships were observed in a non-intermediate host (e.g., not in the vectors of the associated disease), (iii) hosts were animals, to prevent any bias due to multiple definitions of virulence between the plant and animal disease literature, (iv) the raw data were available either because they were provided in the paper, by the author, or they could be extracted digitally from the published figures, and (v) the main text was written in English.

The trade-off hypothesis inspired empirical work in a wide variety of host–parasite systems that do not necessarily meet the model's simplifying assumptions. Moreover, out of these studies just a small proportion have measured virulence, transmission, and recovery rates in units consistent with the theoretical models. This leads to a dichotomous clustering of views on how to empirically test the trade-off hypotheses predictions. To account for these views, we conducted two complementary analyses.

First, we conducted a set of conservative analyses in which we included studies of host–parasite systems with horizontal contact transmission. In this conservative analysis, we also restricted inclusion of studies that measured transmission and virulence in units consistent with the theory (Day 2002). For instance, we considered studies that measured replication in ways that describe the amount of pathogen load in the host (e.g., viral load, virus titer, and plaque forming units [PFU]). We also considered studies in which virulence was measured in units related to survival in ways that reduce the length of the infectious period (e.g., time to death, survival time, and LT50, which describes the time at which 50% of the hosts die). We also included in this conservative analysis studies that directly measured transmission rates or proportion of infected individuals.

Second, we also conducted a complementary inclusive analyses where, in addition to the studies that met the conservative criteria, we also included studies of vector-borne host–parasites systems (as long as transmission was horizontal) and studies that measured virulence or transmission using proxies (Tables S1 and S7). For instance, we included studies in which a sublethal measure of virulence was used as a proxy, such as minimum live weight, weight loss, or red blood density, assuming that these proxies are ultimately related to a potential decrease in survival and hence, also a decrease in the length of the infectious period. With regards to transmission rates, we also considered studies in which maximum or total gametocytemia or parasitemia were used as proxies for transmission rate, but only in systems where empirical data show that the proxies are correlated linearly with transmission rates (i.e., Mackinnon and Read 1999; Ben-Ami 2017). The corresponding data collected given the conservative and inclusive frameworks were analyzed to quantify whether the literature supports each of the hypothesized relationships associated with the virulence trade-off hypothesis.

STATISTICAL ANALYSES

Our analysis resembles a two-stage individual patient data (IPD) meta–analysis in the medical literature. While standard meta-analyses pool effect sizes and other summary statistics, an IPD directly extracts and analyzes the raw data from published papers (Mengersen et al. 2013). These data were accessed in

permanent archives, made available by the corresponding author or were digitally extracted from the published study using Plot Digitizer (http://plotdigitizer.sourceforge.net). In an IPD meta-analysis, raw data from multiple studies are re-analyzed individually using the same approach. The effect sizes are extracted from these models, which reduces among-study heterogeneities stemming from variability in modeling approaches (Stewart and Parmar 1993; Berlin et al. 2002; Simmonds et al. 2005). This was our method of choice because it allowed us to test the fit of a polynomial model describing the relationship between variables in each study (e.g., Ben-Ami 2017) and analyze the partial relationships of the slopes as our effect sizes (e.g., Mackey and Currie 2001; Dukic et al. 2013). Mittelbach et al. (2001) applied a similar approach to test multiple functional responses between species richness and productivity in one of the few examples of IPD meta–analyses in ecology and evolution.

A quantitative synthesis of the trade-off theory requires that studies are analyzed in a uniform and systematic way. As a first step, we scaled and centered the raw data extracted from each study. The SD used to scale the available data is based on the experimental design and/or availability of pathogen strains in each individual study. Thus, the SD of the general population may be more or less than that measured. We cannot offer a correction with regard to this and, thus, assume that the experiments have reasonably sampled the population at large. Then, we fitted a polynomial model of the form $y = b_0 + b_1 x_1 + b_2 x_1^2$, where y is the response variable and x the predictor (e.g., in the virulence–transmission relationship y represents transmission and x represents virulence) to the data of each study. There are other potential decelerating functions that can be fitted to the data such as Michaelis–Menten, Chapman–Richard's growth function, and other type II or type III functions. Yet, the polynomial model provides a flexible and general approach that captures multiple functional forms in the data including the decelerating relationships predicted by the theory. The parameters b_1 and b_2 represent the effect sizes that describe the strength of the partial relationships of a linear and polynomial term, respectively. We analyzed these parameters, their associated uncertainty, and within-study sample sizes in our meta-analysis (see below). An estimate of $\hat{b}_1 > 0$ and $\hat{b}_2 = 0$ would capture a positive linear relationship. An estimate of $\hat{b}_1 > 0$ and $\hat{b}_2 < 0$ (polynomial model) would capture the predicted decelerating function (Ben-Ami 2017; Fig. 1). Thus, this modeling approach allows for a quantitative estimate of support for each of the hypothesized relationships.

