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Review Article

A meta-analysis highlights the idiosyncratic nature of tradeoffs in laboratory models of virus evolution

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Abstract

Different theoretical frameworks have been invoked to guide the study of virus evolution. Three of the more prominent ones are (i) the evolution of virulence, (ii) life history theory, and (iii) the generalism–specialism dichotomy. All involve purported tradeoffs between traits that define the evolvability and constraint of virus-associated phenotypes. However, as popular as these frameworks are, there is a surprising paucity of direct laboratory tests of the frameworks that support their utility as broadly applicable theoretical pillars that can guide our understanding of disease evolution. In this study, we conduct a meta-analysis of direct experimental evidence for these three frameworks across several widely studied virus–host systems: plant viruses, fungal viruses, animal viruses, and bacteriophages. We extracted 60 datasets from 28 studies and found a range of relationships between traits in different analysis categories (e.g., frameworks, virus–host systems). Our work demonstrates that direct evidence for relationships between traits is highly idiosyncratic and specific to the host–virus system and theoretical framework. Consequently, scientists researching viral pathogens from different taxonomic groups might reconsider their allegiance to these canons as the basis for expectation, explanation, or prediction. Future efforts could benefit from consistent definitions, and from developing frameworks that are compatible with the evidence and apply to particular biological and ecological contexts.

Keywords virus evolution;tradeoffs;virulence;life history theory;niche breadth

Introduction

The biological diversity of viruses—in morphology, natural history, and molecular mechanisms—can limit the development of theories that apply across diverse contexts. Nonetheless, the development of theoretical frameworks for virus evolution has helped set expectations, generate hypotheses, and to make more informed predictions. Three commonly used frameworks include (i) the evolution of virulence, (ii) life history theory, and (iii) the generalism–specialism dichotomy.

The evolution of virulence is perhaps the most popular framework in the field of pathogen evolution. The premise is that the use of within-host resources devoted to virus replication imposes a fitness cost (virulence) that necessarily increases host mortality, thereby reducing the virus' ability to between hosts (Anderson and May 1982, Ewald 1991, Frank 1996). This framework has transformed how professionals across several fields—from the evolutionary sciences to clinical medicine—study the constraints surrounding the harmful viruses cause to their hosts. Several studies have shown evidence of virulence-transmission tradeoffs (Sacrist´an and Garc´ıa-Arenal 2008, Froissart et al. 2010, Goldhill and Turner 2014), while others have suggested this tradeoff is not always evident (Bull 1994, Levin and Bull 1994, Ebert and Bull 2003, Alizon et al. 2009, Doumayrou et al. 2013, Bull et al. 2014). The evolution of virulence remains a widely adopted framework that guides our expectations for how pathogens evolve towards more or less pathogenicity/virulence in host populations.

Life history theory is another framework that has been used to study virus evolution. It focuses on constraints on virus evolution driven by tradeoffs between traits associated with survival and reproduction, analogous to the expected tradeoffs in organisms imposed by energetic limitations (Stearns 1976, 1989), and has been applied to viruses. For example, "reproduction" or "fecundity" relates to the number of virus particles produced from infection and replication. Several studies have demonstrated a tradeoff between such traits and those associated with virus survival [virus particle stability outside the host, sometimes termed as free-living survival (Paepe et al. 2006, Heineman et al. 2012, Brandon Ogbunugafor et al. 2013)], while other studies have not found evidence for this tradeoff (Goldhill and Turner 2014, Han-

del et al. 2014). Even though life history theory was not developed specifically for the study of virus pathogens, its strength lies in its widespread application, which helps to bridge gaps between subfields of evolutionary biology.

The generalism–specialism dichotomy has a long history in the evolutionary ecology literature, applying to organisms across the biosphere (Futuyma and Moreno 1988, Poisot et al. 2011, Leggett et al. 2013). Specialists utilize relatively fewer resources, whereas generalists can access greater resources but are assumed to use them less efficiently (i.e. a "jack-of-all-trades is master of none"). In the context of pathogens, the theory is applied to host niche breadth, the range of hosts that a pathogen can successfully infect and propagate in (Garnick 1992, Fodor 2011). This frame- work has been useful because of its resonance with the ecological challenges associated with viral disease emergence, often defined by a viruses' evolved ability to infect novel hosts (as in zoonosis or host shifts) (Elena et al. 2014, Longdon et al. 2014, Roche et al. 2014). Studies in viruses have demonstrated tradeoffs between generalism and specialism in some circumstances, where fitness decreases when a virus switches or expands its host range (Duffy et al. 2006, Elena et al. 2009, Moreno-P´erez et al. 2016, Bera et al. 2018). Like life history theory, this framework comes with the benefit of a long history applied to other organisms, which means that terms and metrics have not been invoked solely for the study of infectious diseases.

Though these frameworks are well-developed, there is dubious support for their continued relevance to virus evolution. Studies of tradeoff models are also limited by imprecise definitions for key terms, which affect how traits are measured and analyzed. For example, in plants, virulence is often defined as "aggressiveness" (Sacrist´an and Garc´ıa-Arenal 2008, Montarry et al. 2012), while in animals, virulence is defined as the harm done to the hosts (Bull 1994, Read 1994). Meanwhile, in theoretical studies, virulence is often defined as the mortality of host organisms (Anderson and May 1982, Day 2002b). While these differences may seem subtle, they can have powerful consequences for how we understand virulence evolution (Surasinghe et al. 2024). Unfortunately, the imprecision of these terms is often taken for granted in our collective adoption of the evolution of virulence (and other frameworks) in application to medicine and health policy.

In this study, we examine direct evidence from laboratory studies for the three tradeoff frameworks. Specifically, we examine the relationships between traits associated with the different frameworks: transmission versus virulence (evolution of virulence), survival versus reproduction (life history theory), and single-host fitness versus multiple host fitness (generalism-specialism dichotomy) frameworks. We focused only on experimental data and excluded data from theoretical, clinical studies, and observational studies because conditions in laboratory settings can be controlled and traits measured with relative ease. With regards to virus–host type, we focused on viruses that infect plants, animals, fungi, and bacteria, as these are systems where most experiments of this sort have been conducted. We demonstrate that direct support for any particular relationships between traits in these frameworks are scant, often specific to virus–host type and based on subjective definitions of traits (and methods used to measure them). Summarizing, we suggest that tradeoff frameworks applied to viruses be re-evaluated and used with caution in efforts to interpret or predict the direction of pathogenicity in virus evolution.

Methods

Note on the definition of "direct evidence"

Studies of virus evolution are widespread, and include studies from clinical and field settings (Holmes et al. 2005, Fraser et al. 2007, Fargette et al. 2008, Wasik et al. 2023), as well as mathematical and computational explorations (Gandon et al. 2001, Alizon and van Baalen 2005, Kucharski et al. 2015, Miller-Dickson et al. 2019, Gomez et al. 2020). While studies of various kinds have provided valuable contributions to our understanding, those that directly test the relationships between traits that correspond to various virus evolution frameworks are relatively scant. Our meta-analysis focused on direct evidence—studies where traits were measured, evolved, or manipulated in laboratory settings under controlled conditions. We argue that these studies provide an important window into the basis for these theoretical frameworks, and we sought to test whether such laboratory data supported the assumptions of the three theoretical frameworks under study. We note that observational studies have been very valuable in the study of virus evolution tradeoffs (especially in the evolution of virulence) (Fenner and Marshall 1957, Witter 1997, Fraser et al. 2007, McKay et al. 2020). Observational studies are, however, confounded by the myriad variables that could impact how traits associated with virulence and transmissions evolve and are measured in the natural world.

Systematic review of data for meta-analysis

Between 1 January 2023 and 31 March 2023, we searched for scholarly articles from Web of Science, Pub-Med, and Scopus databases. We focused only on these three databases because of their comprehensive coverage of international scholarship and reduced search biases. Searches using the Web of Science were limited to ecology, evolutionary biology, virology, microbiology, infectious diseases, immunology, tropical medicine, and parasitology. Similarly, searches using Scopus were limited to medicine, agricultural and biological sciences, biochemistry, genetics, molecular biology, immunology and microbiology, and pharmacology. The search terms used were "virulence transmission trade-offs viruses," "virulence transmission tradeoffs viruses," virulence transmission trade-offs bacteriophages," "virulence transmission trade-offs bacteriophages," "virulence transmission trade-offs phages," "virulence transmission trade-offs phages," "survival reproduction trade-offs viruses," "survival reproduction trade-offs viruses," survival reproduction tradeoffs bacteriophages," "survival reproduction trade-offs bacteriophages," "survival reproduction trade-offs phages," "survival reproduction trade-offs phages," "host range/expansion/shift and fitness in viruses," "host range/expansion/shift and fitness in bacteriophages," and "host range/expansion/shift and fitness in phages." The studies from our search and those identified from other sources were combined in a spreadsheet. Other sources included several reviews or studies cited in articles from our primary search.

Each study and its metadata were downloaded from the database, and all duplicates were removed, as indicated in the PRISMA chart (Supplementary Fig. S1). Some studies were excluded for various reasons, e.g. studies that did not focus on viruses, studies that were computational/mathematical, and those that were conference/opinion/perspectives or poster abstracts. Notably, many of the studies from our searches were computational/mathematical, where theoretical ideas in infectious disease have been studied for many decades. After this, the

final studies were further screened, and if they did not directly test the frameworks or present data relevant to the frameworks, they were removed.

Our main inclusion criteria for meta-analysis were as follows: (i) the study served as primary literature and was published in a peer-reviewed academic journal, (ii) the study tested or measured the relationships within one or more of the three frameworks described for viruses (evolution of virulence, life history theory, generalism–specialism dichotomy), and (iii) correlational data were reported or could be calculated from the data reported. The remaining studies were screened under our inclusion criteria, and only those that met the criteria were used for data extraction. The inclusion criteria were carefully chosen to ensure that the studies in our analysis were relevant to our meta-analysis goal. Extracted data included the following: virus–host type, virus genome type, traits measured, statistics reported, sample size, and study reference (Supplementary Fig. S1).

Categorization of data

We discretized the data into categories based on theoretical frameworks, combined virus–host type, and coupled categories. Datasets were placed in their respective frameworks based primarily on traits measured in the studies used in this meta-analysis. For virus–host type, separate categories were made for animal viruses and bacteriophages. For coupled categories, different analyses were done, as summarized in the results. We analyzed data by taking a gross look at the relationships between traits for all datasets (Supplementary Fig. S2) and then used one of the following methods: (i) a theoretical framework across virus-host types; (ii) a virus-host categorization across theoretical frameworks, and (iii) additional analyses of different coupled categories (Supplementary Fig. S3).

Statistical analyses

All meta-analyses were done following the methods described by previous studies (Schwarzer et al. 2015, Acevedo et al. 2019, Rafaluk-Mohr 2019, Harrer 2021). Correlations were extracted from studies and also calculated in instances where they were not directly reported. Since we were only interested in the relationship effect sizes, we pooled correlation coefficients, and all the meta-analyses were done using random effects models in the metacor function of the meta package (Balduzzi et al. 2019) of R version 4.1.1 (Posit 2023). Random effects models were useful here because, in addition to accounting for the sampling error of the pooled effect sizes, they also account for variation introduced by datasets drawn from different studies (Hedges and Vevea 1998). We used the Sidik–Jonkman (SJ) estimator (Sidik and Jonkman 2005) in the meta package to estimate between-study heterogeneity (R^2) resulting from differences in sample size experimental design and virus–host systems However, regardless of the estimator used in a meta-analysis, there can be bias. To control for this, we used the Knapp–Hartung adjustments (Knapp and Hartung 2003) to calculate confidence intervals of pooled effect sizes because the number of datasets was small and the heterogeneity was high. Visualization of publication biases was conducted using contour-enhanced funnel plots shown in the Supplementary Material (Supplementary Fig. S4). In addition, after each meta-analysis was performed, the find.outliers function was used to identify outliers. After outliers were identified, additional meta-analyses were performed without outliers.

Results

Our meta-analysis examined direct experimental evidence for traits associated with the three theoretical frameworks (evolution of virulence, life history theory, and generalism–specialism dichotomy) of virus evolution. We extracted 60 datasets from 28 studies (Table 1, Supplementary Fig. S1) and compiled direct evidence for tradeoffs between traits measured in studies of virus evolution. Next, we explored a different possibility with regards to tradeoffs: perhaps relationship patterns between traits are less dependent on the model but are specific to virus–host system. To investigate this possibility, we examined tradeoffs between measured traits within virus–host types.

Evolution of virulence

In the evolution of virulence framework, we found a nonsignificant positive correlation between traits associated with transmission and virulence (Fig. 1, Table 2) [$N = 32$; $R = 0.1541$; 95% confidence interval (CI): −0.2178 – 0.4869; $t = 0.84$; P - value = .4068] consistent with an absence of a tradeoff. The test for heterogeneity was high (Q = 384.61; df = 27; P- value < .0001), and the I^2 test was 93.0% with a 95% CI of 90.9–94.6%. Six outliers were identified (datasets 1, 4, 28, 29, 45, 52). Without the outliers, we found a positive correlation ($N = 26$; $R = 0.2209$; 95% CI: −0.0363 to 0.4506; $t = 1.77$; P- value = .0884). While the test for heterogeneity was lower (Q = 89.46; df = 21; P-value < .0001), and the I^2 test was 76.5% with a 95% CI of 64.7–84.4%, these results were highly significant. Distributions of effect sizes are shown in (Fig. 1). In addition, because this framework had multiple studies with a sample size of $N = 3$, we conducted an additional analysis (Supplementary Fig. S5) to demonstrate that removing these studies of $N = 3$ did not affect the overall correlation found in the primary analysis.

Life history theory

Relatively, fewer studies were found for the life history theory framework (Fig. 2a, Table 2). We found a nonsignificant negative correlation between survival and reproduction ($N = 10$; $R = -0.6358$; 95% CI: −0.9266 to 0.1305; $t = -1.93$; P- value = .0863). The test for heterogeneity was high (Q = 173.32, df = 7; P- value = .0001), and the I^2 test was 96.0% with a 95% CI of 93.9–97.3%. Running the analysis without the identified outliers (Datasets 36, 43, 53), we found a significant negative correlation implying tradeoffs between reproduction and survival ($N = 7$; $R = -0.3217$; 95% CI: −0.5504 to −0.0481; $t = -2.86$; P-value = .0288). The test for heterogeneity was reduced (Q = 10.36; df = 4; P- value = .0348), and the I^2 test was 61.4% with a 95% CI 0.0–85.5%.

Generalism–specialism

Studies in this framework showed a significant negative correlation of traits between host shift/expansion and fitness ($N = 18$; $R = -0.5497$; 95% CI: −0.6769 to −0.3905; $t = -6.34$; P- value = .0001) (Fig. 2b, Table 2). The test for heterogeneity was low (Q = 15.90; df = 12; P-value = .1959), and the I2 test was 24.5% with a 95% CI of 0.0–60.8%. No outliers were detected in this framework.