In the second step, we analyzed $i = 1, \ldots, k$ independent effect sizes (i.e., the $b_{1,i}$ and $b_{2,i}$ and their associated uncertainty estimated for each study). Most meta-analyses assume that:

$$z_i = \theta_i + e_i, \tag{3}$$

where z_i represents the observed effect in the i-th study, θ_i represents the unknown true effect, and e_i the sampling error (Koricheva et al. 2013). We also assume that e_i is normally distributed (i.e., $e_i \sim N(0, v_i)$, where v_i represent the sampling variance).

We can account for heterogeneity among studies using a random effects model:

$$\theta_i = \mu + u_i, \tag{4}$$

where true effects are assumed to be normally distributed with mean μ and $u_i \sim N(0, \tau^2)$. The parameter τ^2 describes the residual heterogeneity associated with the random effect. Therefore, we assume that each study is part of a greater population of studies that have been conducted or will be conducted in the future. The theoretical literature predicts that the virulence trade-offs may vary depending on the measures used to quantify the variables (Day 2002). Therefore, we tested for the effect of measurement units driving the size of the true effect using a mixed-effects model, such that:

$$\theta_i = \beta_0 + \beta_1 x_{i1} + \beta_2 x_{i2} \cdots + u_i, \tag{5}$$

where $u_i \sim N(0, \tau^2)$ and x_{ij} describes the value of the j moderator of study i. With the exception of the conservative analysis on replication and virulence, each of the relationships tested had at most two types of moderators that needed to be explicitly accounted for in the analysis. The moderator variable for replication had two categories: (1) "parasite load", which included studies that measured replication as PFU, total parasite spore load, parasite density, spores at death, or DNA copies per million, and (2) "parasitemia", which included studies that measured replication as total parasitemia and maximum asexual parasitemia. These measures of parasitemia were common in human and mice malaria studies, where parasitemia is quantified as the proportion of infected red blood cells. Some studies reported their measure of replication in the log scale (e.g., log(PFU)), while others in the natural scale. To make the comparison consistent among studies, we exponentiated the data from studies that reported their results in a log scale. The moderator variable for virulence included four categories: (1) "expected lifespan", which included studies that measured virulence as time to death, life span, or longevity, (2) "sublethal", which included studies that measured virulence as severity and duration of inflammation, weight, or red blood count loss, (3) "mortality", which included those studies that measured virulence as mortality rate or percent mortality, and (4) "LD_x", which included studies that measured virulence as lethal dose required to result in the death of x% of the experimental group (Day 2002). To make the trend consistent among studies, we used the opposite of the variable in the cases where the virulence measure correlated negatively with virulence (e.g.,

virulence decreases with body weight, life-span, or longevity). Similarly, we calculated percent mortality from studies reporting percent survival by percent mortality = 100 − percent surviving. The moderator variable for transmission included four categories: (1) "transmission rate", (2) "gametocytemia", (3) "proportion", which included studies that measured transmission as the probability or proportion of individuals infected, and (4) "MID50", which describes a study that quantified transmission as median infectious dose. There was no need to transform any recovery measures because there were not enough studies to conduct a meta-analysis on the $v(\alpha)$ relationship (see below).

To assess the relative influence of each study driving the average effect size, we also performed a leave-one-out sensitivity analysis in which we iteratively performed the analysis leaving one study out. All analyses were conducted in `R` using the package `metafor` (Viechtbauer 2010).

Results

We manually searched a total of 6122 studies of which 29 met our inclusion criteria for at least one of the four studied relationships. These studies comprised a variety of vertebrate hosts including mice, humans, salamanders, fur seals, birds, and rabbits, and invertebrate hosts like crustacean, butterflies, and moths. In turn, these hosts were infected by a variety of parasites including neogregarines, protozoa, hookworms, fungi, bacteria, and virus (Tables S1, S7, S14, and S18). In summary, the available studies were exhaustively searched and the subsequent studies used, although low in number, represent a relatively broad spectrum of host–parasite systems.

REPLICATION AND VIRULENCE

A total of $n = 8$ studies met our conservative inclusion criteria for the replication–virulence relationship (Table S1). The data show strong support for an increasing relationship between replication and virulence ($\hat{b}_1 = 0.62 \pm 0.24$ SE, $P < 0.01$; Fig. 2 A). While the average estimate for the polynomial term suggests that this overall relationship decelerates with increasing replication, the result was not statistically significant ($\hat{b}_2 = -0.10 \pm 0.21$ SE, $P = 0.62$; Fig. 2 B). A test for heterogeneity does not show significant variability among studies in the linear ($Q_{(df=7)} = 4.84$, $P = 0.68$) or polynomial terms ($Q_{(df=7)} = 1.03$, $P = 0.99$). The leave-one-out diagnostic showed that Jensen et al. (2006) and De Roode et al. (2008) had a significant influence on the overall effect on $\hat{b}_1$. When De Roode et al. (2008) is excluded the overall of effect $\hat{b}_1$ becomes weaker and not statistically significant ($\hat{b}_1 = 0.44 \pm 0.31$ SE, $P = 0.16$; Table S3). In contrast, when Jensen et al. (2006) is excluded the overall effect of $\hat{b}_1$ is stronger ($\hat{b}_1 = 0.80 \pm 0.26$ SE, $P < 0.01$; Table S3). There was no study that had a disproportionate effect on $\hat{b}_2$ (Table S4).