Plant viruses

For plant viruses, we found a significant positive correlation between virulence and transmission ($N = 22$; $R = 0.4442$; 95% CI: 0.1838–0.6464; $t = 3.41$; P-value = .0027). A test of the between-study heterogeneity variance was estimated (Q = 110; df = 15; P-value = .0027); I2 = 86.4 with a 95% CI of 79.5–91.0%. Running the

Table 1. Datasets used in meta-analysis

Dataset	Virus type	Host	Genome	Traits	Framework
1	Tomato spotted wilt virus	Plant	ssRNA	Transmission vs. virus titer (Rotenberg et al. 2009)	EoV
2	Tomato spotted wilt virus	Plant	ssRNA	Transmission vs. virus titer (Rotenberg et al. 2009)	EoV
3	Tomato spotted wilt virus	Plant	ssRNA	Transmission vs. virus titer (Rotenberg et al. 2009)	EoV
4	Tomato spotted wilt virus	Plant	ssRNA	Transmission vs. virus titer (Rotenberg et al. 2009)	EoV
5	Tomato spotted wilt virus	Plant	ssRNA	Transmission vs. virus titer (Rotenberg et al. 2009)	EoV
6	Cucumber mosaic virus	Plant	ssRNA	Accumulation vs. infection (Sacrist´an and Garc´ıa-Arenal 2008) effects	EoV
7	Cucumber mosaic virus	Plant	ssRNA	Growth effects on original host (Sacrist´an and Garc´ıa-Arenal 2008)	G/S
8	Cucumber mosaic virus	Plant	ssRNA	Original hosts vs. diversification (Sacrist´an and Garc´ıa-Arenal 2008)	G/S
9	Rice yellow mottle virus	Plant	ssRNA	Virus titer vs. weight loss (Poulicard et al. 2010)	EoV
10	Rice yellow mottle virus	Plant	ssRNA	Virus titer vs. weight loss (Poulicard et al. 2010)	EoV
11	Rice yellow mottle virus	Plant	ssRNA	Virus titer vs. weight loss (Poulicard et al. 2010)	EoV
12	Potato virus Y	Plant	ssRNA	Virus aggressiveness vs. virus loss (Montarry et al. 2012)	EoV
13	Potato virus Y	Plant	ssRNA	Virus aggressiveness vs. virus loss (Montarry et al. 2012)	EoV
14	Cauliflower mosaic virus	Plant	dsDNA	Leaf reduction vs. transmission (Doumayrou et al. 2013)	EoV
15	Cauliflower mosaic virus	Plant	dsDNA	Viral accumulation vs. leaf reduction (Doumayrou et al. 2013)	EoV
16	Cauliflower mosaic virus	Plant	dsDNA	Viral reduction vs. transmission (Doumayrou et al. 2013)	EoV
17	Cauliflower mosaic virus	Plant	dsDNA	Accumulation vs. virulence (Doumayrou et al. 2013)	EoV
18	Cauliflower mosaic virus	Plant	dsDNA	Accumulation vs. virulence (Doumayrou et al. 2013)	EoV
19	Cauliflower mosaic virus	Plant	dsDNA	Accumulation vs. transmission (Doumayrou et al. 2013)	EoV
20	Cauliflower mosaic virus	Plant	dsDNA	Accumulation vs. transmission (Doumayrou et al. 2013)	EoV
21	Cauliflower mosaic virus	Plant	dsDNA	Viral load vs. transmission (Doumayrou et al. 2013)	EoV
22	Cauliflower mosaic virus	Plant	dsDNA	Viral load vs. transmission (Doumayrou et al. 2013)	EoV
23	SpexNPV	Animal	dsDNA	Speed to kill vs. virus yield (Redman et al. 2016)	EoV
24	SpexNPV	Animal	dsDNA	Speed to kill vs. virus yield (Redman et al. 2016)	EoV
25	Cryphonectria hypovirus	Fungi	dsRNA	Colony size vs. sporulation (Brusini et al. 2017)	EoV
26	Cryphonectria hypovirus	Fungi	dsRNA	Colony size vs. spore size (Brusini et al. 2017)	EoV
27	Cryphonectria hypovirus	Fungi	dsRNA	Spore size vs. sporulation (Brusini et al. 2017)	EoV
28	West Nile virus	Animal	ssRNA	Attachment rate vs. alternating host (Deardorff et al. 2011)	EoV
29	West Nile virus	Animal	ssRNA	Survival vs. viral load (Ciota et al. 2013)	EoV
30	Vesicular stomatitis virus	Animal	ssRNA	Fitness vs. transmission time (Elena 2001)	G/S
31	Vesicular stomatitis virus	Animal	ssRNA	Fitness vs. transmission time (Elena 2001)	G/S
32	Vesicular stomatitis virus	Animal	ssRNA	Fitness vs. transmission time (Elena 2001)	G/S
33	Vesicular stomatitis virus	Animal	ssRNA	Fitness vs. transmission time (Elena 2001)	G/S
34	Vesicular stomatitis virus	Animal	ssRNA	Fecundity vs. survival (Brandon Ogbunugafor et al. 2013)	LHT
35	Vesicular stomatitis virus	Animal	ssRNA	Fecundity vs. survival (Brandon Ogbunugafor et al. 2013)	LHT
36	Vesicular stomatitis virus	Animal	ssRNA	Fitness vs. alternating hosts (Turner and Elena 2000)	LHT
37	Vesicular stomatitis virus	Animal	ssRNA	Survival vs. reproduction (Wasik et al. 2015)	LHT
38	Vesicular stomatitis virus	Animal	ssRNA	Generalists vs specialists (Alto and Turner 2010)	G/S
39	Vesicular stomatitis virus	Animal	ssRNA	Generalists vs specialists (Alto and Turner 2010)	G/S
40	Vesicular stomatitis virus	Animal	ssRNA	Generalists vs specialists (Alto and Turner 2010)	G/S
41	Vesicular stomatitis virus	Animal	ssRNA	Generalists vs specialists (Alto and Turner 2010)	G/S
42	Vesicular stomatitis virus	Animal	ssRNA	Robustness vs. thermostability (Presloid et al. 2016)	LHT
43	Vesicular stomatitis virus	Animal	ssRNA	Robustness vs. thermostability (Presloid et al. 2016)	LHT
44	Coliphages	Bacteria	NA	Multiplication rate vs. decay rate (Paepe et al. 2006)	LHT
45	ΦX174	Bacteria	ssDNA	Growth rates vs. attachment rates (Crill et al. 2000)	EoV
46	$Q\beta$	Bacteria	ssRNA	Adsorption rate vs. infectivity (Garc´ıa-Villada and Drake 2013)	EoV
47	Φ6	Bacteria	dsRNA	Fitness vs. attachment rate (Ford et al. 2014)	EoV
48	Φ6	Bacteria	dsRNA	Fitness vs. host range (Ferris et al. 2007)	G/S
49	Φ6	Bacteria	dsRNA	Fitness vs. host range (Ferris et al. 2007)	G/S
50	Φ6	Bacteria	dsRNA	Fitness vs. host range (Ferris et al. 2007)	G/S
51	$Q\beta$	Bacteria	ssRNA	Growth vs. fitness (Domingo-Calap et al. 2010)	LHT
52	T7	Bacteria	dsDNA	Adsorption rate vs. infectivity (Heineman et al. 2012)	EoV
53	P5	Bacteria	dsDNA	Mortality vs. reproduction rate (Dessau et al. 2012)	LHT
54	ID11	Bacteria	ssDNA	Fitness vs. binding affinity (Lee et al. 2011)	EoV
55	ID11	Bacteria	ssDNA	Fitness vs. decay rate (Lee et al. 2011)	LHT
56	$Q\beta$	Bacteria	ssDNA	Fitness vs. thermal adaptation (L´azaro et al. 2018)	G/S
57	$Q\beta$	Bacteria	ssDNA	Fitness vs. thermal adaptation (L´azaro et al. 2018)	G/S
58	$Q\beta$	Bacteria	ssDNA	Fitness vs. thermal adaptation (L´azaro et al. 2018)	G/S
59	Φ6	Bacteria	dsRNA	Generalists vs. specialists (Bono et al. 2015)	G/S
60	Φ6	Bacteria	dsRNA	Generalists vs. specialists (Bono et al. 2015)	G/S

Datasets were not grouped even if they infect the same host or w from the same study. They were analyzed individually and restricted to one framework: evolution of virulence life history theory (LHT), and generalism–specialism dichotomy (G/S) (see "Methods" section). We note that the "Host does not provide any specific detail on species or subtaxa." The hosts used in each study may or may be commonly associated with that virus in nature. We urge interested readers to consult individual those particulars.

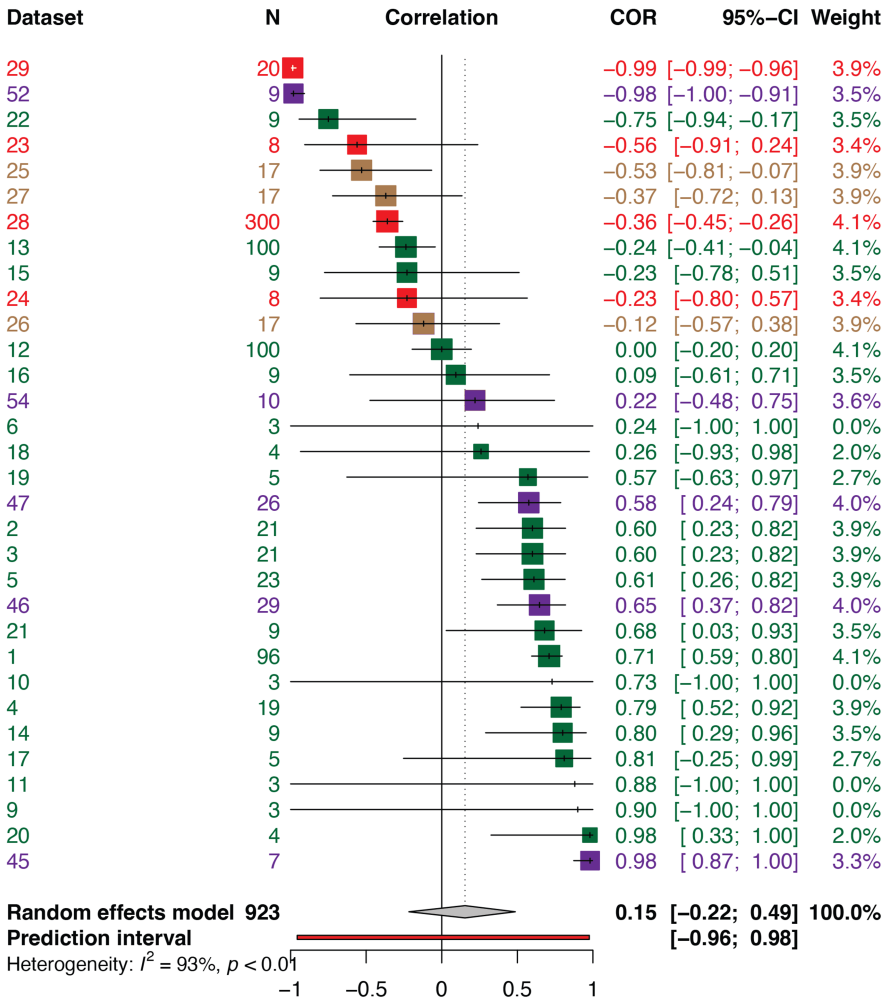

Figure 1. Forest plot showing the distribution of effect sizes for evolution of virulence datasets. The gray diamond represent the pooled correlation for evolution of virulence (*R* = 0.15). The colored squares represent the weight of the study on the pooled correlation, and the vertical black dash inside the colored squares shows the extracted correlation of the dataset. Datasets 6, 9, 10, and 11 have zero weight on overall correlation; therefore, they are not represented by any colored squares in this plot. Horizontal lines represent 95% confidence intervals for the dataset. Negative and positive correlations indicate values for individual datasets. The 95% confidence intervals and the weight of each dataset on the pooled correlation are indicated. The evolution of virulence framework is dominated mainly by plant viruses, represented by datasets 1, 2, 3, 4, 5, 6, 9, 10, 11, 12, 13, 14, 15, 16,17, 18, 19, 20, 21, 22 (green squares). Viruses that infect animals are represented in datasets 23, 24, 28, 29 (red squares), fungi datasets 25, 26, 27 (brown squares), and bacteriophages datasets 45, 46, 47, 52, 54 (purple squares).

test without outliers (Datasets 13 and 22), we found a significant positive correlation (*N* = 20; *R* = 0.5658; 95% CI: 0.3910–0.7013; *t* = 5.88; *P*-value < .0001) with a reduced test of between-study heterogeneity variance (Q = 56.46; df = 13; *P*-value < .0001 and I2 = 77.0% and 95% CI 61.6–86.2%) (Fig. 3a, Table 2). In addition, we performed an analysis of viruses that infect plants with viruses that infect fungi (Table 2, Supplementary Fig. S6). We found a significant positive relationship (*N* = 25; *R* = 0.3150; 95% CI: 0.0417–0.5444; *t* = 2.37; *P*- value = .0264). A test for heterogeneity was also estimated (Q = 129.75; df = 18; *P*- value < .0001; I2 = 86.1 with a 95% CI 79.7–90.5%. Without outliers (Datasets 1, 13, 22, and 25), we found a positive correlation (*N* = 21; *R* = 0.4411; 95% CI: 0.2042–0.6292; *t* = 3.77; *P*-value < .0014; Q = 49.33; df = 14; *P*- < .0001; I2 = 71.6%; 95% CI: 52.2–83.1%).

Animal viruses

For viruses infecting animals, we found a significant overall negative correlation (*N* = 18; *R* = −0.6429; 95% CI: −0.8571 to −0.2392; *t* = −3.10; *P*-value = .0065 (Fig. 3b; Table 2). A test of between-study heterogeneity variance was estimated (Q = 145.07; df = 12; *P*-value = .0001; I2 = 91.7%; 95% CI: 87.7–94.5%). Without outliers (Datasets 29, 36, and 43), we found a negative correlation between traits (*N* = 15; *R* = −0.3990; 95% CI: −0.5417 to −0.2340; *t* = −4.92; *P*-value < .0002). A test of between-study heterogeneity variance was estimated (Q = 13.22; df = 12; *P*-value = 0.3532; I2 = 9.2%; 95% CI: 0.0–47.6%).

Bacteriophages

For bacteriophages, we found a significant negative correlation (*N* = 17; *R* = −0.3535; 95% CI: −0.7294 to 0.1863; *t* = −1.40; *P*-value = .0001. A test for between-study heterogeneity variance was estimated at (Q = 239.22; df = 16; *P*- = .0001), and I2 at 93.3%; 95% CI: 90.7–95.2%. Without outliers (Datasets 45, 46, 47, 52, and 53), we found a significant negative correlation between traits (*N* = 12; *R* = −0.4213; 95% CI: −0.6153 to 0.1792; *t* = −3.69; *P*-value = .0036 and I2 at 47.6%. The test for heterogeneity was reduced (Q = 20.99; df = 11; *P*-value = .335; I2 = 47.6%; 95% CI: 0.0–73.1%). (Fig. 3c, Table 2.)