A total of $n = 14$ studies met our inclusive criteria for the replication–virulence relationship (Table S1). Similar to the conservative analysis, the overall pattern supports an increasing relationship between replication and virulence ($\hat{b}_1 = 0.64 \pm 0.17$ SE, $P < 0.01$; Fig. 2 C). The average estimate for the polynomial term suggests that this overall relationship decelerates with increasing replication; however, the relationship was not statistically significant ($\hat{b}_2 = -0.03 \pm 0.15$ SE, $P = 0.86$; Fig. 2 D). A test for heterogeneity shows no significant among-study variability in the linear ($Q_{(df=18)} = 6.82$, $P = 0.99$) and polynomial terms ($Q_{(df=18)} = 6.04$, $P = 0.99$). This relationship had no significant heterogeneity explained by variation in the measures of virulence used in the studies ($QM_{(df=2)} = 0.27$, $P = 0.87$ for $\hat{b}_1$ and $QM_{(df=2)} = 1.91$, $P = 0.39$ for $\hat{b}_2$). Similarly, this relationship had no significant heterogeneity explained by variation in the measures of replication used in the studies ($QM_{(df=2)} = 0.06$, $P = 0.97$ for $\hat{b}_1$ and $QM_{(df=2)} = 0.13$, $P = 0.94$ for $\hat{b}_2$; Table S2). The leave-one-out diagnostic shows a significant effect of Mackinnon and Read (2004) influencing the overall effect of $\hat{b}_1$. When the study is excluded the overall effect of $\hat{b}_1$ is stronger ($\hat{b}_1 = 0.68 \pm 0.19$ SE, $P < 0.01$; Table S5). There was no study that had a disproportionate effect on $\hat{b}_2$ (Table S6).

REPLICATION AND TRANSMISSION

A total of $n = 4$ studies met our conservative criteria for the replication–transmission relationship (Table S6). The overall pattern supports a strong increasing relationship between replication and transmission ($\hat{b}_1 = 0.95 \pm 0.30$ SE, $P < 0.01$; Fig. 3 A). The average estimate for the polynomial term suggests that this overall relationship decelerates with increasing replication; however, the decelerating term was not significant ($\hat{b}_2 = -0.16 \pm 0.33$ SE, $P = 0.64$; Fig. 3 B). A test for heterogeneity shows no significant among-study variability in the linear ($Q_{(df=3)} = 0.13$, $P = 0.99$) or polynomial terms ($Q_{(df=3)} = 0.04$, $P > 0.99$). This relationship had no significant heterogeneity explained by variation in the measures of transmission used in the studies ($QM_{(df=1)} = 0.08$, $P = 0.77$ for $\hat{b}_1$ and $QM_{(df=1)} = 0.01$, $P = 0.98$ for $\hat{b}_2$). Similarly, this relationship had no significant heterogeneity explained by variation in the measures of replication used in the studies ($QM_{(df=1)} = 0.02$, $P = 0.90$ for $\hat{b}_1$ and $QM_{(df=1)} = 0.02$, $P = 0.89$ for $\hat{b}_2$; Table S8). The leave-one-out diagnostic shows no significant effect of any study influencing the overall effects (Tables S10 and S11).

A total of $n = 10$ studies met our inclusive criteria for the replication–transmission relationship (Table S7). The overall pattern of the average estimates supports a strong increasing relationship between replication and transmission ($\hat{b}_1 = 0.94 \pm 0.04$ SE, $P < 0.01$; Fig. 3 C). The average estimate for the polynomial term suggests that this overall relationship accelerates with increasing replication; however, this term was