Table 2. Summary statistics from all analyses

Analysis	Relationship	Correlation	Correlation P-value	Q	P-value	K
All three frameworks	Negative	–0.1818	.1745	638.07	.0001	60
Evolution of virulence	Positive	0.1541	.4068	384.61	.0001	32
Life history theory	Negative	–0.6358	.0863	173.32	.0001	10
Generalism–specialism	Negative	–0.5497	.0001	15.9	.1959	18
Plant viruses	Positive	0.4442	.0027	110	.0027	22
Plant/Fungal viruses	Positive	0.5444	.0264	129.8	<.0001	25
Animal viruses	Negative	–0.6429	.0065	145.07	.0001	18
Bacteriophages	Negative	–0.3535	.0001	239.22	.0001	17
Evolution of virulence & life history theory	Negative	–0.0487	.7841	597.54	.0001	42
Evolution of virulence & plant viruses	Positive	0.4442	.0043	110.32	.0001	20
Evolution of virulence & animal viruses	Negative	–0.7396	.1692	70.422	.0001	4
Life history theory & animal viruses	Negative	–0.6267	.2996	51.84	.0001	6
Generalism–specialism & animal viruses	Negative	–0.7145	.0002	1.33	.8569	8
Evolution of virulence & bacteriophages	Positive	0.3163	.6816	63.75	.0001	5
Life history theory & bacteriophages	Negative	–0.6717	.2107	68.54	.0001	4
Generalism–specialism & Bacteriophages	Negative	–0.5131	.0069	13.25	.0663	8

Effect sizes (correlations) with their P-values, tests for between-study heterogeneity (Q) with P-values, and the number of datasets combined in the analysis (K) are summarized.

Analysis of coupled categories

The above analyses focus on the relationships between traits organized by tradeoff model or virus–host system. However, there is a possibility of interactions between these categories. That is, a trade-off framework (the evolution of virulence, for example) may have a statistical signature specific to certain virus–host systems Furthermore, we recognize that certain frameworks might be combined due to similarities in how traits are measured [e.g. some studies mention the evolution of virulence and life history theory in single studies (Brandon Ogbunugafor et al. 2013)]. It should be noted that Table 2 summarizes the results from analyses of frameworks and host types in detail and describes the results of datasets of the coupled categories. The analysis column specifies the type of analysis performed, and the nature of correlation is indicated along with corresponding P-values. Because of the different categories analyzed, we included the level of heterogeneity Q and the corresponding p – values with the number of datasets in the respective category. Below, we highlight some of the findings, also summarized in Table 2.

- The analysis of evolution of virulence that was combined with life history theory returned a nonsignificant negative correlation between traits ($N = 42$; $R = -0.0487$; 95% CI: –0.3845 to 0.2985; $t = -0.28$; P-value = .7841; Q = 597.54; df = 35; P-value < .0001; I2 = 94.1%; 95% CI: 92.8–95.3%) (Supplementary Fig. S3).
- An analysis of traits associated with the evolution of virulence in only plant viruses produced a significant positive correlation between traits ($N = 20$; $R = 0.4442$; 95% CI: 0.1674 – 0.6561; $t = 3.24$; P-value = .0043; Q = 110.32; df = 15; P-value < .0001; I2 = 86.4%; 95% CI: 79.5– 91.0%).
- Studies of the evolution of virulence in animal viruses showed a nonsignificant negative correlation between traits ($N = 7$; $R = -0.6036$; 95% CI: –0.8970 to 0.0590; $t = -2.26$; P-value = .0649; Q = 72.52; df = 6; P-value < .0001; I2 = 91.7%; 95% CI: 85.5–95.3%).
- Studies using life history theory in animal viruses showed a nonsignificant negative correlation between traits ($N = 6$; $R = -0.6267$; 95% CI: –0.9827 to 0.7161; $t = -1.16$; P-value = .2996; Q = 51.84; df = 3; P-value < .0001; I2 = 94.2%; 95% CI: 88.3–97.1%).
- Those studies examining the generalism–specialism dichotomy analyzed in animal viruses showed a significant negative correlation between traits ($N = 8$; $R = -0.7145$; 95% CI: –0.8312 to –0.5375; $t = -7.17$; P-value = .0002; Q = 1.33; df = 4; P-value < .8569; I2 = 0%; 95% CI: 0.0–79.2%).
- Studies of the evolution of virulence analyzed in bacteriophages showed a nonsignificant positive correlation between traits ($N = 5$; $R = 0.3163$; 95% CI: –0.9393 to 0.9832; $t = 0.44$; P-value = .6816; Q = 63.75; df = 4; P-value < .0001; I2 = 93.7%; 95% CI: 88.2–96.7%).
- Life history theory in bacteriophages showed a nonsignificant negative correlation between traits ($N = 4$; $R = -0.6717$; 95% CI: –0.9851 to 0.6741; $t = -1.59$; P-value = .2107; Q = 68.54; df = 3; P-value < .0001; I2 = 95.6%; 95% CI: 91.6–97.7%).
- The generalism–specialism dichotomy analyzed in bacteriophages produced a significant negative correlation of traits ($N = 8$; $R = -0.5131$; 95% CI: –0.7268 to –0.2089; $t = -3.78$; P-value = .0069; Q = 13.25; df = 7; P-value = .0663; I2 = 47.2%; 95% CI: 0.0–76.5%).

Discussion

In this study, we examined direct (experimental, laboratory-based) evidence for tradeoffs between traits across three frameworks: evolution of virulence, life history theory, and the generalism–specialism dichotomy. We find that evidence for relationships between traits differ according to the framework examined and host–virus type. In general, the direct evidence for tradeoffs between traits in any of the frameworks is mixed, with some evidence for correlation and anticorrelation depending on the tradeoff model and virus–host system. More broadly, our findings suggest that while such theory may be sound in conception, direct experimental evidence does not point to a singular framework offering consistent insight across a suite of virus–host types or scientific disciplines. That is, the support for tradeoff frameworks is highly idiosyncratic.

The evolution of virulence

This is likely the most widely adopted framework in the evolution of infectious disease is the evolution of virulence. Thus, it may qualify as a main focus of our study, as virulence studies constitute the majority of direct tests of tradeoff models focusing on some aspect of virus evolution. Because the evolution of virulence