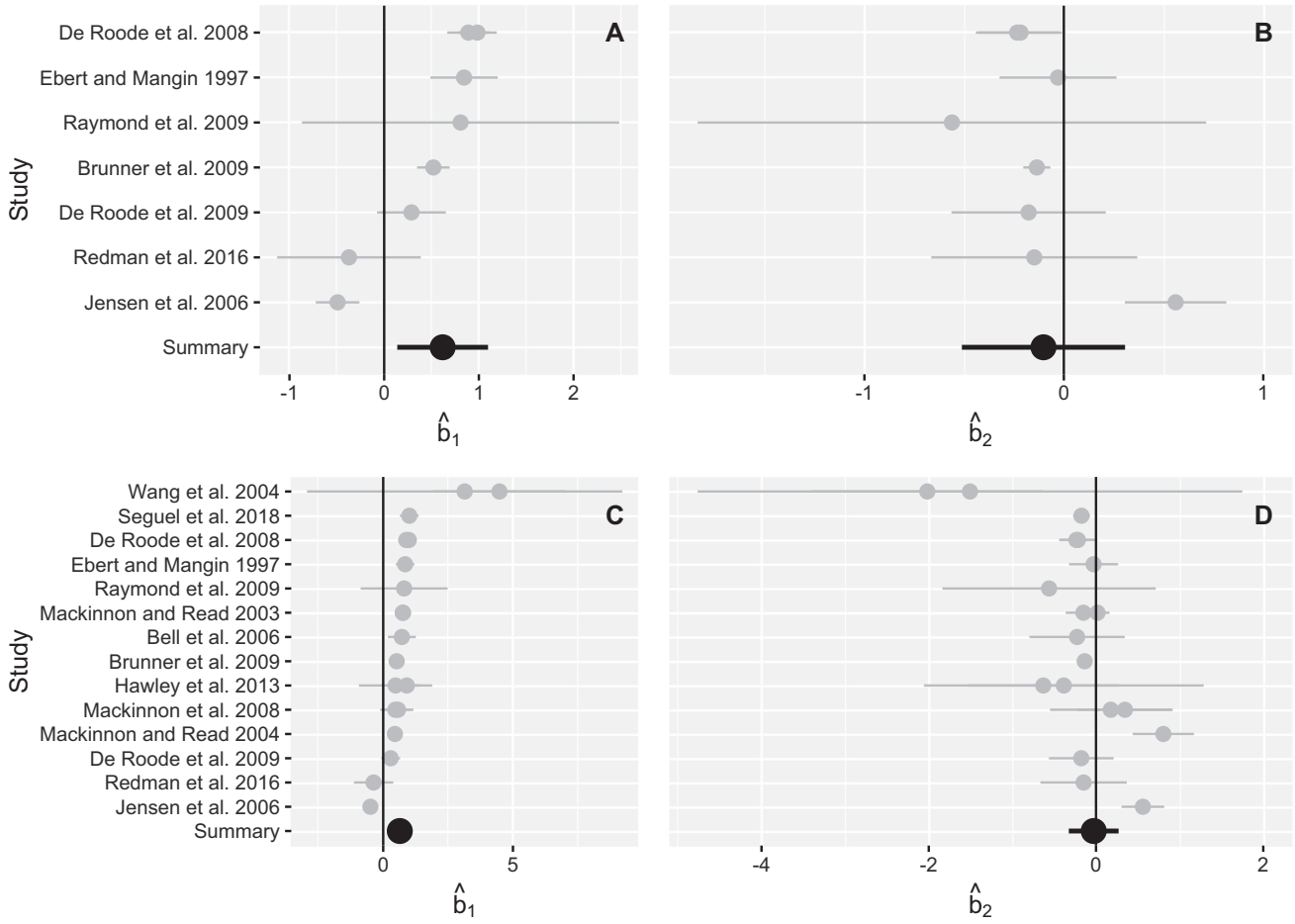

Figure 2. Distribution of effect sizes (with 95% confidence interval) for studies describing the relationships between replication and virulence following a (A and B) conservative or (C and D) inclusive criteria. Parameter $\hat{b}_1$ represents the linear term and $\hat{b}_2$ the polynomial term. The black symbols represent the overall effects. Studies with multiple dots represent those that included more than one data set in the same study.

not significant ($\hat{b}_2 = 0.14 \pm 0.189$ SE, $P = 0.46$; Fig. 3D). A test for heterogeneity shows no significant variability among studies in the linear ($Q_{(df=12)} = 2.42$, $P > 0.99$) and polynomial terms ($Q_{(df=12)} = 5.04$, $P = 0.96$). There was no significant heterogeneity explained by variation in the transmission measures used in the studies ($QM_{(df=3)} = 1.44$, $P = 0.70$ for $\hat{b}_1$ and $QM_{(df=3)} = 3.85$, $P = 0.28$ for $\hat{b}_2$). Similarly, there was no significant heterogeneity explained by variation in the replication measures used in the studies ($QM_{(df=5)} = 1.68$, $P = 0.89$ for $\hat{b}_1$ and $QM_{(df=5)} = 2.81$, $P = 0.73$ for $\hat{b}_2$; Table S9). The leave-one-out diagnostic shows a significant effect of Dwyer et al. (1990) influencing the overall effects $\hat{b}_1$ and $\hat{b}_2$. When the study is excluded the overall effect of $\hat{b}_1$ is less strong, but still significant ($\hat{b}_1 = 0.77 \pm 0.25$ SE, $P < 0.01$; Table S12), and the overall effect of $\hat{b}_2$ changes direction suggesting deceleration but still not significant ($\hat{b}_2 = -0.16 \pm 0.25$ SE, $P = 0.53$; Table S13).

VIRULENCE AND TRANSMISSION

We did not find enough studies meeting the conservative criteria. In fact, Dwyer et al. (1990) is generally considered to meet a similar conservative inclusion criteria; however, *Myxoma virus* can also be vector-borne transmitted, which violates the direct transmission assumption from the classical model. Note that Bolker et al. (2010) considered Jensen et al. (2006) and Fraser et al. (2007) as meeting a similar conservative inclusion criteria; yet, the original studies quantified replication that they transformed into transmission; therefore, we included them in the replication–virulence or replication–transmission relationships. Therefore, no conservative analysis could be conducted for virulence and transmission. A total of $n = 9$ studies met our inclusive criteria for the virulence–transmission relationship (Table S14). The overall pattern of the average estimates supports a decreasing relationship between virulence and transmission ($\hat{b}_1 = -0.02 \pm 0.29$ SE, $P = 0.94$) that decelerates with

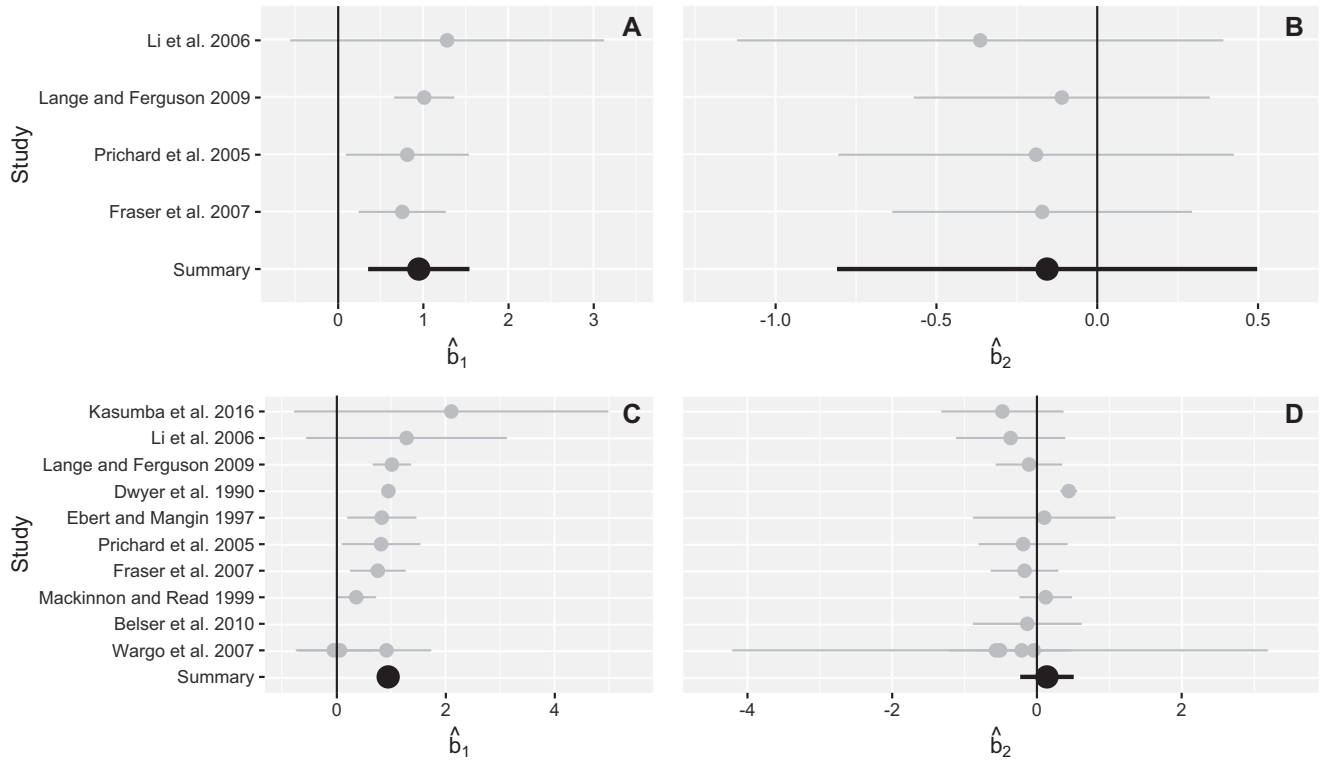

Figure 3. **Distribution of effect sizes (with 95% confidence interval) for studies describing the relationships between replication and transmission following a (A and B) conservative and (C and D) inclusive criteria. Parameter $\hat{b}_1$ represents the linear term and $\hat{b}_2$ the polynomial term. The black symbols represent the overall effects. Studies with multiple dots represent those that included more than one data set in the same study.**