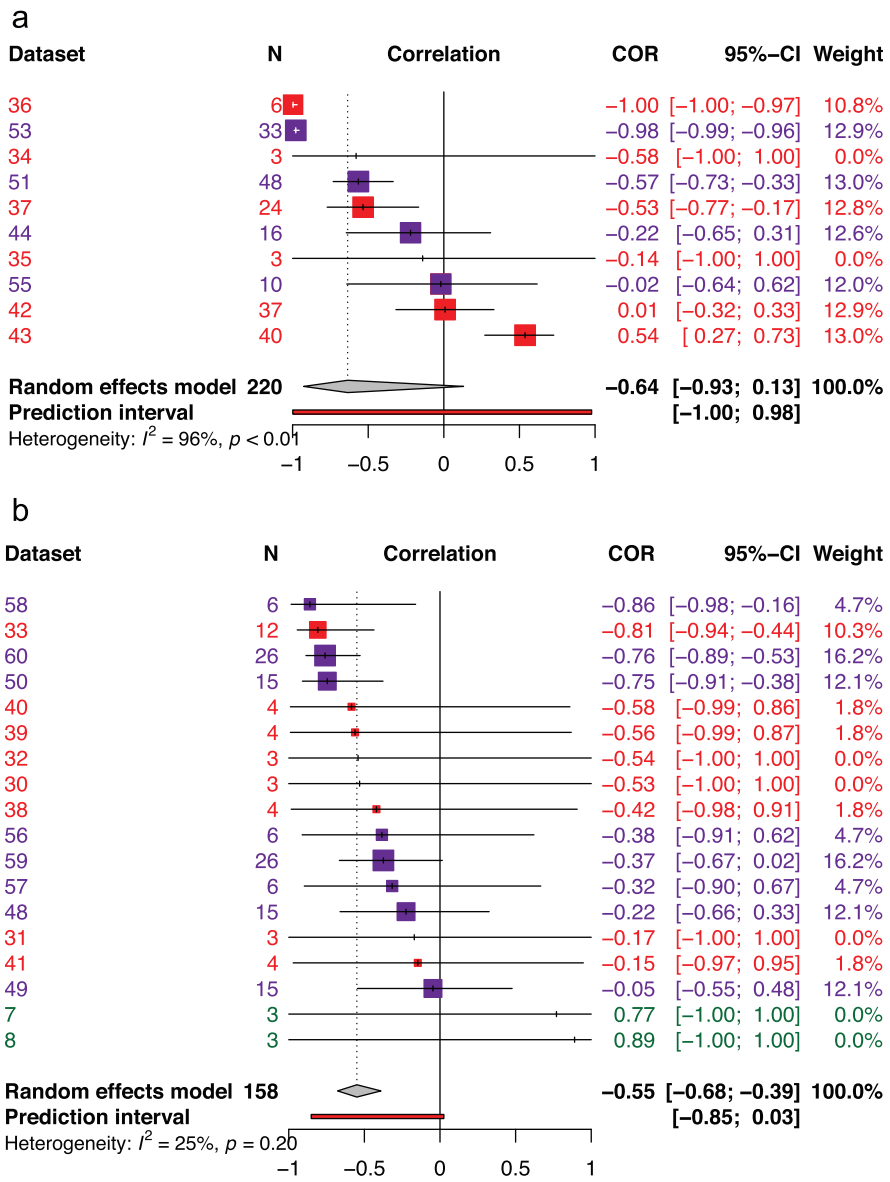

Figure 2. Forest plots displaying the distribution of effect sizes (correlations) for the life history theory (a), and the generalism–specialism dichotomy (b). The gray diamonds represent the pooled correlations, evolution of virulence (R = 0.15), life history theory (R = –0.64), and the generalism–specialism dichotomy (R = –0.55). The life history framework is mainly represented by negative correlations, which implies a tradeoff between survival and reproduction with animal viruses represented by datasets 34, 35, 36, 37, 42, and 43 and bacteriophages represented by datasets 44, 51, 53, and 55. Animal viruses in the generalism–specialism dichotomy are represented by datasets 30, 31, 32, 33, 38, 39, 40, and 41 while bacteriophages are represented by datasets 48, 49, 50, 56, 57, 58, 59, and 60. Datasets 7 and 8 represent plant viruses. Across panels, bacteriophage data sets are color-coded in purple, animal viruses in red, and plant viruses in green.

is applied so broadly, the direct evidence for it demonstrated great variation with respect to how traits (associated with virulence and transmission) are measured. For example, in Cauliflower mosaic virus (CMV), dry weight reduction in host stems and leaves is used as a proxy for virulence (Doumayrou et al. 2013), while in Tomato spotted wilt virus (TSWV), virulence is measured as the estimated number of virus titers in Frankliniella occidentalis (western flower thrips); similarly, transmission is also measured by the thrips ability to transmit viruses (Rotenberg et al. 2009). Until now, the evolution of virulence paradigm has often treated virulence as a trait driven by the biology of the pathogen and may undervalue the role of host biology (e.g. via the immune system), medical interventions, coinfection, and many other modulators (Casadevall et al. 1999). Virulence could be considered a complex trait composed of factors from the pathogen, host, other modifiers, and interactions between them. Therefore, theory governing how virulence evolves could be modernized to include these parameters. Relatedly, scientists could better explain how traits are measured and be more consistent with measures across systems.

Our findings reflect this complexity. As summarized in Table 2, the nature of relationships between traits differs, and our analyses show mixed results depending on the category of analysis performed. For example, in plant viruses, relationships between virulence and transmission appear positive; in animal viruses, negative; in bacteriophages, positive. That is, there is no consistent pattern for how virulence and transmission relate. Moreover, it may be specific to the system and setting. In addition,