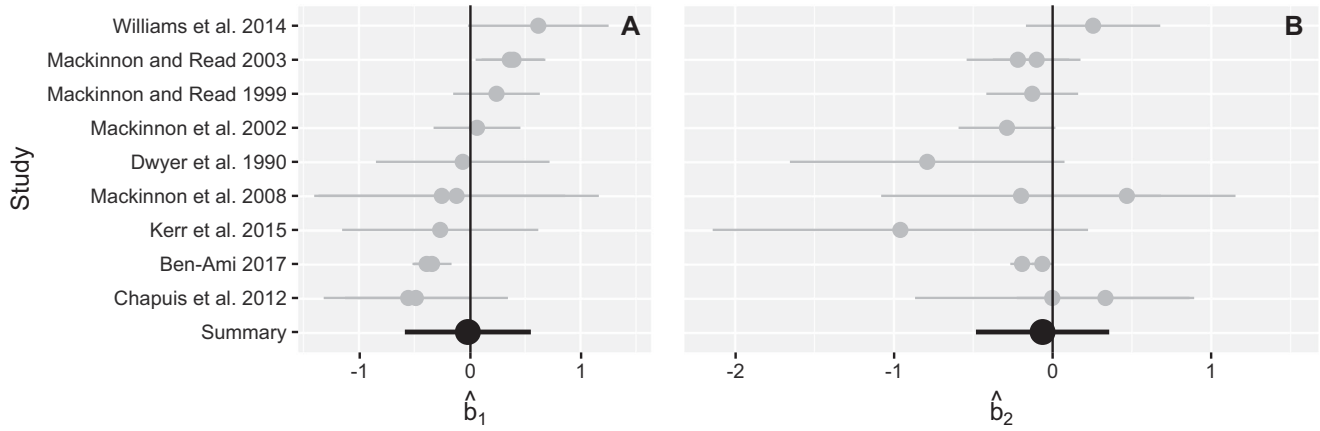

Figure 4. **Distribution of effect sizes (with 95% confidence interval) for the (A) linear and (B) polynomial term of the analysis of studies meeting the inclusive criteria describing the relationships between virulence and transmission. The black symbols represent the overall effects. Studies with multiple dots represent those that included more than one data set in the same study.**

increasing virulence ($\hat{b}_2 = -0.06 \pm 0.21$ SE, $P = 0.77$); however, these patterns are not statistically significant (Fig. 4). A test for heterogeneity shows no significant variability among studies in the linear ($Q_{(df=12)} = 1.89$, $P > 0.99$) or polynomial terms ($Q_{(df=12)} = 1.89$, $P = 0.99$). There was also no significant heterogeneity explained by variation in the virulence measures used in the studies ($QM_{(df>2)} = 1.45$, $P = 0.48$ for $\hat{b}_1$ and $QM_{(df=2)} = 0.55$, $P = 0.78$ for $\hat{b}_2$). Similarly, there was also no significant heterogeneity explained by variation in the transmission measures used in the studies ($QM_{(df=2)} = 0.88$, $P = 0.64$ for $\hat{b}_1$ and $QM_{(df=2)} = 0.46$, $P = 0.79$ for $\hat{b}_2$; Table S15). The leave-one-out diagnostic did not show a study having a

disproportionate effect on $\hat{b}_1$. However, Ben-Ami (2017) had a significant effect on $\hat{b}_2$. When the study is removed, the estimate becomes slightly weaker ($\hat{b}_1 = -0.06 \pm 0.25$ SE, $P = 0.80$; Tables S16 and S17).

RECOVERY AND VIRULENCE

Only one study met our inclusive criteria for the recovery–virulence relationship (Anderson and May 1982), which is not enough to meaningfully synthesize. We found four more studies that did not met our inclusion criteria but may be useful in future syntheses (Mackinnon and Read 2003; Williams et al. 2014; Samuel et al. 2018; Ben-Shachar and Koelle 2018). We included them in the Supporting Information (Table S18).

Discussion

More than 30 years have passed since the formulation of the virulence–transmission trade-off hypothesis that predicts that transmission rates increase at a decelerating rate with a cost of increased virulence to the host (Fig. 1; Anderson and May 1982). We conducted a meta-analysis to quantitatively and rigorously assess the empirical generality of the four core relationships of this hypothesis and to identify areas to prioritize future research. All in all, we found strong support for an increasing relationship between within-host replication and virulence, and between within-host replication and transmission. While this is an important first step toward the generalization of theory, more empirical studies are needed to better understand if these relationships eventually decelerate. Moreover, many more studies are needed to understand the relationship between virulence and transmission, which is the cornerstone of the trade-off hypothesis, and recovery and transmission. Overall, after more than three decades, even though we are standing on firmer ground with regard to Anderson and May (1982)'s predictions, there is much more empirical work to be done.

Each of the relationships varied in degree of support. In the replication–virulence, and the replication–transmission relationships, the mean estimates for both the conservative and inclusive analyses predicted an overall increasing relationship that decelerates. This result is predicted, by theory, in systems in which there is high association between virulence and competition among parasite genotypes (Frank 1996). However, the decelerating polynomial terms were highly uncertain due to relatively high within-study variability, which limits the generalization of this result. Nevertheless, finding strong support for an increasing relationship between replication and virulence in addition to replication and transmission is necessary to assess the generalizability of the trade-off hypothesis. It would have been difficult to justify the remaining predictions without support from this increasing relationship.

Even though the virulence and transmission-rate decelerating functional relationship is the cornerstone of the trade-off hypothesis, just a handful of empirical studies addressed the quantitative relationship between virulence to the main host and variation in transmission rates. However, in multiple instances the mean effect sizes support a decelerating relationship, our analyses show large overall confidence intervals that overlap zero, which suggest that many more studies are needed. The original proponents of the trade-off hypothesis argue that the trade-off curve will be less common in vector- and water-borne diseases because the potential of virulence decreasing host mobility becomes less relevant when vectors are the ones transmitting the pathogen between hosts (e.g., Ewald 1994). However, more than half of the studies that met our criteria were vector-borne transmitted parasites. However, these results may indicate more data are needed or that the hypothesis should be refined, the results definitely point to one of the major research gaps in the study of virulence evolution.

Even when the relationship between virulence and recovery rate was empirically shown in Anderson and May's (1982), now seminal paper, this was the only study we found that met our inclusion criteria. The lack of empirical tests on this relationship represents one of the key gaps in our understanding of the trade-off hypothesis. A decrease in the recovery period has been predicted as the ultimate cost of increased virulence due to the exhaustion of host resources for replication. Without a decrease in the recovery period it would be hard to predict a decelerating relationship between virulence and transmission rate.

A meta-analysis is a powerful tool to generalize the wealth of data produced by individual studies. Application of meta-analysis to understand virulence driven trade-offs is challenging for various reasons. First, because these types of analyses aim to generalize over multiple studies that were not necessarily designed to be analyzed together, we were carefully selective in the studies that were included in the synthesis to ensure consistency. This is a common issue in meta-analysis when conclusions are made on a representative subset of studies that may not necessarily represent the whole breadth of natural systems. Second, the virulence trade-off hypothesis makes qualitative (not quantitative) predictions about the relationship between variables. For instance, the trade-off hypothesis predicts that the relationship between virulence and transmission rate will increase and later decelerate, but it does not specify what type of decelerating function will best describe the relationship (Alizon et al. 2009; Bolker et al. 2010). The functional form that best described the relationship between variables may vary in each host–pathogen system. This makes a quantitative test of the generality of the predictions particularly challenging. To meet this challenge, we opted for an IPD meta–analysis approach that allowed us to use a general polynomial model that would fit the data without making many assumptions about a specific shape of the nonlinear function. Alternatively,

we could have fit a different function to each study (e.g., Bolker et al. 2010); yet, making quantitative syntheses of these would be unfeasible. Overall, our approach can be considered conservative because it requires multiple studies showing a consistent decelerating relationship to result in parameter estimates with small confidence intervals and enough power (replicability). Therefore, our approach provides a robust and conservative means of testing the generality of the predictions of the virulence trade-off hypothesis. As more studies with accessible raw data become available, these conclusions can be easily updated by conducting a similar analysis.

In general, testing the predictions of the trade-off hypothesis is logistically challenging. Some authors argue that lack of empirical evidence for the trade-off hypothesis comes from the fact that measuring virulence and transmission are particularly challenging (Alizon et al. 2009). First, experimental systems require variation in virulence that can be measured, such as, having multiple strains of the pathogen with varying virulence. This virulence also needs to decrease the host's fitness in a way that eventually decreases the infectious period. Second, rigorously measuring transmission rates is difficult and often subject to multiple sources of variation. There is individual variation in immune response along with individual variation in susceptibility and environmental factors that affect transmission rate measures. Moreover, transmission rates are quantified at the population level that captures many aspects of individual variation. Some studies turned to proxies for transmission such as gametocytemia. Even though Mackinnon and Read (1999) showed that for rodent malaria, which is the most representative system in the virulence and transmission rate relationship, gametocytemia and transmission rates are linearly related, these variables do not necessarily follow a linear relationship with true transmission rates in other systems (Bolker et al. 2010).

We found some support for the predictions of the trade-off hypothesis, but the models fitted with the limited empirical data had large unexplained variance. There are multiple potential causes for this large variance. First, there is large intrinsic variation in observed magnitude of virulence in host individuals due to variations in age, nutrition, immunity, or interactions with other pathogens (Grech et al. 2006). For instance, a recent study found an important role of incomplete immunity driving the selection of higher virulence (Fleming-Davies et al. 2018). As noted by Ebert and Bull (2003), the trade-off hypothesis reduces the relationship between variables to two dimensions when multiple variables may interact to influence, for example, the relationship between virulence and transmission rate. For instance, observed virulence may be the result of both the pathogen-replication rate and the host-immune response (André et al. 2003). Pathogen host range might influence the trade-off curve. When comparing generalist and specialist pathogens, generalists may have lower within-host replication rates, but a broader host range. In fact, empirical evidence suggests that virulent pathogens often have narrow host ranges resulting in a trade-off between host-range and within-host reproduction (Leggett et al. 2013). As more data become available, future synthesis efforts will be able to consider the role of life-history strategies and transmission modes driving the trade-off predictions. This will allow us to move beyond the two-dimensional relationships that form the basis of the trade-off hypothesis.

Since the publication of Anderson and May (1982), which has been cited close to 1000 times, the virulence trade-off hypothesis has been a foundational cornerstone in how ecologists and evolutionary biologists try and understand disease transmission dynamics. Given our results, it is clear that more studies are needed to better understand if these theoretical relationships proposed by Anderson and May ultimately decelerate. While it would be best if these additional experimental studies addressed one or more parts of the hypothesis head on, these studies do not necessarily have to be stand alone research projects but can be easy add-ons to current experiments. For instance, in examining the papers that were not included under the replication–virulence portion of the meta-analysis, the vast majority of these papers reported data on the replication of the virus within the host at a variety of time points. With a little additional work, measures of virulence can also be recorded during the experiment whether based on morbidity or mortality associated with the pathogen. Additionally, to ensure nonlinearities are detected when present, experiments should be carried out using a regression-based as opposed to an ANOVA-based experimental design (Inouye 2001; Gotelli and Ellison 2004). That is, instead of having multiple replicates at relatively few treatment levels, we should strive for more treatment levels and less replication, which will better allow for detecting nonlinear dynamics. Thus, by expanding an experiment to collect new data via project add-ons and designing an experiment using a regression-based approach, new insight into how well the empirical data back general theoretical predictions can be gained.