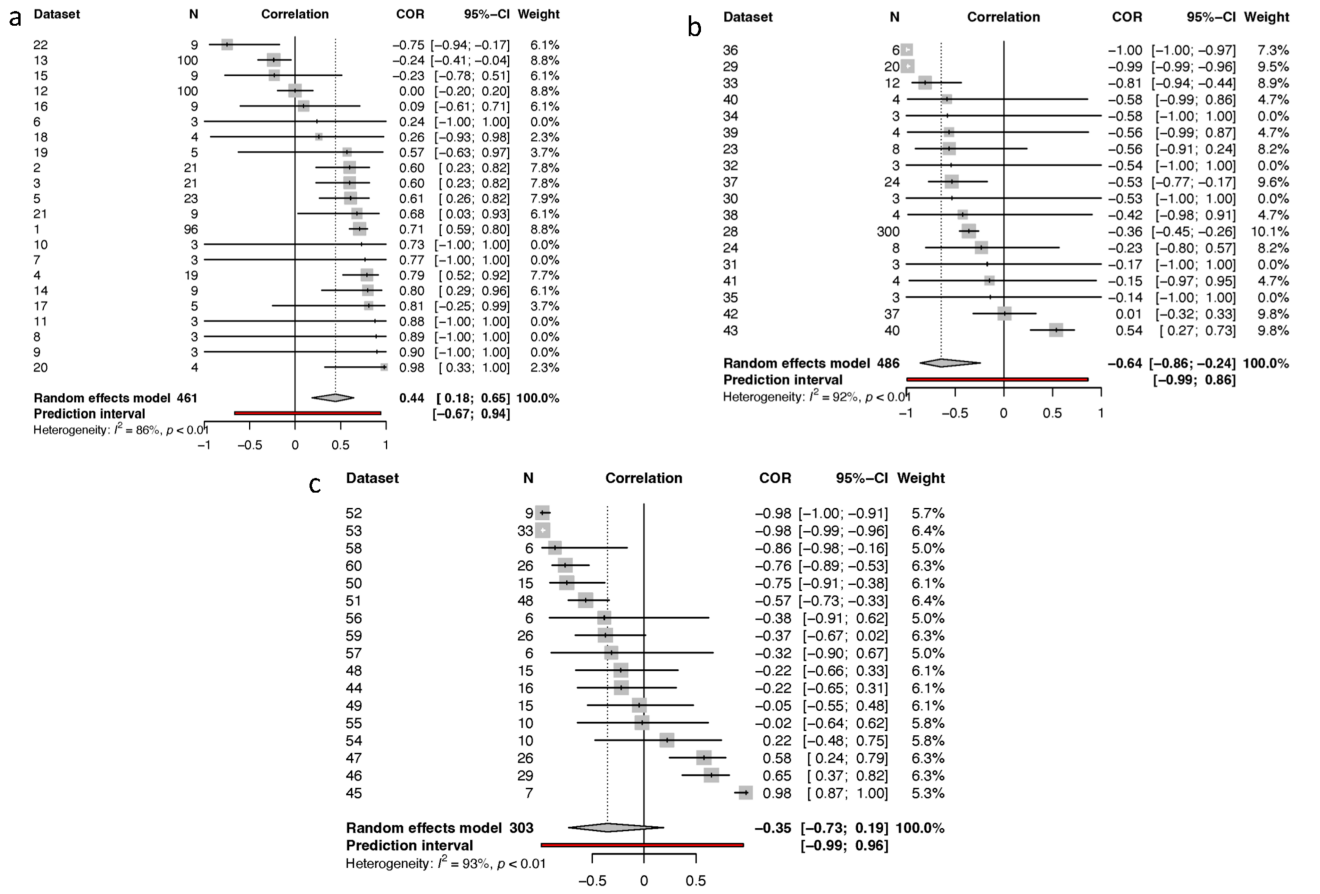

Figure 3. Forest plots showing the distribution of effect sizes for virus–host categories. Forest plot showing the distributions of effect sizes for viruses infecting plants (a), animals (b), and bacteriophages (c). The diamond indicates the pooled correlations. Viruses that infect plants indicate an overall positive correlation, which implies a positive relationship between virulence and transmission ($R = 0.44$). Most plant viruses tested the evolution of virulence framework, except for datasets 7 and 8 which tested the generalism–specialism dichotomy. No datasets explored life history theory in this category. Viruses that infect animals represented negative correlations with a pooled correlation of $R = -0.6429$. This category had a reasonable distribution of datasets across all three frameworks. The evolution of virulence is represented by datasets 23, 24, 28, and 29. The life history theory category is represented by datasets 34, 35, 36, 37, 42, 43, while the generalism–specialism dichotomy is represented by datasets 30, 31, 32, 33, 38, 39, 40, and 41. The pooled correlation for bacteriophages is $R = -0.35$ with most datasets identified for the generalism–specialism dichotomy (datasets 48, 49, 50, 56, 57, 58, 59, and 60). The evolution of virulence is represented by datasets 45, 46, 47, 52 and 54, and life history theory represented by datasets 44, 51, 53, and 55.

generalism–specialism has a significant (negative) relationship, the only framework with a strongly significant global pattern. When we look at other host–virus systems, more subtle patterns emerge, indicative of the differences in biology: plant viruses show a positive relationship, while bacteriophages and animal viruses show negative relationships. This might reflect differences in how frameworks are used and how data collection methods are applied across different host–virus systems. Furthermore, we show the presence of publication bias and significant between-study heterogeneity. These features all highlight the differences in how frameworks are considered and tested.

Life history theory

Traits associated with life history theory are relatively easy to define and measure, as "survival" and "reproduction" can be described and quantified as less ambiguous than traits involved in tradeoffs in other frameworks. However, there were few experimental tests of life history theory tradeoffs of viruses in our search and analysis. Most tests we analyzed in this study had negative correlations between traits, with wide variance across studies. Notably, one of the strengths of life history theory is how it includes the evolution of the ability to survive outside a host (free-living survival and fomite transmission). Indirect or fomite transmission can be relevant in natural histories where viruses must spend much of their life cycle in the extra-host environment. In this sense, there is a conceptual overlap between life history theory and the evolution of virulence, as "survival" in a life history framing relates to "transmission" in the evolution of virulence (Brandon Ogbunugafor et al. 2013).

The generalism–specialism dichotomy

Of the three frameworks we examined, the generalism–specialism dichotomy contains the data with the least heterogeneity between datasets. However, this may be due to one virus–host system being overrepresented in the experimental data: vesicular stomatitis virus (VSV). VSV is widely used in evolution studies due to its wide host range and relative tractability under experimental conditions (Hanson 1952, Turner and Elena 2000). Considering the potential disease implications (especially for humans and animals), one can expect studies of animal viruses to be overrepresented. Nonetheless, the relative consistency in measurements from study to study are not necessarily reflective of the

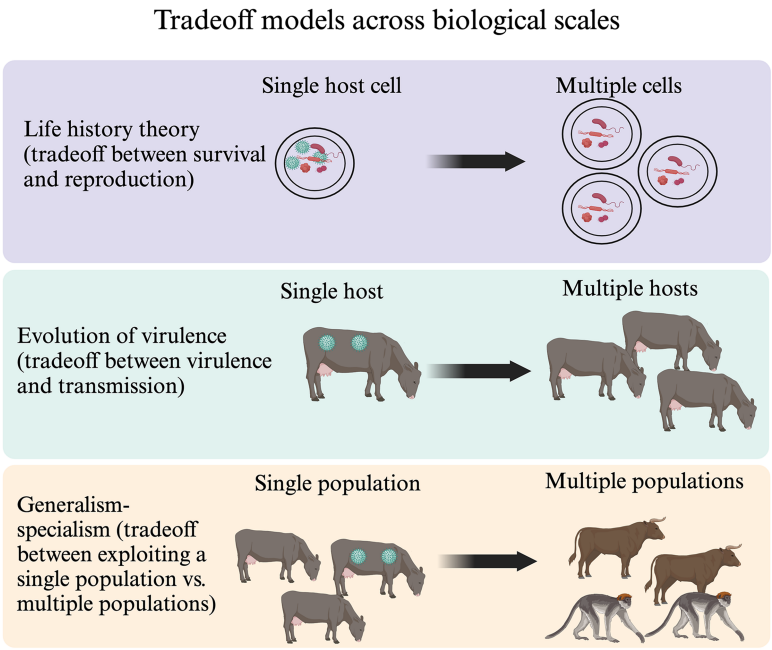

Figure 4. A hypothetical illustration of the evolutionary frameworks at different biological scales. Here, we present a scenario where the existence of three frameworks analyzed in this study–life history theory, evolution of virulence, and generalism–specialism–could be reconciled through application to different scales of virus–host interactions. Life history theory could be applied to the problem of reproduction within a cell versus surviving transmission between cells. The evolution of virulence might apply to virulence traits associated with growth within a host at the organismal scale, and transmission to other (generally conspecific) hosts. Lastly, for the generalism–specialism dichotomy, the purported tradeoff exists between growth fitness within a single species or population and the ability to propagate across different species or populations. This entire model represents a speculative view on tradeoff frame- works whereby they could all operate and have relevance for our efforts to understand the constraints underlying virus evolution.

relative robustness of the theory across virus–host systems The results of the more granular analysis of the generalism–specialism dichotomy (organized by virus–host type) demonstrate that, overall, there is a negative relationship between traits, indicative of a tradeoff (Fig. 1, Table 2). However, the low number of studies and the lack of virus–host diversity across studies caution against drawing large conclusions about the applicability of this framework.

Analyses by virus–host type

There are several noteworthy patterns that appeared in the analysis of particular virus–host types. Overall, datasets from studies of plant viruses were the most numerous, most of which examined tradeoffs within the evolution of virulence. This is appropriate, given the vastness of plant life in the biosphere (Bar-On et al. 2018). These studies demonstrated evidence for a positive relationship between transmission and virulence consistent with other studies (Lipsitch and Richard Moxon 1997, Sacrist´an and Garc´ıa-Arenal 2008). Patterns in animal viruses were profoundly influenced by data from VSV, which revealed a negative relationship between traits across tradeoff frameworks. Datasets of bacteriophages contained the most outliers and showed a significant negative relationship after they were removed. The presence of multiple outliers in these datasets here could be derived from studies where phages were exposed to widely different stressors. The stressors include elevated heat (Dessau et al. 2012), urea (Heineman et al. 2012), and differing times spent outside the host (Garc´ıa-Villada and Drake 2013), among other variables. Such diverse stressors can also obscure signals of correlational traits and differences in how traits are measured. In addition, another key contributing factor to the overall correlation may be the molecular basis of the viral genome (e.g. RNA vs. DNA, single-stranded vs. double-standard), which impacts life history traits of bacteriophages, particularly reproduction and survival (Kindt et al. 2001, Smith et al. 2001). Such differences can further confound our application of tradeoff frameworks in virus evolution.

Limitations

The limitations of our study reflect challenges in the greater literature of virus evolution that have offered unclear definitions and inconsistent measurements of traits. In particular, studies of this sort can suffer from being underpowered. We have attempted to address this issue by providing a transparent data pipeline and a detailed discussion of the inclusion criteria. Through this, we were able to extract datasets that facilitated a meaningful analysis that we believe contributes to the greater literature on virus evolution.