From an empirical standpoint, these experiments could be conducted on host–pathogen systems with short-generation times and high-genetic diversity in order to quickly yield data that would provide a direct test to the theory. For organisms with longer generational times, we may be able to take advantage of long-term data sets for systems that are driven by host–pathogen interactions. There may also be organisms that fit both of the categories—short-generation times and long-term datasets (e.g., lepidopteran forest defoliators; Liebhold and Kamata 2000; Elderd et al. 2008). This would allow researchers to explicitly link short-term laboratory experiments with long-term data sets showing how trade-offs may drive population cycles (Barraquand et al. 2017). In turn, this future work could help to connect the theoretical construct of the virulence trade-off hypothesis directly to the empirical world.

The ideal pathway to move forward with this research agenda will include stronger interactions between theoreticians and empiricists. The historic distance between theoretical and empirical studies is a major barrier to the advancement of ecology and evolution (Scheiner 2013). This is particularly true for the constitutive theory of virulence evolution in which the classical models make many simplifying assumptions that are difficult to meet in empirical studies. As Alizon et al. (2009) emphasized, the theory needs to be expanded to account for some of the complexity inherent in empirical systems. Ideally, empirical studies would also be designed around these more realistic theoretical constructs quantifying variables in units that are consistent with the models. Therefore, the future of this research agenda rests in our ability to develop meaningful interdisciplinary collaborations in which theoreticians contribute to the design of laboratory and field experiments and empiricists contribute to the development of more realistic theoretical models.

While some have argued that the data associated with the virulence–transmission hypothesis may be too noisy to serve as a good framework (Ebert and Bull 2003), others maintain that the framework is sound and a good start for understanding the evolution of virulence (Alizon et al. 2009; Leggett et al. 2013). To our knowledge, we provide the first quantitative synthesis of the empirical evidence testing four core relationships of the trade-off hypothesis. Recent qualitative reviews suggest that empirical evidence supporting the virulence trade-off hypothesis is accumulating and we are close to being able to validate it as a general theory. Our results showing strong evidence for an increasing relationship between replication and virulence, and replication and transmission reinforce, in part, these qualitatively driven reviews (Alizon et al. 2009; Leggett et al. 2013). At the same time, our results suggest that many more studies are needed to better understand the virulence and transmission rate, and the virulence and recovery rate relationships. More studies are also needed to accurately assess if these relationships decelerate as predicted by the theory. As more studies are conducted, the uncertainty in these relationships may decrease. If these relationships continue to be cloudy, the theory forming the basis for the virulence trade-off hypothesis should be carefully refined or reconsidered.

AUTHOR CONTRIBUTIONS

M.A.A., F.P.D., A.J.F., M.J.F., and B.D.E. designed the study and collected the data. M.A.A. analyzed the data. M.A.A. and B.D.E. wrote the manuscript with contributions from all authors.

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Supporting Information

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

Table S1. Studies included in the meta-analysis of replication-virulence prediction of the parasite virulence trade-off hypothesis.
Table S2. Results from the moderator analysis in the replication-virulence (inclusive) relationship.
Table S3. Results from the leave-one-out analysis of the linear parameter (b_1) in the replication-virulence relationship using conservative inclusion criteria.
Table S4. Results from the leave-one-out analysis of the polynomial parameter (b_2) in the replication-virulence relationship using conservative inclusion criteria.
Table S5. Results from the leave-one-out analysis of the linear parameter (b_1) in the replication-virulence relationship following an inclusive inclusion criteria.
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Table S7. Studies included in the meta-analysis of replication-transmission prediction of the parasite virulence trade-off hypothesis.
Table S8. Results from the moderator analysis in the replication-transmission (conservative) relationship.
Table S9. Results from the moderator analysis in the replication-transmission (inclusive) relationship.
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Table S11. Results from the leave-one-out analysis of the polynomial parameter (b_2) in the replication-transmission relationship of the studies that met the conservative inclusion criteria.
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Table S14. Studies included in the meta-analysis of virulence-transmission prediction of the parasite virulence trade-off hypothesis.
Table S15. Results from the moderator analysis in the virulence-transmission relationship.
Table S16. Results from the leave-one-out analysis of the linear parameter (b_1) in the virulence-transmission relationship of studies that met our inclusive inclusion criteria.
Table S17. Results from the leave-one-out analysis of the polynomial parameter (b_2) in the virulence-transmission relationship.
Table S18. Studies with potential data for future syntheses on the recovery-virulence relationship. Note that of these only Anderson and May (1982) met our inclusion criteria; however, we included other studies in this table for future reference.
Figure S1. Figure shows the distribution of studies that did not met the inclusive criteria for the replication–virulence relationship.
Figure S2. Figure shows the distribution of studies that did not met the inclusive criteria for the replication–transmission relationship.
Figure S3. Figure shows the distribution of studies that did not met the inclusive criteria for the virulence–transmission relationship.
Figure S4. Figure shows the distribution of studies that did not met the inclusive criteria for the recovery–virulence relationship.