This specific issue aside, a more general question remains: what makes a study worth including in meta-analyses? We have attempted to explain the technical aspects of this study in the "Methods" section, but more broadly, the usefulness of a study relates to the metrics used in measuring tradeoffs and the clarity of the data reported in the study. Though translating theoretical variables into measurable laboratory parameters can be challenging, scientists can aim for consistency, clarity, and transparency in measuring and reporting experimental data. Relatedly, it is crucial that diverse sample of viruses are included in laboratory-based studies, ideally not solely viruses of clinical or agricultural importance to humans.

Our study aimed to test experimental evidence for the evolution of virulence, life history theory, and the generalism–specialism dichotomy frameworks by analyzing data

across virus–host systems. We were focused only on experimental data where traits associated with the frameworks were directly tested. This is a stringent but necessary constraint. This criterion eliminates hundreds of studies of virus evolution (mostly with respect to the evolution of virulence) in theoretical, field and clinical research, many rigorously examining traits associated with, for example, clinical outcomes (virulence) and compound metrics related to transmissibility like the basic reproductive ratio (also known as the R0) (Chowell et al. 2004, Yang et al. 2009, Althaus 2014, Grubaugh et al. 2019, Althouse et al. 2020). These studies are important, and many offer insight into the practical manifestations of virus evolution (and clarify the shape of epidemics). Thus, our study does not imply that findings from these natural settings are irrelevant or incorrect. Within the definitions and structures outlined in those studies, patterns of virulence evolution very well may operate according to existing canon. Instead, our study aimed to test whether these virus evolution frameworks sensu stricto (many animated in field and clinical studies) are based on rigorous laboratory-derived findings. Future work may take an analogous approach to ours but based on clinical or field-based studies. Also, our study focuses strictly on viruses. There are, of course, analogues to the questions that we have asked in other pathogen types, where some studies have examined how virulence or cost of infection can vary based on tradeoffs with other factors (Budischak et al. 2018. Turner et al. 2021). While our study is a meta-analysis that aims to cover a breadth of studies, we recognize the vastness of the literature on tradeoff models in pathogen evolution. Though this study is not a review, we have attempted to engage a large breadth of literature on the application of tradeoff models in virus evolution. Nonetheless, there are many review, opinion, and perspective articles that have argued multiple perspectives on virulence and other frameworks, some challenging the paradigms not unlike ours (Day 2002a, 2003, Day and Proulx 2004, Alizon et al. 2009, Cressler et al. 2016, Acevedo et al. 2019). We encourage those interested to engage with this corpus.

Other study limits apply to specific virus-host systems, to different kinds of biases that may skew the literature, how they may impact the literature, and, by extension, the data analyzed in the meta-analysis. For example, many plant viruses in this study affect hosts of economic interest to humans and are not representative of the vast diversity of plant viruses in nature. More generally, studies of this sort are influenced by publication biases of various kinds (see Supplementary Fig. S4). Our meta-analysis did not aim to formally test hypotheses about the nature or direction of biases but rather analyze data from the literature based on a set of clear criteria (see "Methods" section). However, we did use funnel plots to assess the presence of publication bias in our meta-analysis. But our results highlight that published data isn't necessarily reflective of the natural world, but rather, how the process of science may select certain sorts of objects (virus-host systems in our case) for data collection. This is a common challenge in meta-analyses, which undoubtedly influences data and the inferences drawn from them.

Lastly, we should highlight what might be a glaring omission: we did not include tradeoffs in the evolution of resistance to antivirals, antibodies, and their associated evolutionary processes because resistance is often associated with reduced replication. There is a large and growing literature in these arenas, especially on HIV (Pennings 2013), influenza (Holmes et al. 2021), and SARS-CoV-2 (Segala et al. 2021). But while tradeoffs associated with resistance are of immense biomedical significance, these apply to specific circumstances of viruses evolving against small molecules or other antagonists, and do not qualify as a grander explanatory theory of virus evolution in the same way as the three foci of this study. In fact, one can argue that the evolution of HIV resistance to antiviral drugs (for example) is a special case of virus evolution that could be modeled using one of the three large frameworks in our study (evolution of virulence, life history theory, the generalism–specialism dichotomy).

Ideas and speculation

While our findings introduce skepticism that may undermine our confidence in existing frameworks, we emphasize that this could start a productive effort to generate new perspectives, theories, and tests. Future work could offer ways for the three frameworks examined in this study to be compatible. One hypothetical reconciliation between the different frameworks and our findings involves the notion that different frameworks apply to different scales of the interaction between virus and host. Previous studies have suggested how the evolution of virulence may manifest differently across scales of the disease emergence process (Geoghegan and Holmes 2018, Visher et al. 2021) and other temporal aspects of infection (Day 2003). We can extend this concept to include settings other than the disease emergence process. Figure 4 offers one such hypothetical scenario: that life history theory may apply to viruses in the intra-host setting, that the evolution of virulence dictates constraints at the between-host scale, and generalism–specialism operating at the between-species scales. We acknowledge that this overview is pure conjecture: we offer no data-driven reason to suggest any specific relationship between frameworks and scales exists. We offer it to suggest the perspectives that might explain our results and the general complexity of theory in virus evolution. Future efforts can directly test these theories or establish new multiscale theories.

Conclusion

The quest for unified theoretical frameworks to understand and predict virus evolution has a long history, a robust present, and a promising future. Current technological and computational advances offer new ways to measure virus traits at a large scale, with accompanying tools allowing scientists to uncover the genetic bases for such changes (Grubaugh et al. 2019, Black et al. 2020, Vogels et al. 2021). However, large data approaches alone cannot serve as a replacement for theoretical ambiguity. Scientists who study topics related to the evolution of infectious diseases can challenge themselves with basic questions about assumptions built into the theoretical frameworks used to study and discuss virus evolution.

Our findings indicate that the picture offered from direct tests of virus evolution frameworks is cloudy. This ambiguous picture implores us to revisit and possibly reconfigure theories that guide the study of virus evolution. Such a new picture could consider how variation in host–parasite systems, definitions of terms, measurements, analysis methods, and modeling approaches can profoundly complicate our expectations for virus evolution. Theory that accommodates this pluralism might (paradoxically) clarify our picture of virus evolution, with implications for evolutionary biology, disease ecology, evolutionary medicine, and epidemiology.

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Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Author contributions

Project conception: K.K., C.B.O, Collected data: K.K, Analyzed data: K.K., C.B.O, Integrated and interpreted results: K.K., W.C.T., P.E.T., C.B.O, Supervision: C.B.O. Writing, first drafts: K.K., C.B.O, and Revisions, editing, and commenting: K.K., W.C.T., P.E.T., C.B.O.

Supplementary data

Supplementary data is available at *VEVOLU* online.

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Data availability

Data and code can be found on GitHub: https://github.com/OgPlexus/MetaKetty1

Supplementary information

In the Supplementary Information, we offer additional figures and analyses that support findings from the main text. These include the PRISMA flow diagram (Supplementary Fig. S1), an analysis of all datasets from the meta-analysis (Supplementary Fig. S2), an analysis of the evolution of virulence and life history theory combined (Supplementary Fig. S3), an analysis of publication bias (Supplementary Fig. S4), an analysis of the evolution of virulence framework without N =3 datasets (Supplementary Fig. S5), an analysis of plant viruses combined with mycoviruses (viruses that infect fungi) (Supplementary Fig. S6), statistics from all analyses (Table 1), forest plots with very high contrasts of analyses of frameworks, and virus–host types (Supplementary Figs S7–9).

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